Identification and characterization of ice nucleation active bacteria isolated from precipitation

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Abstract

Since the 1970s, a growing body of research has suggested that bacteria play an active role in precipitation. These bacteria are capable of catalyzing the formation of ice at relatively warm temperatures utilizing a specific protein family which aids in the binding of water molecules. However, the overall biodiversity, concentration, and relationship of ice nucleation active (ice+) bacteria with air mass trajectories and precipitation chemistry is not well studied. Precipitation events were collected over 15 months in Blacksburg, VA and ice+ bacteria were isolated from these samples. From these samples, 33,134 total isolates were screened for ice nucleation activity (INA) at −8 °C. A total of 593 of these isolated positively confirmed for INA at the same temperature in subsequent tests. The precipitation events had a mean concentration of 384±147 colony forming units per liter. While the majority of confirmed ice+ bacteria belonged to the gammaproteobacteria, a well-studied class of bacteria, including ice+ species of *Pseudomonas*, *Pantoea*, and *Xanthomonas*, two isolates were identified as *Lysinibacillus*, a Gram-positive member of the Firmicute phylum. These two isolates represent the first confirmed non-gammaproteobacteria with INA. After further characterization, the two isolates of *Lysinibacillus* did not appear to use a protein to freeze water. Instead, the *Lysinibacillus* isolates used a secreted,

nanometer-sized molecule that is heat, lysozyme, and proteinase resistant. In an attempt to identify the mechanism responsible for this activity, species type strains were tested for INA and UV mutants were generated to knock out the ice+ phenotype. Based on these results, only members of the species *L. parviboronicapiens* exhibit INA and the genes responsible for the activity may lie within a type-1 polyketide synthase/non-ribosomal peptide synthase gene cluster. This gene cluster is absent from the genomes of all non-ice+ strains of *Lysinibacillus*, and contains mutations in five of the nine ice nucleation inactive mutants generated from the rain isolated strain. To better understand the phylogenetic relationship among ice+ *Lysinibacillus*, a comprehensive reference guide was compiled to provide the most up-to-date information regarding the genus and each of its species. This reference will be available to other researchers investigating *Lysinibacillus* species or other closely related genera.

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General Audience Abstract

It is a common misconception that water freezes at 0°C (32°F). In clouds, water may remain liquid until -37 to -40°C (-35 to -40°F). At temperatures warmer than this, water molecules must collect around small particles that can help form ice, called ice nuclei. Numerous ice nuclei have been identified, ranging from dirt and dust, to volcanic ash, and even to pollen, fungi, and bacteria. One of these bacteria, *Pseudomonas syringae*, was identified as an ice nucleus in the 1970's when it was discovered that it was increasing susceptibility of corn to frost damage. Since then, other *Pseudomonas* species as well as other bacteria within the same family of bacteria have been shown to have the ability to freeze water at relatively warm temperatures utilizing a specialized protein. Despite numerous studies on how these bacteria can exist in the atmosphere and how they can freeze water, the extent of this freezing ability, the concentration of bacteria in precipitation, and how cloud chemistry affects these bacteria has not been widely studied. In this study, precipitation was collected over the course of 15 months and the bacteria found within the collected precipitation were checked to see if they could act as ice nuclei. We found many of the previously described bacterial ice nuclei in the precipitation samples, but also identified a previously unidentified bacterium capable of freezing water. This bacterium, *Lysinibacillus parviboronicapiens*, does not

use the same method of freezing as the other described bacterial ice nuclei. As such, we set out to determine the method it uses. We have determined that this bacterium utilizes a heat-stable, nanometer-sized particle that is not a protein. To better understand this molecule, representative strains of each species of this genus of bacteria were tested for their ability to freeze water, however, only this species has the ability. To further identify the molecule, UV radiation was used to disrupt the bacteria's ability to freeze water, and the genes responsible were identified. Based on these results, we have tentatively identified the responsible genes as part of a polyketide synthase gene cluster. This gene cluster is responsible for producing small molecules that provide some survival advantage for the bacteria, in our case, possibly the ability to freeze water. As a final step, and to help serve other researchers, a comprehensive analysis of the entire *Lysinibacillus* genus has been performed and a reference guide has been generated to help describe and distinguish individual species.

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Attributions

Noam Eckshtain-Levi and Sophie LeBlanc of the Department of Plant Pathology, Physiology, and Weed Science at Virginia Tech prepared some preliminary complementation of the UV mutated *Lysinibacillus parviboronicapiens* VT1065.

Richard Helm of the Department of Biochemistry at Virginia Tech performed several preliminary tests to identify the *Lysinibacillus* ice nucleation active molecule.

Hinrich Grothe of the Institute of Materials Chemistry at the Vienna University of Technology ran infrared spectroscopy on the *Lysinibacillus* ice nucleation active molecule preparations and tested the ice nucleation activity of the preparations using his oil immersion method.

Christopher Lefevre of the Institut National de la Recherche Agronomique, France provided scanning electron microscopy pictures of *Lysinibacillus* and the *Lysinibacillus* ice nucleation active molecule preparations.

Haijie Liu of the Department of Plant Pathology, Physiology, and Weed Science at Virginia Tech served as a mentor and successfully transformed a plasmid into *Lysinibacillus parviboronicapiens* VT1065.

Caroline Monteil of the Institut National de la Recherche Agronomique, France designed the methods and began the collections of the precipitation sample collection experiments. Dr. Monteil

also served as a mentor throughout this project, a project lead and corresponding author on Chapter 2, and a co-author on Chapters 3 and 4.

Julia Sanchez Martin del Campo of the Department of Biochemistry at Virginia Tech ran the *Lysinibacillus* ice nucleation active molecule preparations on the HPLC and performed ultraviolet spectroscopy on the preparation.

David Schmale III of the Department of Plant Pathology, Physiology, and Weed Science at Virginia Tech, served as a committee member and co-author on Chapter 2. He also provided financial support through the National Science Foundation (NSF) Grant Numbers DEB-1241068 (Dimensions: Collaborative Research: Research on Airborne Ice-Nucleating Species (RAINS)).

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Long Tian of the Department of Genomics, Bioinformatics, and Computational Biology at Virginia Tech provided bioinformatic analyses on the *Lysinibacillus* whole genome sequences and provided many of the comparisons required to identify the putative ice nucleation gene cluster.

William Whitman of the Department of Microbiology at the University of Georgia offered the chance to compose the chapter on *Lysinibacillus* for the Bergey's Manual of Systematics of Archaea and Bacteria (Chapter 5).

Chapter One: Literature review

Introduction

Bacteria are all around us, on our telephones (Rahi *et al.* 2017), on our skin (Grice *et al.* 2009), within creeks (Strahan *et al.* 2011) and, in fact, in every facet of the water cycle (Morris *et al.* 2008). While many of these bacteria are affected by the environment where they reside, a growing body of evidence suggests that some of these bacteria are capable of changing their environment to better suit their own needs. One example of this phenomenon is bacterial ice nucleation, whereby certain bacteria are capable of freezing water to their advantage.

The presence of microbial ice nucleators has been extensively studied on plants beginning in the 1970's with the identification of *Pseudomonas syringae* as the causative agent of frost damage on corn (Arny *et al.* 1976) and as the ice nucleation active (ice+) portion of decaying plant matter (Maki *et al.* 1974, Vali *et al.* 1976). More recently, however, *P. syringae* has been isolated from rainfall (Lu *et al.* 2016), snow (Christner *et al.* 2008) and snowpack (Monteil *et al.* 2012), and cloud water (Amato *et al.* 2005, Amato *et al.* 2007, Joly *et al.* 2013); it has been demonstrated that *P. syringae* can exist in every compartment of the water cycle (Morris *et al.* 2008). This idea was supported by David Sands in the 1980's, suggesting that bacteria are passively transported throughout the water cycle. However, some bacteria, like *P. syringae*, demonstrate the ability to catalyze the formation of ice at relatively warm temperatures and can actively contribute to the water cycle by producing precipitation in the cloud layer (Sands *et al.* 1987). The ice crystals generated around ice+ bacteria continue to grow in size as they impact neighboring water molecules until the upward air currents are unable to sustain the crystals and gravity causes the crystals to fall as precipitation (Weidman 2015). Since the discovery of *P. syringae* as an ice nucleator, several other γ-proteobacteria have been identified with varying degrees of ice

nucleation activity (INA), including members of the genera *Pantoea* (Abe *et al.* 1989, Gurian-Sherman *et al.* 1986) and *Xanthomonas* (Zhao and Orser 1988). Both genera have been isolated from plants, and like *Pseudomonas* may be associated with the water cycle. However, the presence of ice+ bacteria besides *P. syringae* in precipitation has not been extensively studied. Likewise, no non-gammaproteobacteria had been confirmed as ice+ prior to this work.

This work will focus on the discovery and characterization of a novel ice nucleation mechanism in bacteria of the species *Lysinibacillus parviboronicapiens* as well as an overall description of the genus in order to fully describe each published species and establish a phylogeny of the genus.

Cloud and precipitation formation

In order for clouds to form, water vapor present in the atmosphere must first condense around a small particle, molecule, or organism called a cloud condensation nuclei (CCN). These CCN serve as a surface for the water molecules to adhere to and can remain suspended in the atmosphere. Because of the small size of the CCN and associated water molecules, the size of the combined CCN and water droplets is initially too small (0.2μm of diameter) for these droplets to fall to Earth as precipitation. Once enough of these CCN associate together, they are capable of forming a cloud, however, the water in these clouds still exists in droplets too small to fall as precipitation. Only when water droplets reach a size of 20μm in diameter do they have a mass large enough to form precipitation (Kerminen *et al.* 2012, Weidman 2015).

In order for the water within the clouds to form precipitation, one of two events occur based on the latitude of the cloud. In tropical zones, the clouds are comprised entirely of water droplets, as such these cloud droplets undergo collision-coalescence until they reach the size of rain droplets.

In this system, turbulent air causes larger water droplets to impact and absorb other, smaller droplets. However, in temperate and polar regions, clouds exist as a heterogenous mixture of water and ice crystals at most elevations. In these clouds, precipitation forms following the ice-crystal process (Weidman 2015). Without any ice nuclei to help freeze the water, pure water does not spontaneously form ice crystals until it reaches temperatures as low as -36°C to -40°C (Debenedetti and Stanley 2003, Moore and Molinero 2011, Wolber 1993). Instead, it exists in a supercooled state until an ice nucleus interacts with the water. For water at -40°C, an ice embryo of critical size requires approximately 70 molecules to form. At -5°, it requires 45,000 molecules in order to overcome the energy barrier of ice formation (Joly *et al.* 2013). However, when water molecules in the droplet associate with a non-water surface that can act as an ice nucleus, the energy requirements diminish, allowing for the formation of ice at higher temperatures.

Several inorganic substances are capable of nucleating ice, such as silver iodide, soot, mineral dusts, and metallic particles (Atkinson *et al.* 2013, O'Sullivan *et al.* 2013, Teller *et al.* 2012). These inorganic particles are capable of freezing water as warm as -20°C. Additionally, organic molecules such as amino acid crystals, monolayers of long chain alcohols, plant synthesized terpenes, and other organic compounds are capable of freezing water, some even as warm as -4°C (Joly *et al.* 2013). As these particles freeze, they generate a dry zone around the ice crystal (Nath and Boreyko 2016), in turn, more humid air shifts to equilibrate the dry halo and is in turn incorporated into the growing ice crystal. As long as the air is sufficiently humid and the rate of condensation onto the crystal is greater than the evaporation rate, the ice will continue to grow in size. A study performed in 1994 showed that cirrus clouds, while incapable of producing precipitation, do show distinct regions of ice production, ice growth, and sublimation (Heymsfield and Miloshevich 1995). This model can be used to describe precipitation bearing cloud as well, as

the varying temperatures and pressures within these clouds coincide with the data presented by Heymsfield and Miloshevich (Weidman 2015).

Biological ice nucleators

Organic ice nucleators are capable of freezing pure water at the highest temperature ranges of any identified ice nucleator. Most biological ice nucleators are able to nucleate ice above -14°C, with *P. syringae* being able to nucleate ice at a temperature as high as -1°C (Morris *et al.* 2004). Certain plant species, including *Lobelia telekii*, contain compounds in their flowers that are active as ice nuclei at -4.5°C (Krog *et al.* 1979). Additionally, proteinaceous ice nuclei have been discovered in several additional plant species, including *Prunus* spp. (Gross *et al.* 1988) and winter rye (Brush *et al.* 1994). Ice nucleation proteins (INPs) have also been identified in animal species, primarily in fish, reptiles, and amphibians. It is hypothesized that these animals use the INPs in the blood as adaptive freezing tolerance (Costanzo and Lee 1996) while the plant species utilize it to control the freezing of water within the cells to prevent large scale frost damage and improve overall frost tolerance (Brush *et al.* 1994, Gross *et al.* 1988, Krog *et al.* 1979). In a 2015 study, it was shown that not only can plants control the freezing of water internally, but microcrystalline cellulose particles can serve as atmospheric ice nuclei. These particles can nucleate ice at a significant level at -21°C and colder. While this is significantly colder than bacterial or fungal ice+ strains, it does fall within the range of some pollen types as well as many inorganic ice nuclei (Hiranuma *et al.* 2015). However, the majority of plant ice nuclei come in the form of pollen, which utilize an exopolysaccharide, or other unknown extracellular molecule rather than a protein to induce ice nucleation activity (Pummer *et al.* 2012). In addition to plant and vertebrate species, several members of the fungal genus *Fusarium* are capable of nucleating ice as warm as -5°C, and

several fungi and algae associated with lichen are also capable of ice nucleation warmer than -5°C (Huffman *et al.* 2013, Humphreys *et al.* 2001). However, the most abundant, and well-studied microorganisms are the bacterial ice nucleators. First independently identified in 1976 by Gabor Vali (Vali *et al.* 1976) and Steve Lindow (Arny *et al.* 1976), bacterial ice nucleators are able to freeze water at the highest recorded temperature of -1°C (Morris *et al.* 2004).

In 1971, Vali noted that there was a higher quantity of ice nucleators capable of freezing water between -2°C and -5°C on decaying plant matter (Vali 1971); In 1974, colleagues at the University of Wyoming discovered that the causative agent of the freezing was *P. syringae* (Maki *et al.* 1974). Utilizing the research by Maki *et al.*, Vali concluded that *P. syringae* produces highly active ice nuclei at relatively warm temperatures, and that the bacteria were ice+ on a wide variety of growth media. He also suggested that it was the bacteria that are the nuclei. He concluded that *P. syringae* may have the capability to play a role in the atmospheric precipitation processes and effect climate (Vali *et al.* 1976).

The same year that Vali published his findings, 1976, Steven Lindow published his own work in which he found *P. syringae* to contribute to frost damage on *Zea mays* (maize) (Arny *et al.* 1976). Lindow found that *P. syringae* was capable of freezing water between -2°C and -5°C, which he suggested caused the explosive crystallization of the intracellular water of corn (*Z. mays*). However, the intracellular water of *P. syringae* did not freeze and cause any damage to the bacteria until approximately -8°C. Two years later, Lindow identified ice+ bacteria from leaf washings from locations in California, Colorado, Florida, Louisiana, and Wisconsin. He determined that conifers, as a group, were unlikely to harbor any ice+ bacteria, and all the ice+ isolates that he identified were either *P. syringae* or *Erwinia herbicola* (later reclassified as *Pantoea agglomerans*) (Lindow *et al.* 1978). Based on his findings, he suggested, as Vali had two years prior, that plant

surfaces may be a primary source of ice nucleators found in the atmosphere. Moreover, due to the bacteria's ability to nucleate ice, pathogenic species can utilize this mechanism to cause frost damage on the host leaf surfaces in order to facilitate host invasion.

Since the identification of *P. syringae* and *Pa. agglomerans* as common ice nucleators, bacterial ice nucleators have been isolated and studied extensively on plant matter and more recently, in the atmosphere and in precipitation. In 2013, Cindy Morris and colleagues of the French National Institute for Agricultural Research (INRA), and leading researchers on bacterial ice nucleation, identified several key ice+ bacteria from cloud water, including *P. syringae*, *Xanthomonas spp.* and *Pseudoxanthomonas spp.*. Morris *et al.* were able to show that these strains were capable of freezing water at a temperature range of -3°C to -6°C, depending on the strain (Joly *et al.* 2013).

In 2005, a study looking at ice nucleation active bacteria in Siberian permafrost identified two previously unknown ice+ bacteria that were not members of the gammaproteobacteria. These two bacteria, *Exiguobacterium sp. 255-15* and *Psychrobacter sp. 273-4*, exhibited ice nucleation activity at -10°C (Ponder *et al.* 2005). However, the INA of these bacteria could not be confirmed in independent follow-up experiments.

Bacterial survival and transport in the atmosphere

In 1961, P.H. Gregory described the dispersal of organisms, including bacteria, fungi, and certain viruses, in the atmosphere. In his work, Gregory focuses on the dispersal of these organisms into both, the lower and upper atmosphere. He states that bacteria are not generally detached from their native surfaces by air currents, but instead require either a mechanism for aerosolization, or association with a particle that is more readily aerosolized. Once in the troposphere, bacteria are

exposed to a vast number of environmental stressors, including lower oxygen concentrations, ultraviolet radiation, and limited nutrient availability (Gregory 1961). However, this does not limit the number of bacteria that are present in the atmosphere. It is likely that the vast majority of bacteria isolated in the atmosphere have originated from terrestrial sources including plant, soil habitats, and water sources (Bowers *et al.* 2009). Recent developments in the understanding of the movement of bacteria within the atmosphere have shown, specifically, the importance of plants in the movement of bacteria. As plants emit water vapor via transpiration, microbes are released as well (Morris *et al.* 2014) and carried via air currents into the upper regions of the atmosphere where they are deposited into the cloud layer. Recent evidence has also linked rainsplash as a viable mechanism for the transport of microbes into the atmosphere (Pietsch *et al.* 2017).

While the atmosphere is a harsh environment to bacteria and other organisms, previous studies have shown that not only are bacteria, fungi, yeast, and protozoa ubiquitous in the atmosphere (Bauer *et al.* 2002, Matthias-Maser and Jaenicke 1995), but they are also capable of survival under these harsh conditions. With the increased interest in these airborne organisms, several studies were performed in order to determine if these organisms are simply surviving in the atmosphere, or if any are even capable of growth and replication within the cloud layer. A study in 1996 demonstrated that certain bacteria, fungi, and yeasts are capable of utilizing fog droplets as a culture media to promote both their growth and survival while suspended with in banks of fog. It is hypothesized that bacteria at higher elevations can utilize similar methods to extend their longevity within the clouds (Fuzzi *et al.* 1997). This hypothesis was shown to have merit in a 2001 study that demonstrated that supercooled water droplets in the cloud layer can in fact harbor actively growing bacteria (Sattler *et al.* 2001).

However, once bacteria and other organisms have reached the atmosphere, they are trapped until they can be deposited out of the atmosphere again. Several mechanisms can be utilized in order to precipitate from the atmosphere. Gregory states that impaction, turbulent deposition, and electrostatic deposition are the primary means for biological aerosols to settle out of the atmosphere, while several minor mechanisms also contribute to the process. While a bacterial cell can settle out of the atmosphere due to the effects of gravity, deposition occurs more commonly through the above-mentioned mechanisms due to the small size and mass of the bacterial cells. In order for the bacteria to undergo deposition via impaction, the bacteria must associate with a much larger object, such as leaves, twigs or wind-blown material. However, higher wind speeds result in more efficient impaction rates. For turbulent deposition, the bacteria must be blown over a relatively horizontal surface, but also encounter turbulent airflow, which causes the spontaneous deposition of a large number of particles. This process has been demonstrated effectively in wind tunnel experiments in his work, but is hypothesized to occur naturally as well (Gregory 1961). This hypothesis was later shown to be correct when a study was performed at the Keiner Feldberg in Taunus, Germany. This study demonstrated that clouds with higher wind velocities contained larger quantities of biological particles (Matthias-Maser *et al.* 2000). Gregory also states that electrostatic deposition is another primary means of deposition, but is used primarily by negatively charged fungal spores, but not by bacteria. Finally, he addresses some minor deposition mechanisms, including rain-washing; for rain-washing to affect airborne bacteria, the bacteria must be impacted by a falling water droplet that is between 1mm and 5mm in diameter (Gregory 1961). A recent study performed in collaboration with this work (Appendix 3) has shown that bacteria and fungi can be scrubbed from the atmosphere and can often include ice nucleation active bacteria within such samples (Hanlon *et al.* 2017). However, when Gregory's work was published,

ice+ bacteria had not yet been identified, and, as such, he does not consider possibility of ice+ bacteria using this mechanism as a transportation method in his work. Organisms that exhibit INA, can catalyze the formation of larger ice crystals via INPs (Deininger *et al.* 1988, Wolber *et al.* 1986, Zhao and Orser 1988), extra cellular molecules (Diehl *et al.* 2002, Pouleur *et al.* 1992), or other methods (Du *et al.* 2017) and potentially use those as a means of deposition (Joly *et al.* 2013). However, not all ice+ bacteria are present in the cloud layer; bacteria in the troposphere can also be carried by this wash out, and would not contribute to precipitation formation as suggested by Hanlon *et al.* (Hanlon *et al.* 2017).

To prove that bacteria are capable of surviving long journeys in the cloud layer, a study was performed utilizing a cloud simulation chamber. In this study, *P. syringae* was introduced into a simulated cloud and the survival of the bacteria was observed over time. It was concluded that, given optimal wind and meteorological conditions, bacteria such as *P. syringae* could survive in the cloud layer for thousands of kilometers, enough to transverse the Atlantic Ocean (Amato *et al.* 2015). However, *P. syringae* cannot form any type of protective endospore, suggesting that spore-forming bacteria could exist in these cloud layers for an even longer period, as they can better protect themselves from UV radiation and other harsh conditions.

Bioprecipitation theory

David Sands postulated in the 1980s that ice+ *Pseudomonas* and *Xanthomonas* live as epiphytes on the surfaces of vegetation and/or associate with small particles. In turn, these bacteria are transported via upward air currents into the atmosphere as suggested by Gregory in 1961. However, not all of these bacteria are simple “passengers” that are transported through the atmosphere. Several genera may be active players in forming precipitation via the formation of ice

crystals in clouds (Morris *et al.* 2014, Sands *et al.* 1987). Precipitation then carries these bacteria back to relatively nutrient-rich environments, such as plant leaves and soil. However, not all bacteria in the atmosphere are ice nucleators; these species are passively transported throughout the entire water cycle relying on turbulent airflow and impaction to be transported back to the earth's surface. Because these bacteria cannot catalyze their return to Earth, they may be subject to lower nutrient conditions for extended amounts of time.

Numerous studies have been performed to determine how ubiquitous *P. syringae* is in the environment. *P. syringae* has been isolated from rain, snow, freshwater, and clouds (Amato *et al.* 2007, Christner *et al.* 2008, Goodnow *et al.* 1990, Monteil *et al.* 2014). Because of its abundance, it was later shown that *P. syringae* can be linked to every facet of the water cycle (Morris *et al.* 2008), and its ability to nucleate ice (Arny *et al.* 1976, Maki *et al.* 1974) gives further evidence for the bioprecipitation theory.

This theory provides a framework for the study of local and global dispersion of microbes. However, the extent to which bacteria are capable of living in and being transported through the atmosphere is not well known. It is much easier to track the movement of host-specific obligate pathogens, such as several crop pathogenic fungi including *Botrytis cinerea* (Monteil *et al.* 2014) and *Fusarium graminearum* (Schmale *et al.* 2012), since the location of their emission corresponds to agricultural fields on which their hosts are cultivated. By identifying the emission source, the amount of time spent in the atmosphere can be back-calculated in order to better understand the microbial dispersal patterns (Purdy *et al.* 1985). For non-obligate biotrophs such as all known ice+ bacteria, which can live practically everywhere, it is much more difficult to elucidate the original emission source. Therefore, their overall dispersal and movement in the atmosphere is significantly harder to track (Monteil *et al.* 2014). With technological advancements in unmanned aerial

vehicles (UAVs), however, it may become easier to monitor and track the emission and dispersal of these organisms (Schmale *et al.* 2012). Despite the advancements in tracking capabilities, identifying emission sources and overall time bacteria are transported within the cloud layer remains an open realm of investigation.

Bacterial INA Genes

In 1986, DNA fragments containing the INA genes of *P. syringae* and *E. herbicola* were cloned and expressed in *E. coli*. It was determined that both *ice* genes (later renamed *inaZ* and *iceE*) showed substantial homology to each other (Orser *et al.* 1985). The same year, a region of DNA coding for 122 imperfect repeats of a consensus octapeptide pattern was identified. It was suggested that this region was responsible for ice nucleation activity in bacteria, but more specifically, each repeated peptide contributed to the overall INA as a deletion analysis showed that up to 68 repeats could be removed, however, a drop in INA was observed with each repeat removed (Green and Warren 1985). The protein product of the cloned *inaZ* gene from *P. syringae* was overexpressed in *Escherichia coli* the following year and was used to compare the amino acid composition to that of the predicted sequence of the gene. It was demonstrated that the protein contained a Met-Asn-Leu-Asp-Lys-Ala-Leu-Val-Leu- N-terminal region, which matched perfectly the predicted gene sequence (Wolber *et al.* 1986). Later that year, the *inaW* gene from *P. fluorescens* was also identified (Warren *et al.* 1986). The InaZ protein was shown to accumulate during stationary phase, leading to the ice nucleation active phenotype; however, the InaW protein was expressed during all stages of *P. fluorescens* growth, and ice nucleation activity correlated with protein abundance (Deininger *et al.* 1988). In 1990, the *inaX* gene from *Xanthomonas campestris* was sequenced and a large 153 octapeptide repeating region was identified. When the

sequence was compared to that of the *inaW*, *inaZ*, and the *iceE* gene, and it was found that there was a 65.8-68.8% sequence homology between the *inaX* and the other three genes (Zhao and Orser 1990). In 2012, another variant of the INP was discovered in *P. syringae*, named InaQ, which shares sequence homology with three other *P. syringae* INPs: InaK, InaZ, and InaV (Li *et al.* 2012).

Upon further study of the proteins, it was found that INPs can exist in three different confirmations: Class A, B, and C (Kajava and Lindow 1993). Based on the results of the study, it was suggested that the Class C structure is the most common. Proteins with a Class C structure can acquire a Class B structure, and further change to a Class A structure. Proteins with a Class C structure are responsible for freezing water at the coldest temperatures (warmer than -7.6°C ± 0.9), Class B proteins are the mid-range freezing proteins, ranging from -4.6°C to -5.6°C, and finally, Class A proteins are responsible for freezing at the warmest temperatures (warmer than -4.3°C ± 0.3). It was also found in this study that the proteins are highly affected by pH. Class A proteins denatured below pH 5.5, while Class B was able to withstand pH as low as 4.5, and Class C was stable until pH 3.5. At lower pH values the INP was irreversibly damaged and activity could not be regained.

In order to determine where the protein localizes, *P. syringae* and *E. coli* containing the transformed gene *iceC* were broken apart. Both membranes were tested to see if any ice nucleating particles existed on either fraction. It was determined that the INP associates with the outer membrane due to the portioning of the 3-ketodeoxyoctanoate (Lindow *et al.* 1989). In 1993, the first three dimensional model of the INP was proposed (Kajava 1995). Molecular modelling predicted that the protein is largely planar with one side associating with the cell membrane and the other side arranged such that the water molecules could associate with the protein to facilitate

the formation of the ice embryo. In a similar study, Graether and Jia (*2001*) demonstrated that the INP of *P. syringae* contains large repeating units that fold to form a β-helical protein. In addition, they determined that the protein had a structure similar to that of the antifreeze protein (AFP), which prevents the formation of larger ice crystals. However, the distinguishing feature between these two protein classes is the overall size of the structures that interact with the water molecules. The AFPs have a small active site, which is capable of binding the water to prevent further expansion of the ice crystal structure by forming a hexagonal bipyramidal crystal (Kobashigawa *et al.* 2005), while the INPs have a much larger active surface which acts as the template for the formation of the ice crystal lattice. The INP has an overall structure with non-repetitive N- and C-terminal domains and a large, repetitive central domain that has a conserved 16 amino acid repeating pattern of AGYGSTxTAxxxSxLx. This pattern is repeated three times in each of the 48 larger repeating residues in the central domain. The N- and C-terminal residues are considerably smaller (175 and 49 residues, respectively) and are hypothesized to anchor the protein to the membrane (Graether and Jia 2001), supporting Lindow's previous results (Lindow *et al.* 1989). Garnham et al, further refined the model and proposed a novel protein structure that has bridged active sites within the larger β-helical protein. In this study, they relied on the same 50 to 80 16-amino acid tandem repeats found by Graether and Jia (GYGSTxTAxxxSxLxA), however, unlike the previous models which utilize a planar array of anti-parallel β-strands and a left-handed helix, the proposed model utilizes a right-handed helix that is stabilized by internal serine and glutamine ladders and is dimerized using a highly conserved tyrosine ladder. The proposed structure proposes similar binding of water molecules at specific distances in order to facilitate the formation of the ice embryo. Garnham *et al.* proposed that the TQTA consensus residues found within the 16 amino acid repeat are responsible for the binding of water through a TxT residue found in both

INPs and AFPs (Garnham *et al.* 2011). Further studies have suggested that the water binding domains are not the only factor that play a role in ice nucleation. Pandey *et al.* (2016) suggested that the hydrophobic regions interspersed within the water binding domains assist in further ordering the water molecules and removing latent heat from the nucleation sites.

Polyketides and Polyketide Synthases

Near the turn of the 20th century, a young chemist by the name of James Collie managed to discover a new set of synthetic pathways (Collie and Meyers 1893). After several more years of work, Collie predicted that a class of chemicals may exist that is synthesized by cyclization of poly-β-carbonyl (polyketo) acids (Light 1970, Staunton and Weissman 2001). Recent versions of this hypothesis propose that the polyketo acid chains can be formed by condensation of one acetyl and several malonyl-thiol-esters in a method similar fatty acid biosynthesis (Birch 1968, Light 1970, Staunton and Weissman 2001).

Despite being proposed in 1907, the first polyketide wasn't officially published until 1951 with the discovery of the macrolide, Pikromycin (Brockmann and Henkel 1951). However, it wasn't for another three decades until the genes responsible for polyketide synthesis were identified. Two groups of researchers identified the polyketide synthase (PKS) genes for erythromycin in *Saccharopolyspora erythraea* (*Streptomyces erythreaus*) using vastly different yet complementary approaches (Cortes *et al.* 1990, Weber and Losick 1988). Following the publication of these two works, numerous follow-up studies were performed to better understand the gene cluster responsible for erythromycin production. Leonard Katz, who helped pioneer the 1988 work, and his colleagues demonstrated that the gene cluster coded for six repeated units which act like fatty acid synthase (FAS)-like enzyme. They proposed that each unit encodes for a

functional synthase unit and each synthase unit is responsible for one of the six steps of the FAS-like elongation steps for the polyketide formation (Donadio *et al.* 1991). In another study, they were able to knock out one specific gene and predict what changes it would cause in the final product produced by *S. erythraea*. By demonstrating this, they were able to further show that they could exhibit some degree of control over the final product produced by the bacteria by changing the PKS, and not the molecule its self (Katz 1997).

PKSs can be divided into three main classes, based on how they assemble the polyketide products. Type I PKSs are large, highly modular proteins that are further divided into modular and iterative PKSs. Modular PKSs contain a sequence of separate modules that do not repeat any domains, whereas iterative PKSs reuse the domains in a cyclic fashion. Iterative PKSs can be nonreducing PKSs which produce true polyketides, partially reducing PKSs, or fully reducing PKSs which produce fatty acid derivatives. Type II PKSs are aggregate of monofunctional proteins which carry a single set of iteratively acting activities. Type III PKSs do not utilize an acyl-carrier protein, and are also known as chalcone synthase-like PKSs. These proteins are iteratively active, homodimeric condensing enzymes. Both type I and II utilize an acyl-carrier protein to activate the acyl-CoA substrates and control the synthesis of the growing polyketide whereas type III can act directly on the acyl-CoA substrate.

Polyketides are a class of secondary metabolites produced by many organisms, including bacteria, fungi, plants, and animals, and typically provide the organism with an additional mechanism for survival. Most well-known polyketides are used as antimicrobials, either targeting fungi or certain classes of bacteria.

One of the most abundant classes of polyketides, the macrolides, contains compounds such as erythromycin, azithromycin, and pikromycin. However, not all polyketides are beneficial to us,

aflatoxin B1 is one of the most carcinogenic substances known and is produced by *Aspercillus flavus* and *A. parasiticus*. Aflatoxin B1 is derived from a FAS followed by a type I iterative PKS. However, most other classes, such as polyenes, polyethers, and tetracyclines all have either antibacterial or anti-fungal properties. While antimicrobial activity is one of the primary uses of these molecules, other pharmacologically important polyketides include compounds used for controlling cholesterol, immunosuppressants, and antitumor agents. Several other polyketides have shown anthelmintic or insecticidal properties, expanding the range of uses of polyketides outside of human health.

Methods of bacterial identification

One of the current difficulties facing bacterial taxonomy is the current lack of a well-defined method for differentiating bacterial strains and species. In 1952, Sergei Winogradsky disavowed the idea of bacterial taxonomy, stating that a phylogenetic classification was "impossible to apply to bacteria" (Sapp 2005, Winogradsky 1952). This sentiment was later shared by several other taxonomists throughout the years, including C. B. van Niel (van Niel 1955) and S. T. Cowan, who stated "The microbial species does not exist; it is impossible to define except in terms of a nomenclatural type, and it is one of the greatest myths of microbiology" (Cowan 1962). Attitudes on the situation began to shift however as DNA-DNA hybridization (DDH) was developed in the late 1960's and the genetic similarity of bacterial strains could be compared. Eventually, the International Union of Microbiological Societies settled on an arbitrary cutoff for species at 70% DDH similarity. Unfortunately, due to the difficulty of performing DDH and its propensity for errors, only a handful of laboratories could perform the analysis well, thus leading to the search for better methods of identifying species (Gevers *et al.* 2005, Stackebrandt *et al.*

2002). In 1990, that method finally appeared when Carl Woese proposed the new domains of life and utilized the 16S rRNA gene to differentiate bacteria and archaea from eukaryotes (Woese *et al.* 1990). As the number of 16S rRNA sequences exploded, a method for using it to differentiate species was required. By comparing 16S rRNA sequence similarity to DDH similarities it was determined that a 97% similarity corresponded to the 70% DDH value (Stackebrandt and Goebel 1994). This 97% similarity was later increased to 98.5% as further studies were completed (Stackebrandt and Ebers 2006).

Due to the low resolution that 16S rRNA sequencing can provide (because of codon redundancy), new methods were required to distinguish closely related bacteria. Utilizing several, highly conserved housekeeping genes provided such a method. Multilocus Sequence Typing (MLST) analysis allowed for distinguishing members of the same genus or species. Additionally, the benefit of MLST over other, similar, molecular typing methods proved to be its accessibility: sequences could be uploaded to NCBI or other online databases and were publically available to any researcher interested in the work (Maiden *et al.* 1998). Despite the increased accuracy of MLST, the ease of sequencing only one gene kept 16S rRNA sequencing as the primary method of identification of novel bacteria.

Around the same time as the proposition to move to 98.5% 16S sequence similarity, another set of studies began looking into utilizing the full genome of a bacterium to better establish its evolutionary relatedness to similar species. One of these studies, performed by K. T. Konstantinidis and J. M. Teidje looked at utilizing the average amino acid identity (AAI) to give inside into how comparable the various ranks are between organisms (Konstantinidis and Tiedje 2005). The most recent shift in the taxonomic gold standard for bacteria occurred in 2009, with the final shift from the 70% DDH value to an average nucleotide identity (ANI) corresponding to

95-96% (Goris *et al.* 2007, Richter and Rossello-Mora 2009). This most recent shift has enabled more specific analyses to be performed, however, while the price of bacterial genome sequencing has continued to decrease, many laboratories still rely in part on 16S rRNA sequencing to assist in identification and taxonomic classification of isolates/strains.

In an attempt to further resolve the longstanding issues of how to classify bacterial strains and species, the Life Identification Number (LIN) platform has become available. This platform expands on the idea set forth by Haitham Marakeby *et al.* (Marakeby *et al.* 2014) and utilizes ANI to generate hierarchical clustering of bacterial strains. The LINs system has been shown to be capable of handling viral outbreak data (Weisberg *et al.* 2015) as well as bacterial phytopathogen outbreaks (Vinatzer *et al.* 2017). The platform utilizes a code system based on the average nucleotide identity of all species currently in the LINbase (linbase.org) database. By assigning codes based on ANI, the output is a significantly more representative of the overall phylogeny of the genus in question. Due to the sensitivity of LINs, even strains with >99.999% similarity can be distinguished, allowing this system to be ideal for analyzing the overall relatedness of every available genome within a single genus. By having this specificity, any issues with naming conventions or mistakes in other databases can be addressed and corrected.

While full genome analyses can distinguish among similar strains and isolates, a bacterium's phenotype still plays a critical role in identifying members of species within a genus. The utilization of polyphasic taxonomy has been key to the classification and publication of novel species since it was first utilized to describe the relationships within the genus *Vibrio* (Colwell 1970). Since then, several other studies have been undertaken to examine the efficacy and benefit of utilizing polyphasic taxonomy as opposed to relying solely on morphological, physical, and biochemical characterizations (Vandamme *et al.* 1996). As such, the introduction of LINs as a key

method of bacterial identification does not look to eliminate the utilization of phenotypes, but instead looks to be supplemented by available phenotypic characterizations.

The genus *Lysinibacillus*

The genus *Lysinibacillus* was differentiated from other rRNA group 2 Bacillaceae via characterization of it cell wall (Ahmed *et al.* 2007). Unlike other group 2 Bacillaceae, the cell well contains L-lysine or D-ornithine in the peptide subunit of the cell wall. The genus was established in 2007 with the proposal of the novel bacterium *L. boronitolerans* and the transfer of two *Bacillus* species (*B. fusiformis* and *B. sphaericus*) to the novel genus. Members of this genus are characterized to be Gram positive, motile, rod-shaped, endospore forming bacteria with cell wall compositions containing lysine and aspartic acid, the major fatty acid iso-$C_{15:0}$, and a G+C content between 35 and 38 mol%. The endospores present in these bacteria are ellipsoidal or spherical which lie at the terminal end of the cell (Ahmed *et al.* 2007). The genus utilizes MK-7 as its dominant respiratory lipoquinone, and test positively for both catalase and oxidase activity, but tests negatively for indole and H2S production, nitrate reduction, and β-galactosidase activity. Ahmed *et al.* (*2007*) list three polar lipids (diphosphatidylglycerol, phosphatidylglycerol and ninhydrin-positive phosphoglycolipid) as the major polar lipids in *Lysinibacillus*. This description was later emended to better reflect the variability of the catalase activity within the genus (Jung *et al.* 2012), and more recently to reflect the wider variability of the main respiratory quinone (now including MK-7 H_2) fatty acid composition (anteiso-C15:0 and iso-C16:0 now present), and the increased range of G-C content (35-44%) (Zhao *et al.* 2015). The genus currently comprises 28 species, including four species that were transferred from the genus *Bacillus* (Ahmed *et al.* 2007, Jung *et al.* 2012). Members of the species have been isolated from a variety of sources including

soil (Ahmed *et al.* 2007, Liu *et al.* 2013, Miwa *et al.* 2009, Sun *et al.* 2017), plants and associated plant matter (Duan *et al.* 2013, Hayat *et al.* 2014), and water (Failor *et al.* 2017, Kampfer *et al.* 2013). In addition to living in a wide variety of ecosystems, several species have been shown to have insecticidal (Berry 2012, Kellen *et al.* 1965), and nematocidal (Bone and Tinelli 1987, Yang *et al.* 2012) properties.

While the genus is still relatively small in terms of published type species, several issues have arisen within the genus, specifically regarding the naming of strains and species. Xu *et al.* (2015) examined 28 total genome sequences spanning three *Lysinibacillus* species and five *Bacillus* species, and argued that the genus *Lysinibacillus* was genetically divergent enough to warrant their own species within the genus (Xu *et al.* 2015). Gomez-Garzon *et al.* (2016) examined a similar issue within the *Lysinibacillus* genus: members of the *L. sphaericus* phenotypic group may contain more than one published species. In this work, a single 16S clade was determined to contain a unique entomopathogenic phenotype, though not every member of this clade demonstrated the toxic phenotype. Further genomic comparisons determined that the toxic strains are similar, comprising a nearly identical lineage (Gomez-Garzon *et al.* 2016). These findings, which showed that genomic classifications are more discriminatory than 16S sequence phylogenies, corroborate with the work presented herein.

Research Objectives

Objective 1

Sample culturable bacteria deposited with precipitation to improve the understanding of the concentrations and genetic diversity of ice+ bacteria linked to precipitation. This objective is based

on the lack of knowledge of which ice+ bacteria, besides *P. syringae* are present in precipitation and hopes to identify previously unknown ice+ bacteria.

Objective 2

Identify the genes and molecule responsible for ice nucleation activity to better understand non-proteinaceous, biogenic ice nucleation active molecules. This objective is based on the preliminary findings presented in Chapter Two.

Objective 3

Create a complete analysis of each *Lysinibacillus* type strain to produce an authoritative description of the species diversity within the genus. The genus description will provide descriptions of the taxonomy, systematics, ecology, physiology, and other biological properties of all described species and will serve as a reference tool for other *Lysiniibacillus* researchers.

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Chapter Two: Ice nucleation active bacteria in precipitation are genetically diverse and nucleate ice employing different mechanisms

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Abstract

A growing body of circumstantial evidence suggests that ice nucleation active (Ice^+) bacteria contribute to the initiation of precipitation by heterologous freezing of super-cooled water in clouds. However, little is known about the concentration of Ice^+ bacteria in precipitation, their genetic and phenotypic diversity, and their relationship to air mass trajectories and precipitation chemistry. In this study, 23 precipitation events were collected over 15 months in Virginia, USA. Air mass trajectories and water chemistry were determined and 33 134 isolates were screened for ice nucleation activity (INA) at −8 °C. Of 1144 isolates that tested positive during initial screening, 593 had confirmed INA at −8 °C in repeated tests. Concentrations of Ice^+ strains in precipitation were found to range from 0 to 13 219 colony forming units per liter, with a mean of 384±147. Most Ice^+ bacteria were identified as members of known and unknown Ice^+ species in the Pseudomonadaceae, Enterobacteriaceae and Xanthomonadaceae families, which nucleate ice employing the well-characterized membrane-bound INP. Two Ice^+ strains, however, were identified as *Lysinibacillus*, a Gram-positive genus not previously known to include Ice^+ bacteria. INA of the *Lysinibacillus* strains is due to a nanometer-sized molecule that is heat resistant, lysozyme and proteinase resistant, and secreted. Ice^+ bacteria and the INA mechanisms they employ are thus more diverse than expected. We discuss to what extent the concentration of culturable Ice^+ bacteria in precipitation and the identification of a new heat-resistant biological INA mechanism support a role for Ice^+ bacteria in the initiation of precipitation.

Introduction

Formation of ice embryos from supercooled water in clouds by heterogeneous ice nucleation initiates precipitation (Murray *et al.*, 2012), particularly in ice clouds and mixed-phase clouds (Hoose and Mohler 2012) over land masses of mid-latitudes (Mulmenstadt *et al.* 2015). Circumstantial evidence suggests that ice nuclei (IN) of biological origin may contribute to this process. In fact, some microorganisms can nucleate ice at significantly warmer temperatures compared to non-biological IN. While temperatures below − 12 °C/ − 15 °C (Murray *et al.* 2012), some bacteria and fungi nucleate ice at temperatures up to − 2 °C (Morris *et al.* 2004, Pouleur *et al.* 1992), and pollen at temperatures up to − 10 °C (Pummer *et al.* 2012). Also, as clouds precipitate, the concentration of IN active at − 10 °C (and thus most likely biological) decreases faster than the concentration of particles of similar size (Bigg *et al.* 2015, Stopelli *et al.* 2015) and the majority of IN in precipitation is heat sensitive suggesting that they are in fact of biological origin (Christner *et al.* 2008, Diehl *et al.* 2002, Pummer *et al.* 2012, von Blohn *et al.* 2005). Importantly, ice nucleation active (Ice^+) microorganisms have been isolated from clouds (Attard *et al.* 2012, Joly *et al.* 2013, Sands *et al.* 1982), the lower atmosphere (Garcia *et al.* 2012, Lindemann *et al.* 1982) and precipitation (Constantinidou *et al.* 1990, Hill *et al.* 2014, Monteil *et al.* 2014, Morris *et al.* 2008, Sands *et al.* 1982, Santl-Temkiv *et al.* 2015). Ice^+ bacteria are also common in terrestrial environments such as the phyllosphere (Hirano *et al.* 1985, Lindow 1983), leaf litter (Maki *et al.* 1974, Monteil *et al.* 2012) and soil (Monteil *et al.* 2014). Therefore, ice nucleation activity (INA) has been proposed to be an adaptation of microbes to promote their deposition from nutrient-poor clouds back to nutrient-rich vegetation or soil, a theory called bioprecipitation (Morris *et al.* 2014, Sands *et al.* 1982).

The first-identified Ice$^+$ bacterium was *Pseudomonas syringae* (Arny *et al.* 1976, Maki *et al.* 1974) and the activity was later identified in additional Pseudomonadaceae, Xanthomonadaceae (Kim *et al.* 1987) and Enterobacteriaceae (Lindow *et al.* 1978),which are all members of the Gammaproteobacteria. A small number of studies also reported INA in bacteria outside the Gammaproteobacteria (Mortazavi *et al.* 2008, Ponder *et al.* 2005). However, INA of these bacteria was found to be restricted to lower temperatures and was neither further characterized nor confirmed in additional studies.

All Ice$^+$ bacteria whose INA has been molecularly characterized encode orthologues of a single INP, which is anchored to the outer membrane (Morris *et al.* 2004, Wolber *et al.* 1986) and initiates ice formation by binding water molecules in a conformation resembling an ice lattice (Garnham *et al.* 2011, Kajava and Lindow 1993). It was recently proposed that this is accomplished by a hydrophilic-hydrophobic pattern and through the removal of latent heat (Pandey *et al.* 2016). The molecules that induce ice nucleation in Ice+ fungi and in pollen have not yet been identified, but they have been found to be secreted and to be partially heat resistant (Frohlich-Nowoisky *et al.* 2015, O'Sullivan *et al.* 2015, Pouleur *et al.* 1992, Pummer *et al.* 2015).

Studies of temporal and spatial dynamics of Ice$^+$ microorganisms associated with precipitation are needed to determine the environmental conditions promoting dispersal and precipitation of Ice$^+$ bacteria and may also provide further evidence that Ice$^+$ bacteria influence atmospheric processes leading to precipitation. Monteil *et al.* (Monteil *et al.* 2014) identified chemical features of precipitation and characterized the history of air masses associated with deposition of *P. syringae* with precipitation, while other studies tested effects of these environmental parameters on the survival and INA of *Pseudomonas* species in the laboratory (Amato *et al.* 2015, Attard *et al.* 2012) . However, studies on distribution of Ice$^+$ bacteria in clouds

and rain need to be extended to all Ice^+ microorganisms to obtain a more complete understanding of the link between Ice^+ microorganisms and timing and patterns of precipitation.

Here we extensively sampled culturable bacteria deposited with precipitation to expand our knowledge of the concentration and genetic diversity of Ice^+ species in precipitation, the mechanism by which they nucleate ice, and the conditions linked to their deposition. Besides observing a link between the concentration of IN and the history of air masses, measuring the concentration of culturable Ice^+ bacteria in precipitation, and elucidating the genetic diversity of the dominant culturable Ice^+ populations, we identified two Ice^+ Gram-positive strains belonging to the genus *Lysinibacillus* that produce an INA molecule fundamentally different from the INP produced by Gammaproteobacteria. We discuss how our findings improve current estimates of the frequency of INA among bacterial communities in the atmosphere and the putative role of bacterial IN in contributing to the initiation of precipitation.

Materials and methods

Sampling of precipitation and sample processing

Field studies were conducted from February 2013 to April 2014 in Virginia, USA (see Table 1 for details). Locations and an image of one of the precipitation collection sites are shown in Supplementary Figure S1. The number K of total IN at temperatures warmer than − 8 °C was determined immediately after sample processing by a droplet-freezing assay (Vali 1971). Supplementary Materials S1 provide additional details on collection, sample analysis and inference of backward trajectories and climatic conditions of air masses.

Quantification of culturable bacteria

Each sample was concentrated 100 times with a vacuum filtration system and a nitrocellulose membrane (0.22 µm porosity, GWSP, Millipore, Cork, Ireland) before dilution plating. Seven different types of culture media were used to enumerate total culturable bacteria and to maximize genetic diversity: Columbia Agar and Tryptic Soy Agar (both at 100 and 10% strength), Eosin methylene blue Agar, Marine Agar 2216, Reasoner's 2A Agar (see (Diehl *et al.* 2002) for details). Plates were incubated at 20 ° C for 3–5 days, and then individual colonies were counted, and analyzed for INA. Details are provided in Supplementary Methods S1.

Characterization of bacterial INA

Colonies were tested for their ability to freeze sterile water at − 8 °C with a droplet-freezing assay (Vali 1971): well-isolated individual colonies were deposited in 140 µl of sterile water, incubated at 4 °C for at least 1 h, and then three 30 µl droplets of each suspension were deposited on parafilm floating on a − 8 °C glycerol bath in a cooling thermostat (Lauda Alpha RA24, LAUDA-Brinkmann, Delran, NJ, USA). After 10 min, the number of frozen drops was recorded for each colony and for positive and negative controls: *P. syringae* strain CC94 (Morris *et al.* 2000) and sterile distilled water, respectively. Any isolate with minimal activity (that is, one frozen drop) was considered as a potential ice nucleator. A second step of semi-quantitative INA characterization was performed with standardized suspensions for all strains that had shown at least minimal activity in the first assay: 6 drops of 30 µl suspensions made of 48 h old pure cultures were incubated at 20 °C and adjusted to an optical density (OD) at 600 nm of 0.2 (corresponding approximately to $1.31 \times 10^8 \pm 2.12 \times 10^7$ CFU ml^{-1} on average, based on calibration curves performed with different representative species). Four temperatures were tested: − 4, − 8, − 10 and

− 12 °C. After 10 min of incubation at each temperature, the number of frozen drops was recorded and strains were considered as Ice^+ at a given temperature if at least half of the drops froze at that temperature. See Supplementary Methods S1 for details.

Cumulative nucleus spectra of Lysinibacillus sp. strains

The cumulative IN concentrations per cell of *Lysinibacillus* sp. were determined at every temperature degree from − 2 to − 12 °C. Cumulative IN concentrations were compared to *P. syringae* CC94 after both species were grown under the same conditions. The average number of cells per drop was determined by dilution-plating. The cumulative IN concentrations per cell were determined before and after treatments as described in Supplementary Methods S1.

Identification of Ice^+ bacteria

All strains for which the initial colony showed at least minimal activity were genotyped. First, a fragment of the cts gene was amplified with primers specific to the *Pseudomonas* genus (Sarkar and Guttman 2004). Strains for which the gene was amplified were considered to belong to the genus *Pseudomonas*. For the strains for which no amplification product was obtained, a 16S rDNA fragment was amplified, sequenced and blasted against the non-redundant nucleotide database in GenBank as described in Supplementary Methods S1. A strain was assigned to a species when sequence similarity with a type strain was greater than 99% over a minimum sequence alignment of 99%. Below these thresholds, strains were identified at either the genus or family level (Supplementary Tables S2 and S3). Phylogenetic trees were built with sequences of strains and of the most similar sequences based on the BLAST results. Our data set was finally enriched with 920 sequences of type strain sequences downloaded from the Ribosomal Database

Project (Cole *et al.* 2014) and GenBank. See Supplementary Methods S1 for details on tree construction. All 16S rDNA sequences were deposited in GenBank: KY073884-KY074547.

Statistical analyses

Statistical analyses were performed in R, version 3.1.2 (R Core Team 2014), and are described in detail in Supplementary Methods S1. Bacterial concentrations were log transformed to obtain a normal distribution before statistical analyses. For all biological variables, averages were compared pairwise either by the Student's t-test or by the nonparametric Mann–Whitney U test. Links between concentrations of total bacteria, Ice^+ bacteria, and of IN with precipitation sample features were explored testing correlations between variables by building a linear model and analyzed using Pearson's method or Spearman's method. Differences and correlations were considered significant when P-values were below 0.05.

Results

Concentration of IN, culturable bacteria and Ice^+ bacteria varies between precipitation events and correlates with chemical features of precipitation samples

The concentration of culturable bacteria isolated from precipitation samples ranged from 4×10^3 to 1×10^6 CFU l^{-1} (Table 1). The concentration of culturable bacteria (Table 2) did not change with the type of culture medium except for R2A, on which colony counts were significantly higher compared to EMB (pairwise Welch's t-test, Po0.05, Table 2). The overall mean and standard error of the lognormal distribution was 4.18 ± 0.13 l^{-1} when data for all media types were pooled.

A total of 33 134 colonies were initially tested for INA using the droplet freezing assay. An average of 4 ± 1% of colonies per precipitation sample initiated the freezing of at least one out

of three drops at − 8 °C. The lowest and highest frequencies of culturable Ice^+ bacteria observed in precipitation samples were 1% and 27%, respectively. This corresponds to a range of concentrations of culturable Ice^+ bacteria from 40 to 21 840 $l^{-1}$of precipitation with a mean of 901 ± 240 l^{-1}.

Only 52% of strains that were Ice^+ during the first test (593 out of 1144) were confirmed to be Ice^+ at − 8 °C when purified cultures were re-tested at an OD_{600} of 0.2. Based on the standardized re-test, culturable Ice^+ bacteria active at − 8 °C ranged in concentration from 0 CFU l^{-1} to 13 219 CFU l^{-1}, with a mean of 384 ± 147 CFU l^{-1}. This value was independent of isolation medium (pairwise Mann– Whitney U-tests, Table 2). Interestingly, for an additional 9% and 4%, respectively, of strains that were Ice^+ at − 8 °C in the initial test, INA was still observed at − 10 and − 12 °C in the re-test. Moreover, an additional 17% of the strains still had 1 or 2 drops freeze at − 12 °C suggesting that they had some minimal INA at lower temperatures.

We also quantified the concentration of total IN in precipitation at − 8 °C and found it to range from 11 to above 7.4×10^3 per liter, with a mean of 1117 ± 528 per liter (Table 1), which corresponds to the average value observed in previous studies (Petters and Wright, 2015). The ratio between the concentration of IN and the concentration of confirmed Ice^+ bacteria ranged widely from 0.01 to 6080 with a mean of 195. No statistically significant correlation was found between the concentration of IN and that of Ice+ bacteria. However, the concentration of total IN was significantly correlated with the population size of total culturable bacteria (positively) and the frequency of Ice^+ strains in that population (negatively) for several media. We finally inferred concentrations of IN of bacterial origin in clouds from the concentrations of cultured Ice^+ bacteria in our precipitation samples. With the exception of one sample, the inferred concentration is less than 10 IN m^{-3} of cloud at − 8 °C (Supplementary Table S4), which would not be sufficient to

initiate precipitation (Crawford *et al.* 2012). However, changing some of our assumptions this number would dramatically decrease or increase, revealing how speculative these inferred values are (see a more detailed discussion below).

To learn about the aeroecology of Ice^+ bacteria present in precipitation, we determined if the concentrations of total IN and of culturable Ice^+ bacteria were associated with selected features of air masses and/or sample characteristics. We found that the concentration of IN, the concentration of culturable bacteria and the frequency of Ice^+ bacteria compared to the total number of culturable bacteria were significantly related to (i) several climatic parameters, such as air pressure, relative humidity, radiative fluxes or temperature experienced by air masses along their trajectories, (ii) the distance travelled by air masses and (iii), precipitation water chemistry (see Supplementary Table S5 and Supplementary Results S1). Whenever these relationships were observed, they were consistent for most media types and for the elevations used here to determine air masses trajectories. The most striking result is that we found significant positive correlations between pH, conductivity and concentration in total dissolved solids and concentration of total culturable bacteria and total IN (Pearson's and Spearman's correlation tests, coefficients of 0.62 and 0.79, Po0.05).

INA is widespread among the Gammaproteobacteria and has also emerged in the Firmicutes

Because Pseudomonads are already known to be common Ice^+ bacteria in precipitation and clouds, we chose to screen our entire collection of 1144 strains that were Ice^+ in the initial test by PCR with primers specific to the cts gene of the *Pseudomonas* genus. We then focused our attention on those Ice^+ strains that did not amplify the cts gene, and thus likely belonged to other taxonomic groups with possibly yet unknown Ice^+ genera and species, and sequenced their 16S

rDNA. However, to gain some insight into the genetic diversity of Ice^+ strains belonging to the genus *Pseudomonas*, 215 out of the 719 Ice^+ strains that amplified the cts gene were chosen randomly for 16S rDNA sequencing as well.

Combining results from cts gene amplification and BLAST searches of 16S rDNA sequences (Supplementary Table S3) showed that culturable Ice^+ bacteria that had at least a minimal activity during the initial test belonged to seven different bacterial classes (Figure 1a). Only bacteria belonging to two of these classes were confirmed in the standardized re-test (Figure 1b). A rarefaction curve was built to estimate the richness of these confirmed Ice^+ species (Supplementary Figure S2). Since the curve is very close to reaching a horizontal asymptote, we conclude that only a small number of additional culturable Ice^+ species remain to be discovered in precipitation using the employed approach, at least at our sampling locations.

Culturable Ice^+ strains belonging to the Gammaproteobacteria were present in 96% of the samples and represented up to 86% of the strains that were Ice^+ during the first test. Actinobacteria and Bacilli that were Ice^+ in the initial test, but could not be confirmed during standardized re-resting, were also regularly found in precipitation (74% and 57% of the samples, respectively) representing a total of 10% of all Ice^+ strains. However, strains confirmed as Ice^+ during standardized re-testing were almost exclusively Gammaproteobacteria (99.73%, Figure 1b) and were isolated from all but one of the precipitation samples. Within this class, members of the Pseudomonadaceae were the most frequent Ice^+ bacteria (80.7%), followed by Enterobacteriaceae (17.1%) and Xanthomonadaceae (1.3%). While these three families include previously identified Ice^+ species (Joly *et al.* 2013, Morris *et al.* 2008, Mortazavi *et al.* 2008, Santl-Temkiv *et al.* 2015), we also confirmed two Ice^+ strains in the Firmicute *Lysinibacillus*, a Gram-positive genus not previously known to include Ice^+ bacteria (Figure 1b, Supplementary Table S3).

To better determine the evolutionary relationships between the diversity of Ice$^+$ strains and characterized type strains, phylogenetic trees using 16S RNA gene sequences were built (Figures 2,3,4 and Supplementary Figures S3–S6). Pseudomonadaceae strains for which minimal INA during the first test was observed are located in several clades containing known environmental species. Most of the strains belong to the *P. syringae* complex and, to a smaller extent, to the *Pseudomonas fluorescens* complex (Garrido-Sanz *et al.* 2016) (Figure 2). While the diversity of Ice$^+$ strains is well known in the *P. syringae* complex (Berge *et al.* 2014) and to some extent in the *P. fluorescens* complex (Hill *et al.*, 2014), here we found confirmed Ice$^+$ strains closely related to additional species in the *P. fluorescens* complex (*Pseudomonas* trivalis, *Pseudomonas* costantinii and *Pseudomonas orientalis*) and in the *Pseudomonas mandelii* group (VT0005, closely related to *Pseudomonas* frederiksbergensis and *Pseudomonas lini*). Additional Ice$^+$ strains clustered with the known Ice$^+$ species *Pseudomonas abietaniphila*, *Pseudomonas graminis* and *Pseudomonas* putida (Lee *et al.* 1995), and even in species in the *Pseudomonas* aeruginosa group never described as Ice$^+$ before (closely related to *Pseudomonas alcaligenes*, *Pseudomonas resinovorans* and *Pseudomonas oleovorans*). In the Enterobacteriaceae family, confirmed Ice$^+$ were distributed in the *Pantoea* and *Erwinia* genera and clustered with several species that had already been described as Ice$^+$ (Lindow *et al.* 1978) (Figure 3). Most of the collection was closely related to *Pa. ananatis* and *Pa. agglomerans*, but some strains also clustered with *Pa. brenneri*, *Pa. eucalypti*, *E. rhapontici* or *E. aphidicola*. Because 16S rDNA sequence polymorphism was too low to determine a robust phylogeny, species-level identification of some of these strains may slightly change using additional genes or whole genomes. The phylogenetic tree of the Xanthomonadaceae family showed that most Ice$^+$ strains clustered with *Xanthomonas campestris* (Supplementary Figure S5). However, three strains were closely related to *X. translucens* and one strain clustered with the

genus *Stenotrophomonas*, a genus not previously known to be INA at − 8 °C. Some strains that only showed minimal INA at the first characterization were identified as members of the genera *Luteibacter* and *Luteimonas*, and as relatives of *Pseudoxanthomonas* spadix.

Most of the Bacillaceae strains that did not have reproducible INA were related to the *Bacillus pumilus* group, the *Bacillus megaterium* group and the *Bacillus subtilis* group (Figure 4). The two strains with reproducible INA, VT1065 and VT1066, had identical 16S rDNA sequences and showed over 99% DNA identity to the type strains of *Lysinibacillus parviboronicapiens*, *Lysinibacillus fusiformis*, and *Lysinibacillus sphaericus*.

INA of Lysinibacillus is heat, lysozyme, and proteinase-resistant and secreted

Since INA in Gram-positive bacteria has not been characterized, we sought to determine the nature of the *Lysinibacillus* INA (LINA) molecule in comparison with the well-known INP (INP) of *P. syringae*. First, we showed that when grown on the same culture medium (R2A), and when tested under the same conditions, the *Lysinibacillus* strains produced at least seven orders of magnitude fewer IN active between − 6 and − 3 °C compared to *P. syringae*. However, this difference decreased rapidly when lowering temperatures and became equal below − 11 °C (Figure 5a). To test whether nutrient availability may impact the production of the INP and of the LINA molecule, we compared these ice nucleation spectra with those obtained from TSA cultures. Growing bacteria on TSA, a medium richer in nutrients, lowered the production of INA molecules by two orders of magnitude for both bacterial classes (Figure 5b), which indicate that growth in oligotrophic conditions promotes the and sensitive to heat (>95 °C) and lysozyme production of both types of INA molecules.

INA of Ice^+ Gammaproteobacteria is due to the INP, which is anchored to the membrane and sensitive to heat (>95 °C) and lysozyme treatment (Christner *et al.* 2008, Morris *et al.* 2004). As expected, by (i) heating cells, (ii) cleaving INP, which is anchored to the membrane peptidic chains with Proteinase K, (iii) breaking its activity is heat resistant. This resistance to heat strongly suggests that LINA is not a protein. This was confirmed with cell suspensions incubated with Proteinase K, after which INA was even higher by one order of magnitude between − 7 and − 12 °C (Figure 5b). Lysozyme treatment slightly reduced INA of *Lysinibacillus* at all temperatures. Therefore, part of LINA may either depend on glycosydic bonds, or lysozyme treatment of *Lysinibacillus* releases molecules that interfere with INA. Finally, to get an indication of the size of the LINA molecule, the 0.22 μm filtrate was filtered through a 100 kDa pore-sized membrane. Retentate from the 100 kDa pore-sized membrane (which retains molecules larger than 30–90 nm) showed an INA spectrum similar to that of the original cell suspension, but the filtrate did not (Figure 5c). Therefore, the LINA molecule appears to be larger than 30–90 nm or the molecule strongly interacts with the membrane interfering with its passage.

Discussion

Although Ice^+ bacteria have been well known to be the most active IN at warm temperatures since the 1970s (Maki *et al.* 1974), information about their diversity and the conditions associated with their deposition with precipitation is limited. To fill this knowledge gap, Ice^+ bacteria were isolated from 23 precipitation samples in Virginia on seven different media types. This study revealed that the most common culturable Ice^+ bacteria in precipitation are Gammaproteobacteria. For this class, INA had been observed previously and the underlying

mechanism is well characterized (Morris *et al.* 2004). However, additional Ice^{+} species and one additional Ice^{+} genus, *Stenotrophomonas*, were found for the first time to nucleate ice at − 8 °C. Although precipitation samples were only collected in one geographic area, an astonishing species richness (430 OTUs) of known and unknown Ice^{+} species was measured. Importantly, two Ice^{+} *Lysinibacillus* sp. strains provide the first evidence to date of a Gram-positive bacterium exhibiting reproducible INA with magnitudes comparable to *P. syringae* at − 8 °C. Ponder *et al.* (Ponder *et al.* 2005) and Mortazavi *et al.* (Mortazavi *et al.* 2008) had previously reported INA for some Gram-positive bacteria but at lower temperatures and with lower magnitude. Moreover, two *Exiguobacterium* strains and a *Psychrobater* strain identified by Ponder *et al.* (Ponder *et al.* 2005) were inactive under our assay conditions even at − 12 °C (data not shown).

Although the LINA molecule still needs to be identified and purified to determine its structure, we showed that the active molecule is not a protein and is secreted, contrary to the INP produced by Gammaproteobacteria that is part of the outer membrane (with the exception of *Erwinia herbicola* which can shed some IN (Phelps *et al.* 1986)). From an evolutionary perspective, this means that INA emerged at least twice over the course of Eubacteria evolution through two independent mechanisms. This is a remarkable example of convergent evolution, in which INA has evolved in phylogenetically unrelated organisms. Determining the species niche and ecology of Ice+ *Lysinibacillus* will be required to better understand the role of INA in the life history of this organism. Since the Ice^{+} *Lysinibacillus* strains are most closely related to soil-borne bacteria and entomopathogens (Ahmed *et al.* 2007, Miwa *et al.* 2009) and the type strain of *L. parviboronicapiens*, a soil-borne bacterium as well (Miwa *et al.* 2009), is also Ice+ (data not shown), INA in these strains may not have evolved as a primary adaptation to atmospheric

dissemination as proposed in the bioprecipitation theory for INA of *P. syringae* (Morris *et al.* 2014).

The discovery of INA in *Lysinibacillus* underscores the need to research other INA microorganisms and mechanisms. Although the rarefaction curve suggests that only a small number of INA species remain to be discovered (at least at our sample location in Virginia), we may still be underestimating the true biodiversity of Ice^+ bacteria. In fact, many microorganisms are not culturable or only express a small set of phenotypes in culture (Barer and Harwood 1999, Kaeberlein *et al.* 2002). INA is possibly one of these phenotypes, as suggested by the effect of incubation temperature on INA observed by Ponder *et al.* (Ponder *et al.* 2005) in psychroactive permafrost strains and based on studies reviewed by Murray *et al.* (Murray *et al.* 2012). Consequently, discrepancies we observed between results from INA tests of colonies directly plated from precipitation samples and those from purified strains may be explained in part by growth conditions not conducive for the expression of INA, froze at − 8 °C during the initial tests were simply false positives. Moreover, since nutrient limitation induced higher INA in *Lysinibacillus* sp. (Figure 5c) and the same was observed previously for *P. syringae* (Nemecek-Marshall *et al.* 1993), INA molecules might generally be produced in higher amounts in oligotrophic conditions, like those found in the atmosphere. Therefore, detection of new Ice^+ species/mechanisms can probably still be improved by optimizing culture media and growth and incubation conditions.

The size and the chemical properties of the LINA molecule also raises questions about the way abundance of bacterial IN has been estimated in the atmosphere. In fact, because it has been assumed that bacterial IN are exclusively of proteinaceous origin (Morris *et al.* 2004, O'Sullivan *et al.* 2015), bacterial IN are often quantified comparing IN concentrations before and after boiling

samples that have been filtered and concentrated with a 0.22 μm membrane (Christner *et al.* 2008). Yet, the size of the LINA molecule is only nanometers in scale and its activity resists boiling and is independent of lipidic membranes, similar to the ice nucleation particle produced by *Fusarium* (O'Sullivan *et al.* 2015), *Mortierella* (Frohlich-Nowoisky *et al.* 2015) and pollen (Pummer *et al.* 2012). This also means that single cells of these organisms could produce many IN over their residence time in the atmosphere. This differs from the Gammaproteobacteria, for which INA is dependent on cell integrity and for which the number of IN is limited to the number of bacterial cells in the atmosphere. Moreover, because of their size, bacterial LINA-like molecules could represent some of the abundant nanometer-sized Ice^{+} particles active at warm temperatures identified by Vali (Vali 1966) and O'Sullivan *et al.* (O'Sullivan *et al.* 2015) and more recently by Du *et al.* (Du *et al.* 2017) or Šantl-Temkiv *et al.* (Santl-Temkiv *et al.* 2015).

In regard to conditions linked to higher concentrations of total IN active at − 8 °C in precipitation (and thus assumed to be biological), the most striking results were: (i) alkaline and mineralized precipitation water and (ii) precipitation initiated within air masses in which relative humidity was lower and that travelled over larger distances. These results are consistent with other experimental and modelling studies suggesting that the frequency of deposition of biological IN like *P. syringae* increases with alkalinity and conductivity of precipitation that is derived from rapidly travelling air masses (Monteil *et al.* 2014). It is therefore possible that the link between the concentration of IN and the distance travelled by air masses is due to a greater uptake of IN from Earth surfaces by upward air fluxes.

Importantly, do our results support a role for bacterial IN in the initiation of precipitation? Crawford *et al.* (Crawford *et al.* 2012) estimated that a small amount of primary ice, as low as 10 IN per m^3, would be enough to initiate precipitation at temperatures as high as − 8 °C. We thus

estimated the number of IN produced by Ice^+ bacteria in clouds based on the mean concentration of Ice^+ bacteria in our precipitation samples (Supplementary Table S4). Interestingly, we inferred fewer than 10 IN of bacterial origin per m^3 of cloud in all samples but one when we assume that (i) Ice^+ bacteria are neither lost nor scavenged while precipitation falls, (ii) expression of genes that determine INA in the clouds is similar to that under our growth conditions, (iii) all bacteria in precipitation are culturable, and (iv) one Ice^+ colony plated from precipitation corresponds to one IN in the clouds. However, changing these assumptions our estimate increases or decreases by orders of magnitudes. In particular, the number of IN may be an underestimation if many Ice^+ bacteria in precipitation were not culturable or may not have expressed INA genes under the conditions we employed. At the other extreme, inferring the number of IN produced by a single bacterial cell in clouds from our droplet freezing assay, the concentration of IN in clouds would only be 1.3×10^{-4} per m^3, by far insufficient to induce precipitation. Even assuming that the concentration of total IN in our precipitation samples (based on the droplet freezing assay) were all of bacterial origin, there would not be a sufficient number of bacterial IN in clouds to initiate precipitation at − 8 °C (they may be sufficient at lower temperatures but we did not determine concentrations of IN below − 8 °C). Moreover, the droplet freezing assay may underestimate the number of IN (Emersic *et al.* 2015, Whale *et al.* 2015). Therefore, developing laboratory assays that accurately quantify IN under conditions to which IN are exposed in clouds needs to be prioritized to further investigate the role of biological IN in atmospheric processes.

In summary, we expanded our knowledge of the genetic diversity of Ice^+ bacteria and the factors that correlate with their deposition. Most importantly, we uncovered a new INA mechanism in Gram-positive bacteria. This novel mechanism, which is based on a heat-stable and proteinase-resistant secreted molecule, challenges our current view of bacterial INA as being exclusively

proteinaceous and thus heat-sensitive. This discovery of the first new bacterial INA mechanism since *P. syringae* over 40 years ago will require re-interpretation of some previous data on biological and non-biological INA detected in clouds, aerosols and precipitation. Our results also ask for optimization of protocols for detecting and isolating Ice^{+} species and for quantifying IN under cloud-like conditions. Finally, identification and characterization of the LINA molecule and surveys of its concentration (and the concentration of Ice+ *Lysinibacillus*) will be important to determine if Ice+ *Lysinibacillus* bacteria influence atmospheric processes leading to precipitation.

Conflict of Interest

The authors declare no conflict of interest.

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Attributions

Caroline Monteil of the Institut National de la Recherche Agronomique, France designed the methods for, and began the precipitation collection experiments. Dr. Monteil was key to the

statistical analyses presented in this work as well as a key contributor to the authorship of this manuscript.

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Tables

Table 1. Water chemistry and concentrations of total culturable bacteria and Ice+ bacteria in precipitation samples in Virginia, USA.

Site[a]	Date	Time precipitation started	Sample ID	Number of IN/L of water[b]	Total Dissolved Solids (ppm)	Salinity (ppm)	pH	Conductivity (µS.cm)	Concentration of total culturable bacteria[c] (log(CFU/L))							Frequency of INA bacteria[d] (%)							Concentration of culturable Ice$^+$ bacteria[e] (log(CFU/L))						
									CA	CA 10%	EMB	MA	R2A	TSA	TSA 10%	CA	CA 10%	EMB	MA	R2A	TSA	TSA 10%	CA	CA 10%	EMB	MA	R2A	TSA	TSA 10%
Town of Blacksburg																													
	23-02-13	04:45	VTP01	na	na	na	na	na	4.90	na	4.48	4.79	5.03	4.81	na	2.1	na	na	-	-	-	na	3.22	na	na	-	-	-	na
	26-02-13	08:00	VTP02	na	na	na	na	na	4.11	na	4.22	3.89	4.66	4.56	na	1.6	na	0.5	1.0	-	0.9	na	2.31	na	1.94	1.91	-	2.51	na
	24-03-13	10:30	VTP03	na	na	na	na	na	4.17	na	4.12	3.44	4.21	3.92	4.43	-	na	-	-	-	1.0	-	-	na	-	-	-	-	2.45
Kentland Farm, Site A																													
	13-06-13	02:30	VTP07	421	na	na	na	na	5.63	5.79	5.40	5.70	5.79	5.68	5.71	-	-	-	-	-	0.3	-	-	-	-	-	-	3.16	-
	18-06-13	09:30	VTP08	11	na	na	na	na	4.16	3.97	3.82	3.90	4.22	4.03	4.12	0.3	0.3	0.7	1.7	-	-	0.8	1.70	1.51	2.05	2.14	-	-	2.02
	01-07-13	21:00	VTP09	441	na	na	na	na	5.26	5.11	4.98	5.15	5.36	5.41	5.21	-	0.3	0.5	-	-	-	-	-	2.52	2.70	-	-	-	-
	17-07-13	15:00	VTP10	48	na	na	na	na	4.35	4.41	3.97	4.25	4.43	5.47	4.31	2.6	0.5	3.6	2.7	1.6	2.1	1.0	2.77	2.13	2.54	2.67	2.62	2.79	2.32
	20-01-14	17:00	VTP21b	7440	27.50	33.1	7.74	46.3	4.26	4.35	3.93	4.16	4.25	4.25	4.40	-	1.0	0.5	-	-	-	-	-	2.37	1.64	-	-	-	-
	16-03-14	13:00	VTP26b	103	6.20	12.6	6.34	8.7	3.63	3.59	2.90	3.27	4.02	3.51	3.63	-	1.0	5.0	1.0	0.3	1.0	-	-	1.60	1.60	1.29	1.56	1.53	-
	07-04-14	07:15	VTP28b	11	3.70	10.9	6.97	5.3	3.23	3.37	2.94	2.90	3.56	3.18	3.31	4.2	2.6	7.5	6.3	4.9	6.9	6.3	1.85	1.78	1.82	1.70	2.25	2.01	2.11
Kentland Farm, Site B																													
	12-04-13	02:00	VTP05	441	na	na	na	na	4.65	4.72	4.36	4.17	4.81	4.77	4.67	5.4	2.1	5.8	6.1	2.6	3.8	2.8	3.39	3.04	4.12	2.95	3.22	3.35	3.01
	01-11-13	01:00	VTP14b	1352	14.30	9.0	7.28	12.7	4.53	2.98	4.68	4.94	5.36	5.41	4.79	0.5	0.5	1.2	-	-	-	1.0	3.25	3.32	2.76	-	-	-	2.81
Kentland Farm, Site C																													
	06-05-13	09:30	VTP06	na	na	na	6.58	3.5	4.22	4.16	3.90	4.90	4.26	4.07	4.29	12.8	6.3	16.7	12.0	3.6	7.6	10.4	3.18	2.95	3.12	2.98	2.82	2.95	3.31
Virginia Tech, Parking Lot																													
	08-08-13	16:30	VTP11	557	21.80	21.6	7.94	30.9	3.67	3.92	3.56	3.68	3.95	3.81	3.90	1.4	1.4	2.8	-	2.8	2.8	2.1	1.96	2.20	2.15	-	2.54	2.40	2.36
Virginia Tech, Latham Hall																													
	21-09-13	12:00	VTP12	231	12.30	10.2	6.61	10.9	3.66	3.63	3.40	3.23	3.72	3.55	3.87	0.7	2.6	6.1	8.2	1.8	1.1	8.5	1.53	2.04	2.19	2.14	1.79	1.61	2.80
	07-10-13	03:30	VTP13	48	15.30	12.2	7.45	17.2	3.54	3.66	3.30	3.60	3.70	3.61	3.64	1.0	1.0	5.2	-	1.0	5.2	1.0	1.56	1.68	2.02	-	1.72	2.32	1.66
	01-11-13	01:00	VTP14a	1352	44.10	54.8	7.33	76.9	3.98	2.65	4.67	5.11	5.75	5.18	5.54	-	0.4	-	0.5	-	-	-	-	2.86	-	2.82	-	-	-
	06-12-13	09:15	VTP17	na	11.10	4.2	7.88	5.8	4.17	na	4.06	4.11	4.26	4.23	4.08	2.6	na	10.4	13.5	4.2	9.5	1.4	2.58	na	3.07	3.24	2.88	3.20	2.23
	02-01-14	12:30	VTP19	na	13.70	9.8	7.39	13.8	4.10	4.58	4.41	3.98	4.88	4.34	4.10	-	-	1.0	-	-	0.5	-	-	-	2.43	-	-	2.05	-
	20-01-14	23:00	VTP21a	6080	26.40	32.3	7.91	46.6	4.34	4.52	4.18	4.28	4.30	4.44	4.27	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	13-02-14	13:30	VTP24	279	11.60	4.2	6.74	5.9	2.74	3.13	2.97	3.20	3.58	2.76	3.57	-	-	-	-	-	-	0.5	-	-	-	-	-	-	1.29
	16-03-14	12:45	VTP26a	170	6.60	11.0	6.80	8.6	3.58	4.70	2.64	3.42	4.58	4.33	4.89	-	-	1.0	-	0.5	-	1.0	-	-	0.66	-	2.29	-	2.90
	07-04-14	07:30	VTP28a	11	9.80	3.3	6.99	4.6	3.60	4.32	3.45	3.46	4.21	3.52	3.77	1.0	1.0	1.6	4.2	-	2.1	1.0	1.63	2.34	1.65	2.08	-	1.84	1.78

"na" refers to non analyzed data and "-" refers to nul value (under the detection threshold).

[a] GPS coordinates and altitudes for each site are the following: Blacksburg city (37°14'16"N, 80°25'2"W); Kentland Farm - Site A (37°12'9"N, 80°33'51"W); Kentland Farm - Site B (37°11'47"N, 80°34'41"W); Kentland Farm - Site C (37°12'8"N, 80°35'32"W); Virginia Tech - Latham Hall(37°13'29"N, 80°25'22"W); Virginia Tech - Parking Lot (37°13'28"N, 80°25'19"W)

[b] Number of IN active at –8°C

[c] CA, CA10%, EMB, MA, R2A, TSA and TSA10% are the abbrebiations for Columbia Agar, Columbia Agar 10% concentrated, Ethylen-Methyl-Blue Agar, Marine Agar, Reasoner's 2 Agar, Tryptic Soy Agar and Tryptic Soy Agar 10% concentrated, respectively

[d] Corresponds to the frequency of isolates for which ice nucleation activity has been reproduced with purified cultures incubated for 48h at 20°C and tested at –8°C

[e] Corresponds to the concentration of isolates for which ice nucleation activity has been reproduced with purified cultures incubated for 48h at 20°C and tested at –8°C

Table 2. Comparative analysis of different culture media used for isolating total culturable bacteria and Ice+ bacteria from precipitation collected in Virginia, USA.

	Concentration of culturable bacteria (log(CFU/L))				Frequency of Ice+ bacteria[b] (%)				Concentration of culturable Ice+ bacteria (log(CFU/L))			
Medium[a]	Mean	±	SE		Mean	±	SE		Mean	±	SE	
CA	4.21	±	0.16	ab	1.58	±	0.60	ab	1.34	±	0.27	a
CA 10%	4.37	±	0.17	ab	1.11	±	0.34	b	1.70	±	0.26	a
EMB	3.99	±	0.16	b	3.20	±	0.90	a	1.75	±	0.25	a
MA	4.03	±	0.15	ab	2.49	±	0.84	ab	1.13	±	0.27	a
R2A	4.48	±	0.13	a	0.94	±	0.32	b	1.03	±	0.26	b
TSA	4.26	±	0.15	ab	1.91	±	0.59	ab	1.44	±	0.27	a
TSA 10%	4.33	±	0.15	ab	1.86	±	0.64	ab	1.57	±	0.27	a

a CA, CA10%, EMB, MA, R2A, TSA and TSA10% are the abbrebiations for Columbia Agar, Columbia Agar diluted 10 times, Eosin-Methyl-Blue Agar, Marine Agar, Reasoner's 2A Agar, Tryptic Soy Agar, and Tryptic Soy Agar diluted 10 times, respectively. Averages and standard errors were estimated from 23 samples for all media, with the exception of CA10% (n=19) and TSA10% (n=21)

[b] Ice nucleation activity of bacteria has been confirmed with purified cultures incubated for 48h at 20°C and tested at −8°C.

c For each variable, values associated with the same letter are not significantly different (pairwise Student t-test, $P < 0.05$, when data were normally distributed, pairwise Mann Whitney U test, $P < 0.05$, when data were not normally distributed). Population sizes of total bacteria were normally distributed while frequencies and concentration of culturable Ice+ bacteria were not.

Figures

Figure 1. Diversity of culturable Ice+ bacteria in precipitation collected in Virginia, USA. (a) Diversity of culturable Ice+ strains at the level of class for which at least one droplet froze at –8°C during initial tests of colonies directly cultured from precipitation. (b) Diversity of culturable Ice+ strains at the level of genus for which activity was confirmed via semi-quantitative tests with pure cultures.

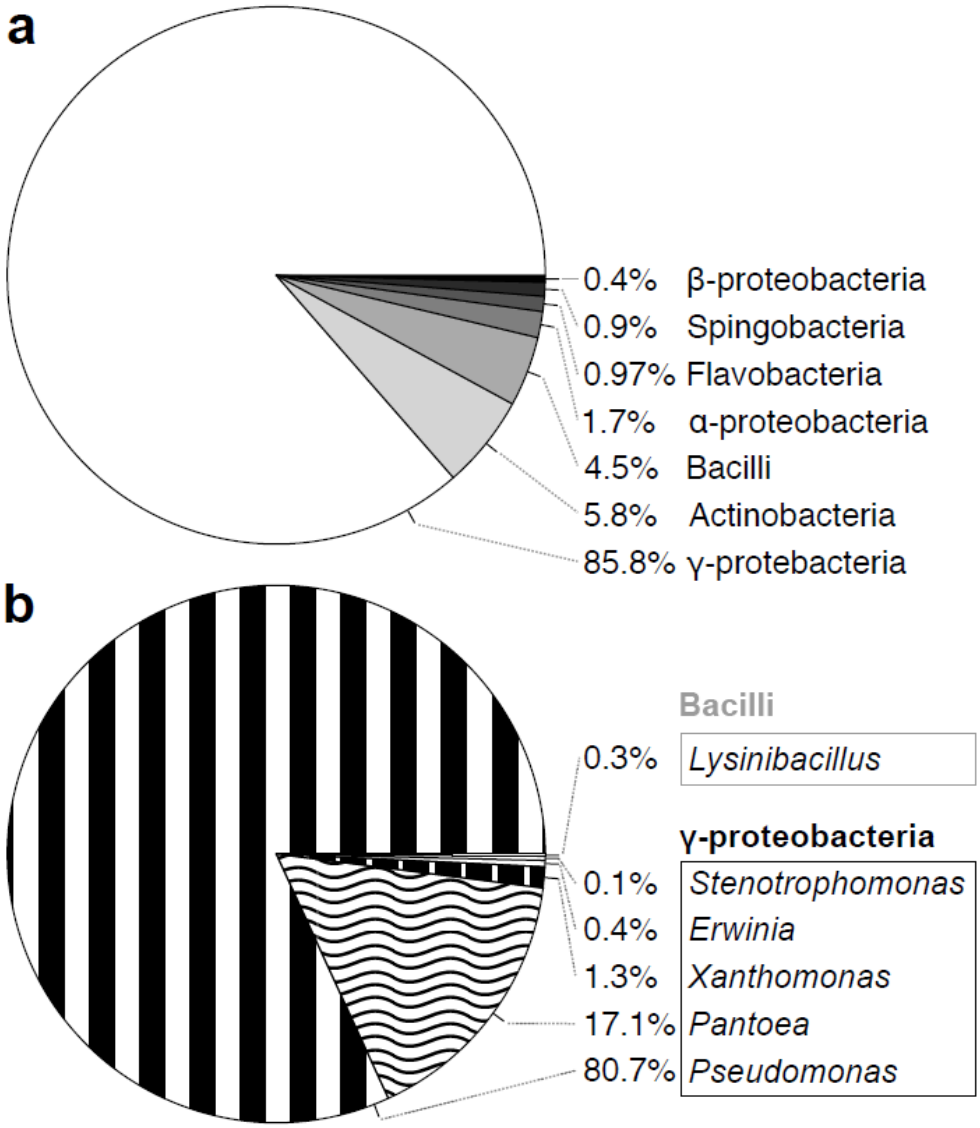


Figure 2. Evolutionary relationships between the culturable strains isolated from precipitation in Virginia, USA and other members of the Pseudomonadaceae family based on the alignment of partial 16S rDNA sequences. A maximum likelihood tree was built from the 930pb-long sequences of 229 strains isolated in this study (labelled "VT#") and 151 type strains downloaded from the Ribosomal Database Project (RDP) (Cole *et al.* 2014) using the GTR model and 100 bootstrap replicates. Gaps were considered as missing data and were partially deleted with a site coverage cut-off of 95%. The strain *Xanthomonas axonopodis* LMG 538-T was used as a root. Only bootstrap values over 50% were included and are symbolized by black dots. Clades and leaves associated with strains that were Ice+ in standardized re-tests at -8°C are labelled in bold and are underlined, those active at -10°C or -12°C are labelled in bold only. When more than one VT# strain had the same 16S rDNA sequence, only the number of strains is indicated and Ice+ strains that were confirmed in repeated tests are labelled "Ice+". RDP names are composed of the species name, the strain code and the accession number in the public database NCBI. The name of the type strain closest to INA strains was chosen to represent collapsed branches. A version of the phylogenetic tree in which all VT# strains are listed is given in Supplementary Figure S3.

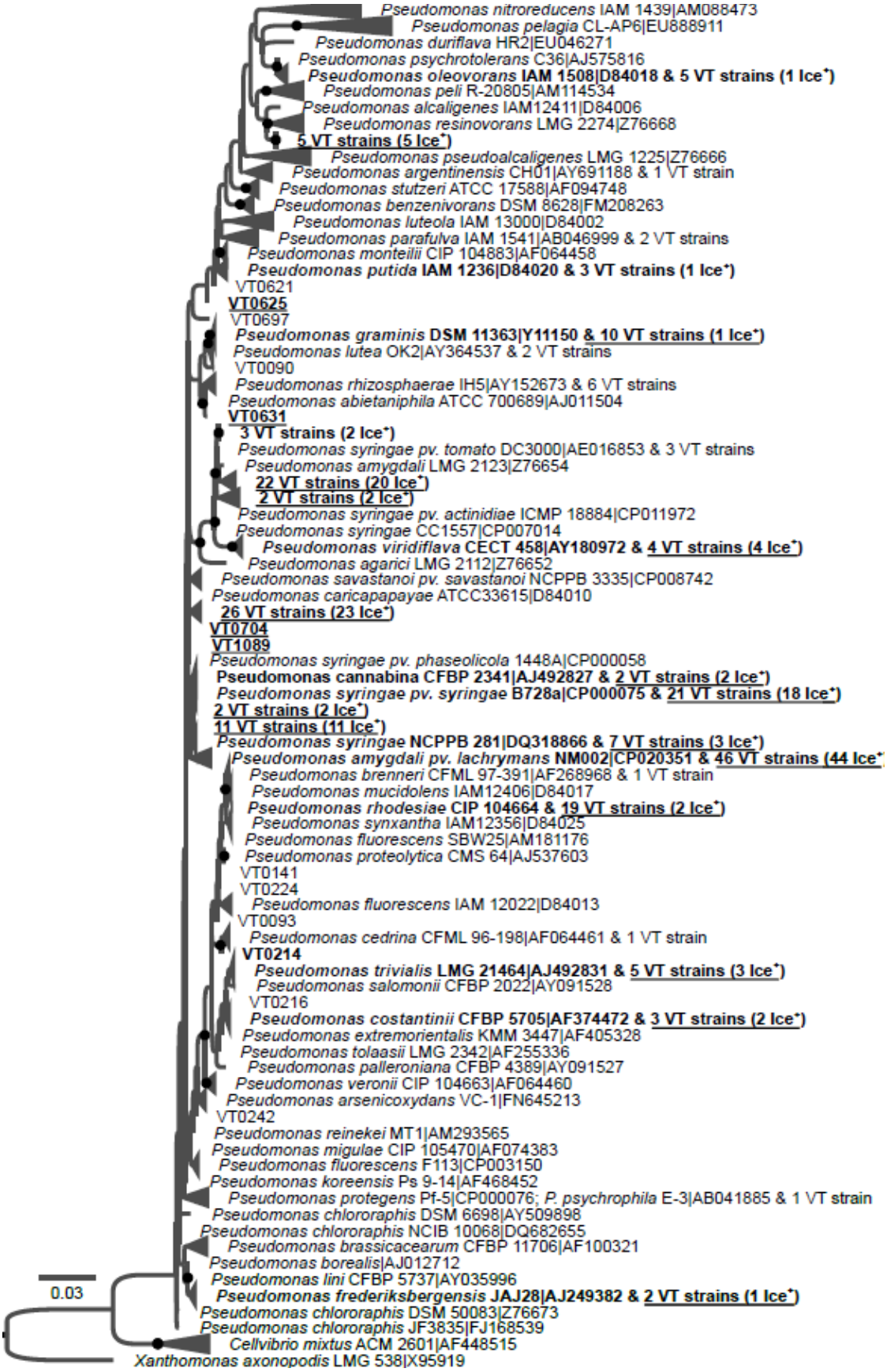

Pseudomonas nitroreducens IAM 1439|AM088473
Pseudomonas pelagia CL-AP6|EU888911
Pseudomonas duriflava HR2|EU046271
Pseudomonas psychrotolerans C36|AJ575816
Pseudomonas oleovorans IAM 1508|D84018 & 5 VT strains (1 Ice+)
Pseudomonas peli R-20805|AM114534
Pseudomonas alcaligenes IAM12411|D84006
Pseudomonas resinovorans LMG 2274|Z76668
5 VT strains (5 Ice+)
Pseudomonas pseudoalcaligenes LMG 1225|Z76666
Pseudomonas argentinensis CH01|AY691188 & 1 VT strain
Pseudomonas stutzeri ATCC 17588|AF094748
Pseudomonas benzenivorans DSM 8628|FM208263
Pseudomonas luteola IAM 13000|D84002
Pseudomonas parafulva IAM 1541|AB046999 & 2 VT strains
Pseudomonas monteilii CIP 104883|AF064458
Pseudomonas putida IAM 1236|D84020 & 3 VT strains (1 Ice+)
VT0621
VT0625
VT0697
Pseudomonas graminis DSM 11363|Y11150 & 10 VT strains (1 Ice+)
Pseudomonas lutea OK2|AY364537 & 2 VT strains
VT0090
Pseudomonas rhizosphaerae IH5|AY152673 & 6 VT strains
Pseudomonas abietaniphila ATCC 700689|AJ011504
VT0631
3 VT strains (2 Ice+)
Pseudomonas syringae pv. tomato DC3000|AE016853 & 3 VT strains
Pseudomonas amygdali LMG 2123|Z76654
22 VT strains (20 Ice+)
2 VT strains (2 Ice+)
Pseudomonas syringae pv. actinidiae ICMP 18884|CP011972
Pseudomonas syringae CC1557|CP007014
Pseudomonas viridiflava CECT 458|AY180972 & 4 VT strains (4 Ice+)
Pseudomonas agarici LMG 2112|Z76652
Pseudomonas savastanoi pv. savastanoi NCPPB 3335|CP008742
Pseudomonas caricapapayae ATCC33615|D84010
26 VT strains (23 Ice+)
VT0704
VT1089
Pseudomonas syringae pv. phaseolicola 1448A|CP000058
Pseudomonas cannabina CFBP 2341|AJ492827 & 2 VT strains (2 Ice+)
Pseudomonas syringae pv. syringae B728a|CP000075 & 21 VT strains (18 Ice+)
2 VT strains (2 Ice+)
11 VT strains (11 Ice+)
Pseudomonas syringae NCPPB 281|DQ318866 & 7 VT strains (3 Ice+)
Pseudomonas amygdali pv. lachrymans NM002|CP020351 & 46 VT strains (44 Ice+)
Pseudomonas brenneri CFML 97-391|AF268968 & 1 VT strain
Pseudomonas mucidolens IAM12406|D84017
Pseudomonas rhodesiae CIP 104664 & 19 VT strains (2 Ice+)
Pseudomonas synxantha IAM12356|D84025
Pseudomonas fluorescens SBW25|AM181176
Pseudomonas proteolytica CMS 64|AJ537603
VT0141
VT0224
Pseudomonas fluorescens IAM 12022|D84013
VT0093
Pseudomonas cedrina CFML 96-198|AF064461 & 1 VT strain
VT0214
Pseudomonas trivialis LMG 21464|AJ492831 & 5 VT strains (3 Ice+)
Pseudomonas salomonii CFBP 2022|AY091528
VT0216
Pseudomonas costantinii CFBP 5705|AF374472 & 3 VT strains (2 Ice+)
Pseudomonas extremorientalis KMM 3447|AF405328
Pseudomonas tolaasii LMG 2342|AF255336
Pseudomonas palleroniana CFBP 4389|AY091527
Pseudomonas veronii CIP 104663|AF064460
Pseudomonas arsenicoxydans VC-1|FN645213
VT0242
Pseudomonas reinekei MT1|AM293565
Pseudomonas migulae CIP 105470|AF074383
Pseudomonas fluorescens F113|CP003150
Pseudomonas koreensis Ps 9-14|AF468452
Pseudomonas protegens Pf-5|CP000076; P. psychrophila E-3|AB041885 & 1 VT strain
Pseudomonas chlororaphis DSM 6698|AY509898
Pseudomonas chlororaphis NCIB 10068|DQ682655
Pseudomonas brassicacearum CFBP 11706|AF100321
Pseudomonas borealis|AJ012712
Pseudomonas lini CFBP 5737|AY035996
Pseudomonas frederiksbergensis JAJ28|AJ249382 & 2 VT strains (1 Ice+)
Pseudomonas chlororaphis DSM 50083|Z76673
Pseudomonas chlororaphis JF3835|FJ168539
Cellvibrio mixtus ACM 2601|AF448515
Xanthomonas axonopodis LMG 538|X95919
0.03

Figure 3. Evolutionary relationships between the culturable strains isolated from precipitation in Virginia, USA, and other members of the Enterobacteriaceae family based on the alignment of partial 16S rDNA sequences. The phylogenetic tree was built from 212 strains isolated in this study and 241 type strains downloaded from the Ribosomal Database Project (RDP) (Cole *et al.* 2014). The strain *Pseudomonas aeruginosa* DSM 50071 was used as a root. See Figure 2 for details about tree construction and labels. A version of the phylogenetic tree in which all VT# strains are listed is given in Supplementary Figure S4.

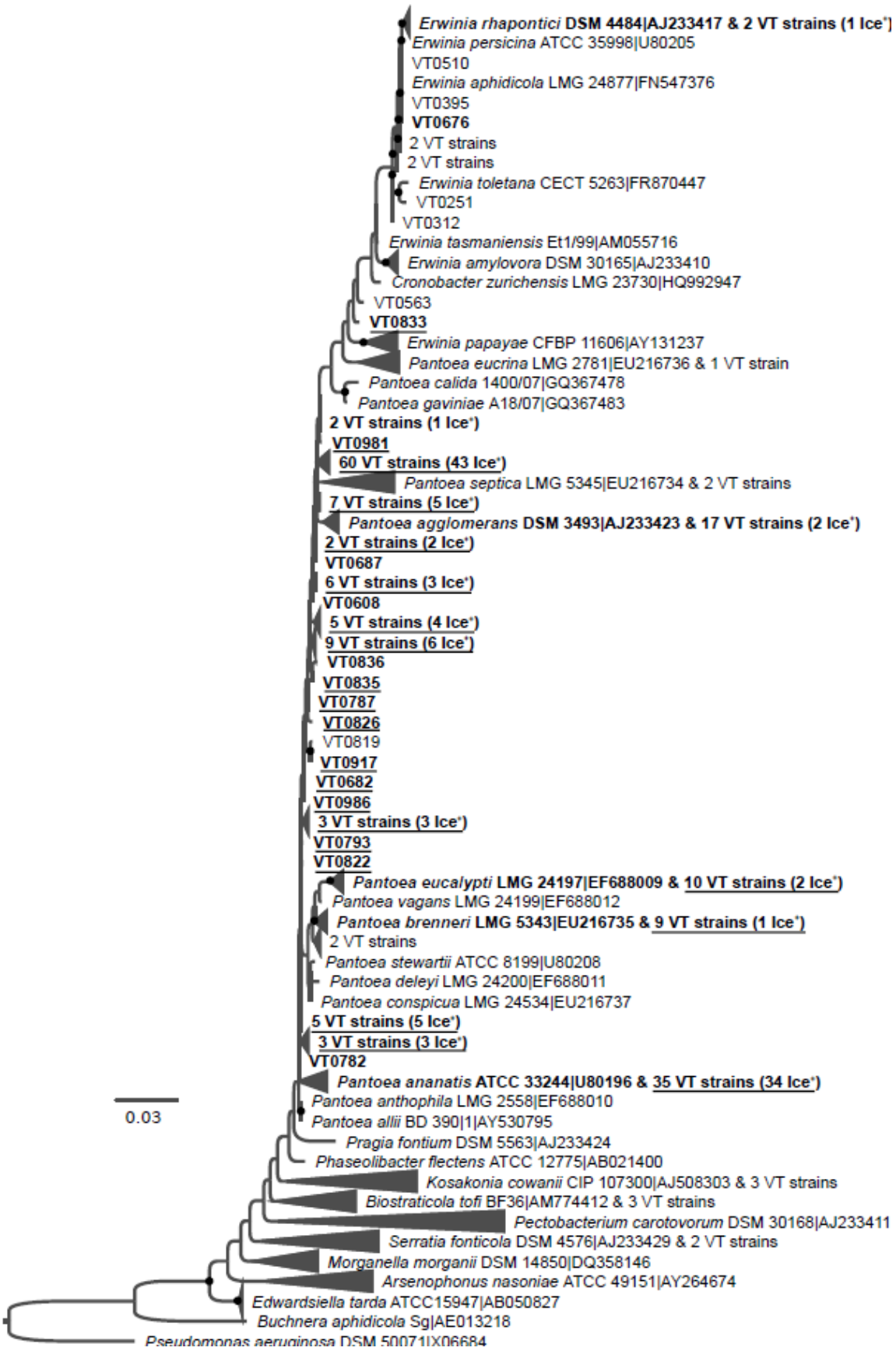

Erwinia rhapontici **DSM 4484|AJ233417 & 2 VT strains (1 Ice⁺)**
Erwinia persicina ATCC 35998|U80205
VT0510
Erwinia aphidicola LMG 24877|FN547376
VT0395
VT0676
2 VT strains
2 VT strains
Erwinia toletana CECT 5263|FR870447
VT0251
VT0312
Erwinia tasmaniensis Et1/99|AM055716
Erwinia amylovora DSM 30165|AJ233410
Cronobacter zurichensis LMG 23730|HQ992947
VT0563
VT0833
Erwinia papayae CFBP 11606|AY131237
Pantoea eucrina LMG 2781|EU216736 & 1 VT strain
Pantoea calida 1400/07|GQ367478
Pantoea gaviniae A18/07|GQ367483
2 VT strains (1 Ice⁺)
VT0981
60 VT strains (43 Ice⁺)
Pantoea septica LMG 5345|EU216734 & 2 VT strains
7 VT strains (5 Ice⁺)
Pantoea agglomerans **DSM 3493|AJ233423 & 17 VT strains (2 Ice⁺)**
2 VT strains (2 Ice⁺)
VT0687
6 VT strains (3 Ice⁺)
VT0608
5 VT strains (4 Ice⁺)
9 VT strains (6 Ice⁺)
VT0836
VT0835
VT0787
VT0826
VT0819
VT0917
VT0682
VT0986
3 VT strains (3 Ice⁺)
VT0793
VT0822
Pantoea eucalypti **LMG 24197|EF688009 & 10 VT strains (2 Ice⁺)**
Pantoea vagans LMG 24199|EF688012
Pantoea brenneri **LMG 5343|EU216735 & 9 VT strains (1 Ice⁺)**
2 VT strains
Pantoea stewartii ATCC 8199|U80208
Pantoea deleyi LMG 24200|EF688011
Pantoea conspicua LMG 24534|EU216737
5 VT strains (5 Ice⁺)
3 VT strains (3 Ice⁺)
VT0782
Pantoea ananatis **ATCC 33244|U80196 & 35 VT strains (34 Ice⁺)**
Pantoea anthophila LMG 2558|EF688010
Pantoea allii BD 390|1|AY530795
Pragia fontium DSM 5563|AJ233424
Phaseolibacter flectens ATCC 12775|AB021400
Kosakonia cowanii CIP 107300|AJ508303 & 3 VT strains
Biostraticola tofi BF36|AM774412 & 3 VT strains
Pectobacterium carotovorum DSM 30168|AJ233411
Serratia fonticola DSM 4576|AJ233429 & 2 VT strains
Morganella morganii DSM 14850|DQ358146
Arsenophonus nasoniae ATCC 49151|AY264674
Edwardsiella tarda ATCC15947|AB050827
Buchnera aphidicola Sg|AE013218
Pseudomonas aeruginosa DSM 50071|X06684
0.03

Figure 4. Evolutionary relationships between the culturable INA strains Lysinibacillus sp. VT1065 and VT1066 isolated from precipitation and other members of the Bacilliaceae family based on the alignment of 16S rDNA partial sequences. The phylogenetic tree was built from 47 strains isolated in this study and 353 type strains downloaded from the Ribosomal Database Project (RDP) (Cole *et al.* 2014). The strain Lactobacillus acidophilus BCRC10695 was used as a root. See Figure 2 for details about tree construction and labels. A version of the phylogenetic tree in which all VT# strains are listed is given in Supplementary Figure S6.

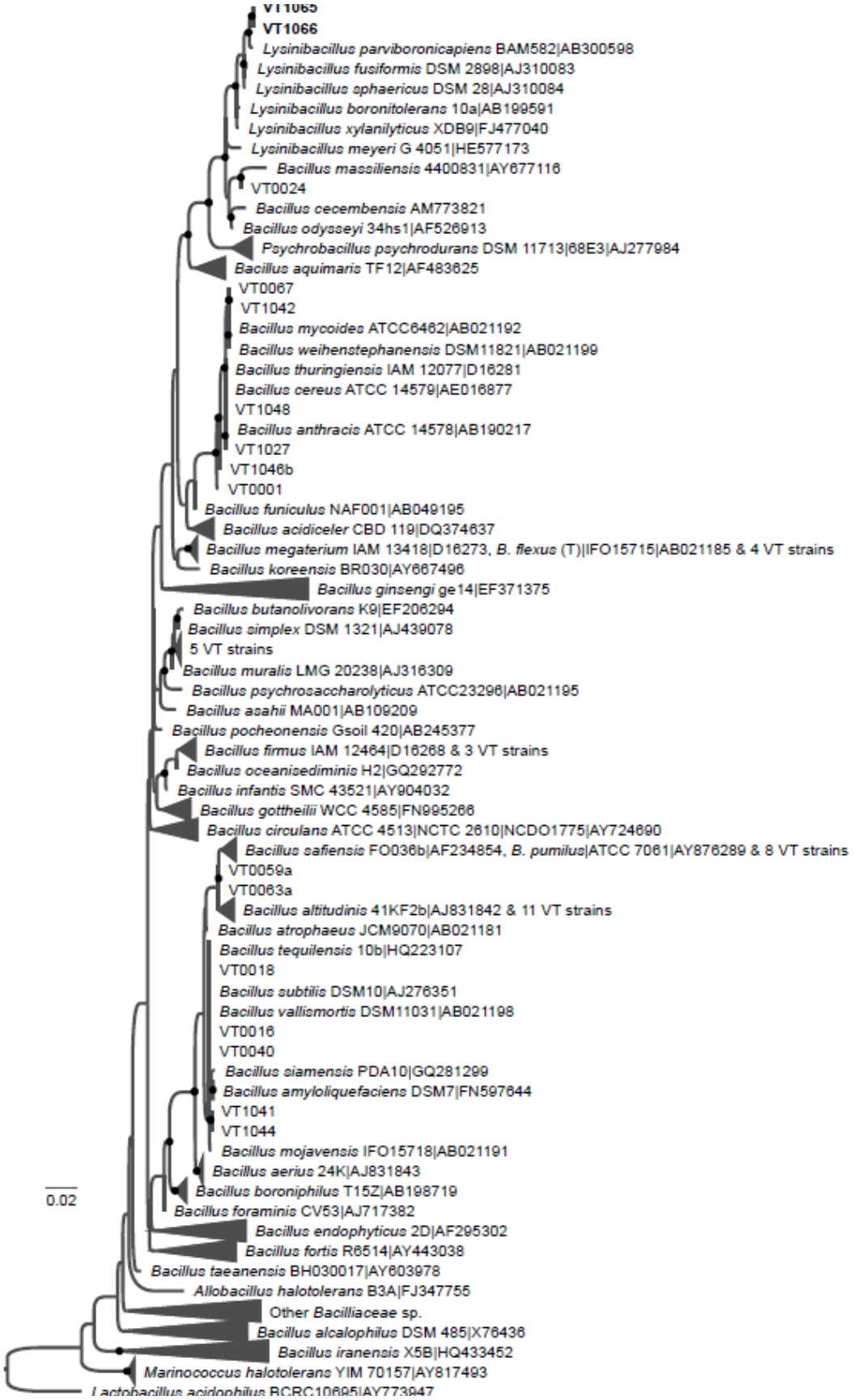
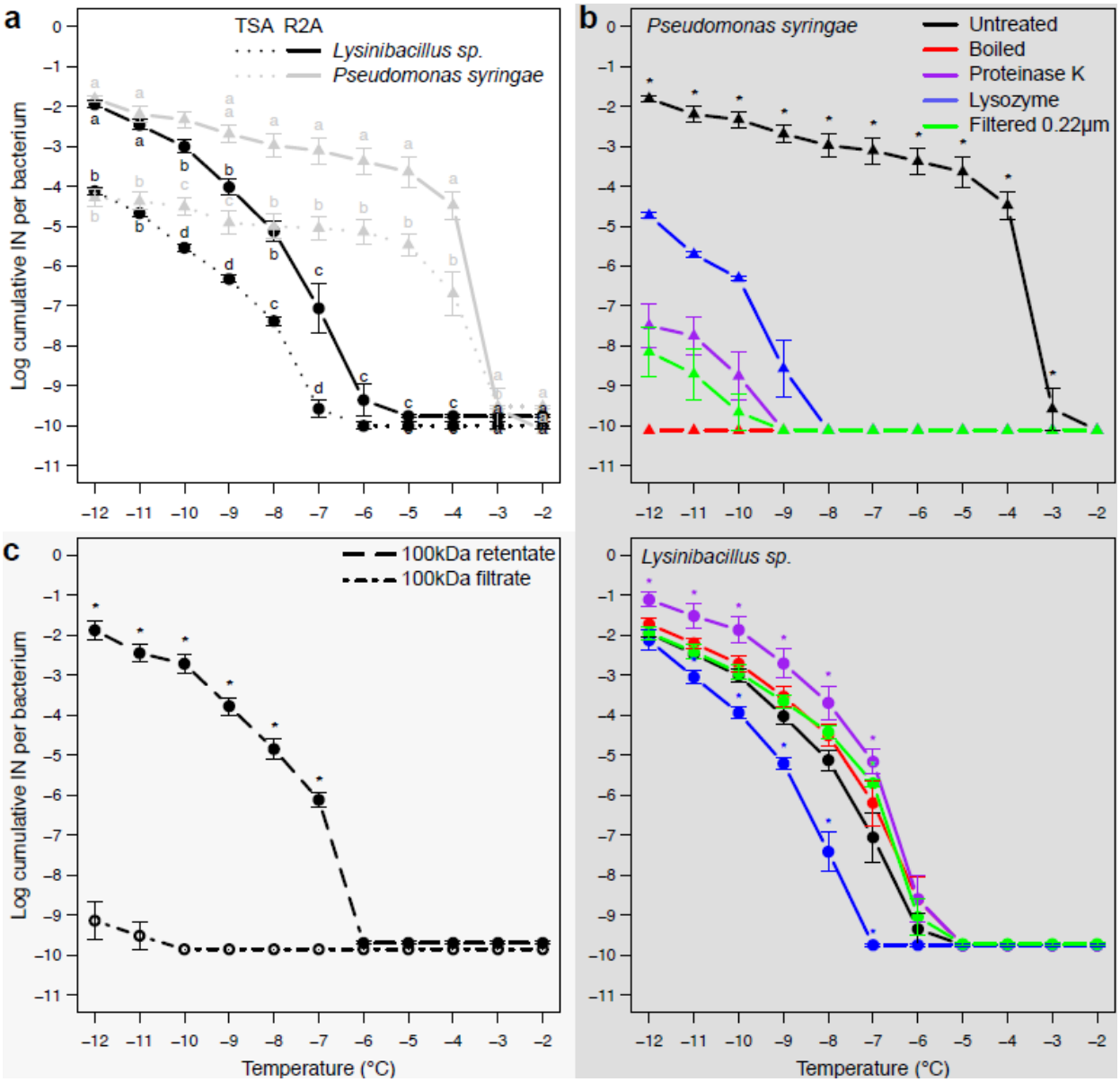

VT1065
VT1066
Lysinibacillus parviboronicapiens BAM582|AB300598
Lysinibacillus fusiformis DSM 2898|AJ310083
Lysinibacillus sphaericus DSM 28|AJ310084
Lysinibacillus boronitolerans 10a|AB199591
Lysinibacillus xylanilyticus XDB9|FJ477040
Lysinibacillus meyeri G 4051|HE577173
Bacillus massiliensis 4400831|AY677116
VT0024
Bacillus cecembensis AM773821
Bacillus odysseyi 34hs1|AF526913
Psychrobacillus psychrodurans DSM 11713|68E3|AJ277984
Bacillus aquimaris TF12|AF483625
VT0067
VT1042
Bacillus mycoides ATCC6462|AB021192
Bacillus weihenstephanensis DSM11821|AB021199
Bacillus thuringiensis IAM 12077|D16281
Bacillus cereus ATCC 14579|AE016877
VT1048
Bacillus anthracis ATCC 14578|AB190217
VT1027
VT1046b
VT0001
Bacillus funiculus NAF001|AB049195
Bacillus acidiceler CBD 119|DQ374637
Bacillus megaterium IAM 13418|D16273, B. flexus (T)|IFO15715|AB021185 & 4 VT strains
Bacillus koreensis BR030|AY667496
Bacillus ginsengi ge14|EF371375
Bacillus butanolivorans K9|EF206294
Bacillus simplex DSM 1321|AJ439078
5 VT strains
Bacillus muralis LMG 20238|AJ316309
Bacillus psychrosaccharolyticus ATCC23296|AB021195
Bacillus asahii MA001|AB109209
Bacillus pocheonensis Gsoil 420|AB245377
Bacillus firmus IAM 12464|D16268 & 3 VT strains
Bacillus oceanisediminis H2|GQ292772
Bacillus infantis SMC 43521|AY904032
Bacillus gottheilii WCC 4585|FN995266
Bacillus circulans ATCC 4513|NCTC 2610|NCDO1775|AY724690
Bacillus safiensis FO036b|AF234854, B. pumilus|ATCC 7061|AY876289 & 8 VT strains
VT0059a
VT0063a
Bacillus altitudinis 41KF2b|AJ831842 & 11 VT strains
Bacillus atrophaeus JCM9070|AB021181
Bacillus tequilensis 10b|HQ223107
VT0018
Bacillus subtilis DSM10|AJ276351
Bacillus vallismortis DSM11031|AB021198
VT0016
VT0040
Bacillus siamensis PDA10|GQ281299
Bacillus amyloliquefaciens DSM7|FN597644
VT1041
VT1044
Bacillus mojavensis IFO15718|AB021191
Bacillus aerius 24K|AJ831843
Bacillus boroniphilus T15Z|AB198719
Bacillus foraminis CV53|AJ717382
Bacillus endophyticus 2D|AF295302
Bacillus fortis R6514|AY443038
Bacillus taeanensis BH030017|AY603978
Allobacillus halotolerans B3A|FJ347755
Other Bacilliaceae sp.
Bacillus alcalophilus DSM 485|X76436
Bacillus iranensis X5B|HQ433452
Marinococcus halotolerans YIM 70157|AY817493
Lactobacillus acidophilus BCRC10695|AY773947
0.02

Figure 5. Ice nucleation spectra of Lysinibacillus sp. in different growth conditions and after different treatments. For each temperature, average values were calculated from at least four independent cultures and bars correspond to standard errors. Lysinibacillus sp. curves were obtained using both strains, VT1065 and VT1066. The minimum detection threshold obtained for P. syringae and Lysinibacillus sp. were 10−10.11 and 10−9.86 IN per bacterium respectively. (a) Ice nucleation spectra of Lysinibacillus sp. ● compared to Pseudomonas syringae strain CC94 ▲, using cultures grown for 24h on either R2A or TSA. Same letters associated to all values for each temperature mean that values were not significantly different according to multiple Student t-tests comparisons. (b) Ice nucleation spectra of cell suspensions obtained from cultures grown on R2A that had been incubated with Proteinase K, lysozyme, filtered through a 0.22µm membrane or heated at 99°C for one hour. For P. syringae CC94, the number of cumulative IN per bacterium was significantly different for all treatments compared to the untreated cell suspension between −12°C and −3°C (values associated to a star). For Lysinibacillus sp., stars were used to label values of treated cell suspensions that were significantly different from the untreated suspension. (c) Ice nucleation activity of the Lysinibacillus sp. 0.22µm membrane filtrate after being either resuspended from a 100kDa membrane (the retentate) or filtered through the 100kDa membrane (the filtrate).


a
TSA R2A
Lysinibacillus sp.
Pseudomonas syringae
Log cumulative IN per bacterium
b
Pseudomonas syringae
Untreated
Boiled
Proteinase K
Lysozyme
Filtered 0.22µm
c
100kDa retentate
100kDa filtrate
Log cumulative IN per bacterium
Lysinibacillus sp.
Temperature (°C)
Temperature (°C)

Chapter Three: Lysinibacillus ice nucleation linked to a type 1 polyketide synthase – Non-ribosomal peptide synthase

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Abstract

Ice nucleation activity (INA) in bacteria was first identified in the phytopathogen *Pseudomonas syringae* in the 1970s. *Pseudomonas syringae* and other ice nucleation active (ice+) bacteria encode a membrane bound, water binding protein, which damages host plant cell walls via ice nucleation and facilitates invasion of plant tissue. Ice+ bacteria may also utilize INA to facilitate return to nutrient rich, terrestrial environments after being carried to the lower atmosphere by upward air currents. A novel ice nucleation mechanism recently identified in *Lysinibacillus* demonstrates that the activity is due to a nanometer-sized, non-proteinaceous, secreted molecule. To identify the biosynthetic genes responsible for INA in *Lysinibacillus*, a combination of comparative and functional genomics approaches was used. *Lysinibacillus* type strains were tested for INA and their genomes were compared, a UV mutagenesis screen was performed, and the transcriptome of ice+ *Lysinibacillus* was determined. A gene cluster exclusively present in ice+ *L. parviboronicapiens* bacteria was found to be mutated in five out of nine ice- mutants and expressed under conditions that favor INA. This gene cluster includes a type-1 polyketide synthase/non-ribosomal peptide synthase. We hypothesize that the polyketide produced by this gene cluster either has INA or is part of a larger multi-molecular INA complex.

Introduction

It is a common misconception that water spontaneously freezes at 0°C. Instead, water can remain liquid until approximately -40°C. Above this temperature, an ice nucleating particle must act on the water molecules to initiate freezing (Debenedetti and Stanley 2003, Moore and Molinero 2011, Wolber 1993). Impurities within the water can serve as ice nucleating particles.

Ice nucleating bacteria were first identified in the mid 1970s with the discovery of ice nucleation activity (INA) in *Pseudomonas syringae* (Schnell and Vali 1972). Since then, several other *Pseudomonas* species and several members of the genera *Erwinia*, *Pantoea* and *Xanthomonas* have been identified to be ice nucleation active (Failor *et al.* 2017, Joly *et al.* 2013, Lindow *et al.* 1978), referred to as "ice+" from here on. These bacteria, all belonging to the gammaproteobacteria, utilize a homodimeric, water binding protein to control the formation of ice crystals in their environment (Garnham *et al.* 2011, Gurian-Sherman *et al.* 1986, Zhao and Orser 1988). Recent studies suggest that the active sites of this protein provide unique hydrophobic-hydrophilic patterns, which impose water molecules to acquire a spatial arrangement that facilitates ice crystal formation (Pandey *et al.* 2016).

We recently uncovered a different ice nucleation mechanism in bacteria, which is present in a *Lysinibacillus* strain, VT1065, isolated from a sample of freezing rain in Blacksburg, Virginia (37°13'29.2"N 80°25'22.0"W). INA in this genus is due to a nanometer-sized, secreted, non-proteinaceous molecule (Failor *et al.* 2017). This study marks the first confirmed ice+ bacteria outside the gammaproteobacteria.

While bacteria are capable of nucleating ice at the warmest temperatures, several other organisms have demonstrated INA as well. Fungi, such as *Fusarium avenaceum and F. acuminatum* have shown activity as warm as -2.5°C, only 1.5°C colder than *P. syringae* (Pouleur

et al. 1992). INA of *Fusarium* is similar to INA of *P. syringae* because it is heat-sensitive and inactivated by proteinases (Fall and Wolber 1995, Humphreys *et al.* 2001, Pouleur *et al.* 1992) but is somewhat more similar to *Lysinibacillus* INA (LINA) since it can be separated from fungal cells (Hasegawa *et al.* 1994). Recently, urideospores of rust fungi have also demonstrated INA. While their activity is not well studied, it is hypothesized that the activity of these fungi is due to a polysaccharide based on the diminished activity after lysozyme treatment (Morris *et al.* 2013). Interestingly, INA in pollen, which nucleates ice at temperatures colder than -18°C, is even more similar to LINA. In fact, Pummer *et al.* (*2012*) report that the ice+ pollen molecule could be separated from the whole pollen grain and is non-proteinaceous due to resistance to heat and enzymatic disruption. These results suggest that two unique classes of ice nucleating particles may exist: proteinaceous compounds like those found in gammaproteobacteria and *Fusarium*, and yet unknown molecules found in rust fungi (Morris *et al.* 2013), pollen, and *Lysinibacillus*.

Taking advantage of the comparatively simple genetics and genomics of bacteria, here we have employed a combination of comparative genomics and functional genetics approaches to identify the genes responsible for LINA with the goal of predicting the chemical composition and structure of the LINA molecule.

Materials and methods

Lysinibacillus selective medium

A *Lysinibacillus* selective medium (LSM) was formulated based on a defined medium used for *Lysinibacillus sphaericus* 2363 (Russell *et al.* 1989). This modified defined medium used to culture *Lysinibacillus* and to aid in the purification of the LINA molecule contains (per liter): Na_2HPO_4, 5.57g; KH_2PO_4, 2.4g; $(NH_4)_2SO_4$, 20mg; $MgSO_4$, 5mg; $MnCl_2*4H_2O$, 400µg;

$FeSO_4*7H_2O$, 810µg; $CaCl_2*2H_2O$, 150µ; H_2SO_4, 0.3µl; sodium acetate, 321.8mg; 25.4mg; biotin, 2µg. Biotin, thiamine, and sodium acetate were prepared in a separate, filter-sterilized stock solution. Mg^{2+}, Mn^{2+}, Fe^{2+}. and Ca^{2+} salts were prepared in a single, acidified (0.03% H_2SO_4 [vol/vol]), filter sterilized stock solution. Both stock solutions were added to the remaining autoclaved solutions after cooling.

Preparation of Lysinibacillus *strains for UV mutagenesis and transformation*

Cultures of the rain-isolated *L. parviboronicapiens* VT1065 were grown on Reasoner's 2A (R2A) plates at 28°C overnight. Overnight cultures of *L. parviboronicapiens* VT1065 were resuspended in water at an $OD_{600}=1.98x10^3$ and incubated for 72hr at 28°C.

UV mutagenesis screening

Ice nucleation inactive (ice-) mutants of *L. parviboronicapiens* VT1065 were generated using 35,000µJ of UV radiation using a modified protocol from the Barrick Laboratory at the University of Texas at Austin (http://barricklab.org/twiki/bin/view/Lab/ProtocolsUVLibrary). 72hr old cultures were resuspended in a 0.9% saline solution at an $OD_{600}=1.56$. 100µl droplets were subjected to 35,000µJ of UV radiation utilizing a UV Stratalinker (Stratagene UV Stratalinker 1800). UV treated cells were plated onto R2A and incubated for 24-48hr at 28°C.

All strains were tested for ice nucleation activity following the protocol from Failor *et al.* 2017. Any putative ice- strains were confirmed in subsequent tests and ice nucleation spectra of each confirmed mutant were generated.

Cumulative nucleus spectra of Lysinibacillus spp.

Cumulative IN concentrations per cell of *Lysinibacillus* type strains and UV mutants were determined following the protocol described previously (Failor *et al.* 2017). Each strain was analyzed from -2°C to -12°C for ten minutes at each temperature. Cumulative IN concentrations were compared to the rain-isolated *L. parviboronicapiens* VT1065 strain after all strains were grown under identical conditions. 30 droplets of 20µl containing six 10-fold dilutions from a starting concentration of 10^8 cells per ml were tested in triplicate. The average number of cells per 20 µl drop *(A)* of *L. parviboronicapiens* VT1065 was determined via dilution plating. The cumulative IN concentration per cell was determined using the equation: $K() = [\ln N_0 - \ln N()]/V$, where V equals A. Error bars were generated using the standard error of the mean.

Treatment of Lysinibacillus *to confirm INA*

Cumulative IN concentrations per cell for each treatment in Figure 2 was performed as above. Treatments followed those previously (Failor *et al.* 2017). Treatments consisted of: (i) boiling suspensions for 1 hour at 99°C, (ii) filtering suspensions through a nitrocellulose membrane (0.22µm porosity, Millipore), and (iii) incubating suspensions for 60 minutes with either 87.4µM of lysozyme (Sigma-Aldrich) at 37°C or with 4.5mM proteinase K enzyme (Roche Diagnostics) at 65°C.

Whole genome sequencing

DNA of all UV generated mutants, *L. parviboronicapiens* VT1065, and the type strains of *L. actophenoni*, *L. composti*, *L. chugkukjangi*, *L. fluoroglycofenyliticus*, *L. jejuensis*, *L. mangiferahumi*, *L. meyeri*, *L. pakistanensis*, *L. parviboronicapiens*, *L. tabacifolii*, and *L.*

xylanilyticus was extracted utilizing a Gram-positive DNA extraction kit (Epicentre, Madison, WI). DNA concentration was determined using both a Nanodrop spectrophotometer and Qubit fluorometer before being sequenced via paired-end Illumina MiSeq. Five UV mutants (UV1, UV11, UV14, UV32, and UV35) were sequenced in a pooled sample while the remaining four (UV131, UV158, UV168, and UV187) mutants were sequenced individually.

Genome analysis

Eight genomes of *Lysinibacillus* type strains were sequenced using Illumina MiSeq and assembled using SPAdes Genome Assembler (Nurk *et al.*, 2013) (Table 1). Genomes of 13 type strains and 29 genomes of non-type strains were downloaded from the NCBI genome database. Pairwise ANI of *Lysinibacillus* genomes were calculated with pyANI (Pritchard *et al.* 2015). Genomes of the *Lysinibacillus* type strains sequenced here and genomes of *L. parviboronicapiens* VT1065 were annotated using the RAST Annotation Server (Aziz *et al.* 2008). GET_HOMOLOGUES was used to perform the comparative genome analysis across these annotated genomes and to identify genes unique to ice+ *L. parviboronicapiens* genomes (Conteras-Moreira and Vinuesa 2013). AntiSMASH was used to predict biosynthetic gene clusters (Weber *et al.* 2015).

LINA molecule preparation and storage

Cultures of the rain isolated *L. parviboronicapiens* VT1065 and UV131 were grown on Reasoner's 2A (R2A) media at 28°C overnight. Overnight cultures were resuspended in water at an $OD_{600}=1.98x10^{3}$ and incubated on LSM for approximately 168hr at 28°C.

Cultures were resuspended at an $OD_{600}=1.56x10^{1}$ in water before centrifugation for 2min at 13,000rpm. The supernatant was filtered through a double 0.8/0.2µm Supor Membrane (Pall Corporation, Cornwall, UK). Filtered samples were then purified using a 100kDa, pre-rinsed,

centrifuge filter unit (Merck Millipore, Ltd. Cork, Ireland). Samples were spun at 5,000 rpm for 10 minutes followed by two wash steps of equal volume to the initial sample. Sample filtrate was discarded after each centrifugation. Filter units were rehydrated in 1ml of molecular biology grade water (HyClone, GE Life Sciences, Utah), before transfer to storage tubes. Purified samples were kept at -20°C or -80°C for maximum shelf-life before being sent for analysis.

Transformation of Lysinibacillus *strains*

5mL of lysogeny broth (LB), were inoculated with *Lysinibacillus* ice- mutant grown on LB agar plates overnight at 28°C. Cultures were shaken overnight at 28°C and 200 rpm. Cultures were re-suspended in 50-ml of LB to an OD of 0.1 and further incubated at 28°C and 200 rpm until an OD of 0.8 was reached (approximately 3-hrs). Cultures were centrifuged for 10 min at 500 rpm and 4°C. The supernatant was discarded and the cells re-suspended with 10 ml of chilled water. Cells were spun and washed with water three more times to clear cells of any residual salts. Following the last centrifugation, cells were re-suspended in 1 mL of 15% glycerol and divided into 100 µl aliquots. Any extra tubes were stored at -80°C for later use. 6 µl of the pHY300PLK vector with our without putative LINA genes were added to 100 µl of cell suspension and allowed to incubate at room temperature for 30 min. The vector-cell suspension was transferred to a chilled electroporation 0.1cm cuvette (Bio-Rad) and a pulse of 2.0-KV was applied for 4.5-5.0 seconds with a resistance of 200Ω and a total capacitance of 25µF using a Gene Pulser (Bio-Rad). Following electroporation, 1-mL of LB was added to the cuvette to mix and facilitate transfer of transformants to a 1.5-mL tube. Transformants were incubated for 4-hrs at 28°C at 200 rpm before plating 600-uL onto a LB agar plate with 10 µg/mL of tetracycline. The culture plate was incubated overnight at 28°C.

Results

INA of Lysinibacillus *species type strains*

Following the identification of the ice+ *Lysinibacillus* strain VT1065 from freezing rain (Failor *et al.* 2017), we wanted to examine the frequency of this phenotype across the *Lysinibacillus* genus. Eighteen type strains were obtained from various culture collections (Table S1) to be tested for INA. Prior to testing, the strain identities were confirmed via 16S rRNA sequencing using the same primer pairs described in Failor *et al.* 2017 (*2017*). All strains were tested in triplicate at a standard OD_{600}=1.56 based on a protocol that provides the highest INA for *Lysinibacillus* strain VT1065 (see Supplemental Methods).

Figure 1a-i shows that INA in *Lysinibacillus* was only observed for strains of *L. parviboronicapiens*. The *L. parviboronicapiens* type strain BAM-582^T begins exhibiting INA as warm as -8 to -9°C. However, the strain produces between 1.5 and 2.9 orders of magnitude fewer ice nuclei active between -8°C and -12°C compared to VT1065 (Figures 1g and 2). While several strains show slight activity at colder temperatures (≤-10°C), their activity is not significantly different from that of pure water, which, under the employed testing conditions, can begin to show freezing of some droplets around these same temperatures.

INA of L. parviboronicapiens is heat, lysozyme, and proteinase resistant and secreted

INA of VT1065 had been determined to be due to a nanometer-sized, secreted, heat-stable, and proteinase and lysozyme resistant molecule (Failor *et al.* 2017). Using the same tests, we determined that INA of BAM-582^T demonstrates the same characteristics, although of slightly different magnitude: BAM-582^T shows a delay in onset of activity by 1°C and a decrease in IN production of two orders of magnitude at -9°C. This difference diminishes as the temperature

decreases, ending at a final difference of 0.7 orders of magnitude at -12°C. Unlike VT1065, BAM-582^T does not present any increase in activity after proteinase K treatment, instead BAM-582^T shows little variation from the untreated samples aside for the delay of onset of activity also seen in the other treatments. Similar to the proteinase K treatment, the addition of lysozyme had minimal effect on the INA of BAM-582^T (<1.0 orders of magnitude difference). The only marked difference between these two tests was the one degree delay in the onset of freezing. Samples that were passed through the 0.22μm filter showed a decrease in activity of 1.5 orders of magnitude at -9°C and the same one degree delay in onset of freezing was seen as with all previous treatments. The decrease in activity diminishes to approximately 0.5 orders of magnitude for the remaining temperatures, ending at a similar concentration as the boiled samples.

Comparative and functional genomics

In order to determine the genes responsible for LINA and to determine the species affiliation of VT1065, the genomes of *L. parviboronicapiens* BAM-582^T and of VT1065 were compared with all other genomes of *Lysinibacillus* strains that were tested for INA above. The genome of VT1065 was found to share a 98.82% average nucleotide identity (ANI) (Konstantinidis and Tiedje 2005) with BAM-582^T. This is well above the generally accepted species threshold of 95-96% ANI (Goris *et al.* 2007, Richter and Rossello-Mora 2009), suggesting that VT1065 belongs to the species *L. parviboronicapiens*. The program Get_Homologues (Conteras-Moreira and Vinuesa 2013) was then used to compare predicted protein repertoires among genomes to identify genes exclusively present in the genomes of ice+ *L. parviboronicapiens* strains and, thus, predicted to be possibly responsible for biosynthesis of the LINA molecule. 239 genes were so identified (Figure 4). In parallel, the transcriptome of VT1065

under LINA-inducing conditions was determined using an RNA-seq approach. Of the 239 genes, exclusively present in ice+ *Lysinibacillus* strains, 131 genes were found to be expressed, further reducing the list of putative LINA biosynthetic genes. Nearly half of these genes encode hypothetical proteins (Table S1).

UV mutagenesis of VT1065 identifies putative ice+ genes

In parallel to the comparative and functional genomics approach, a UV mutagenesis screen was performed to generate loss of function ice nucleation inactive (ice-) strains. 18,416 mutant strains were screened for activity at -8°C, -10°C, and -12°C. Nine mutant strains demonstrated a consistent decrease or loss of activity (Figure 3). UV mutants 1, 11, 14, 32, 35, 131, and 158 all showed a complete loss of activity (no activity detected at higher rates than those seen by the pure water control). VT1065-UV168 exhibited activity starting at -11°C, with a decrease in ice nuclei production of nearly four orders of magnitude at both -11 and -12°C. Similarly, VT1065-UV187 showed a decrease in INA, though not to the same extent as VT1065-UV168. INA of VT1065-UV187 starts as high as -9°C and shows an approximate decrease in activity of two orders of magnitude. The residual activity of these two strains suggests the mutations induced in these strains have an effect on the efficacy of the INA, rather than causing a failure in the production or secretion of the LINA molecule.

Sequencing reads of each mutant were aligned against the wild type (VT1065) genome and non-synonymous mutations, insertions, and deletions were identified. A total of 28 mutations were found between the wild-type genome and the nine mutant strains (Table 2) with an average of 3.89 mutations per ice- mutant. Only one gene was found to be mutated in multiple mutant strains: five mutations occurred in gene 1440 coding for a predicted Malonyl CoA-acyl carrier protein

transacylase in four of the nine mutant strains (mutants 1, 11, 14, and 35). A fifth mutant strain, VT1065-UV131, was found to have a mutation is the neighboring gene, 1439, coding for a predicted non-ribosomal peptide synthase/polyketide synthase (gene 1439). All strains presenting these two mutations showed no INA. Additionally, strain VT1065-UV158 showed no activity, despite having mutations in genes outside of this cluster. Strains VT1065-UV168 and -UV187 present mutations in a pyruvate dehydrogenase E1 component alpha subunit (VT1065-UV168) and in both a sporulation kinase A and sensor histidine kinase gene (VT1065-UV187).

Comparison of the comparative genomics, transcriptomics, and UV mutagenesis results

Finally, the genes identified in the mutant screen were compared with the genes exclusively present in ice+ *Lysinibacillus* genomes that were also found to be expressed in the RNA-seq analysis. From this, it was determined that the majority of the mutated genes (40/47) are expressed but only two of the mutated genes were found to be expressed and, at the same time, to be exclusively present in the ice+ *Lysinibacillus* genomes: genes 1439 and 1440. These two genes were also found to be the only mutated genes that are members of biosynthetic gene clusters when using the antiSMASH secondary metabolite biosynthetic pathway prediction tool (Weber *et al.* 2015). The two genes were found to be part of a three-gene operon together with gene 1438, a putative thioesterase. The operon is predicted to encode a type 1 polyketide synthase/non-ribosomal peptide synthase (T1PKS/NRPS) (Figure 5).

Type-1 polyketide synthase/non-ribosomal peptide synthase operon may have a role in Lysinibacillus *INA*

Based on sequence similarity of the identified T1PKS/NRPS to T1PKS/NRPS enzymes in other organisms, a structure was predicted for the hypothetical polyketide/non-ribosomal peptide produced by VT1065 (Figure 6). The product likely contains an ornithine cysteine dipeptide, whereby the ornithine group may be activated in order to be linked to the polyketide through the delta-amino group, similar to the adenylation domain amino acid activation signature found in the heat-stable antifungal factor (HSAF) (Lou *et al.* 2011, Yu *et al.* 2007). The sequences of HSAF and the *Lysinibacillus* polyketide synthase are 40% identical with an additional 58% showing sequence similarity. Additionally, both enzymes contain the same ketosynthase, acyltransferase, dehydratase, ketoreductase, and acyl carrier protein domain strings, suggesting further similarities between both polyenes. The non-ribosomal peptide synthase may further modify the molecule via an apparent cyclase domain in the cysteine module. This modification suggests that the cysteine is heterocyclized via dehydration to form a thiazoline ring in the resulting molecule (Figure 6b).

Moreover, it appears that the T1PKS/NRPS represents an iteratively acting Type 1 PKS as the gene cluster contains all of the domains necessary to synthesize the polyketide product and it has a high degree of sequence similarity to HSAF and other iterative Type 1 PKS's.

Scanning electron microscopy shows no obvious differences between wild and mutated cells

Scanning electron microscopy (SEM) of both whole cells and prepared LINA molecule preparations showed little variation between the wild-type and mutant cells. Whole cells fall in the normal length for members of the species and present a small inclusion body indicated by the black arrow in wild-type and mutant strains (Figure 7a). Likewise, the LINA molecule preparations for

both wild-type and mutant samples include some remnant of cellular debris or other amorphous material seen in Figure 7b. Abundance of the amorphous material varies between preparations but does not appear to correlate with presence of INA in wild-type and absence of INA in mutants.

Discussion

Currently, the role of polyketides in ice nucleation is almost completely uninvestigated. While several groups have hypothesized that polysaccharides may be linked to ice nucleation (Dreischmeier *et al.*, 2017, Morris *et al.*, 2013, Pummer *et al.*, 2012), polyketides have not been previously shown to have any ice nucleating properties.

Here we have shown that INA of the *L. parviboronicapiens* type strain BAM-582^{T} is due to a secreted, nanometer-sized, heat stable, and lysozyme and proteinase resistant molecule similar to the LINA molecule previously found to be produced by *L. parviboronicapiens* VT1065 (Failor *et al.* 2017). A T1PKS/NRPS gene cluster exclusively present and expressed in ice+ *L. parviboronicapiens* strains was the only biosynthetic gene cluster with mutations in multiple ice- strains obtained during a UV mutagenesis screen. The combination of these results provides strong circumstantial evidence for an ice+ polyketide/non-ribosomal peptide being necessary for LINA. Intriguingly, PKSs have been previously shown to act as nuclei, not for ice, but for the formation of calcium carbonate otolith structures in two separate fish species (Hojo *et al.* 2015, Thiessen *et al.* 2017) suggesting that these types of molecules may provide a scaffold for nucleation. Additionally, recent work has shown that polyvinyl alcohol can aid in the homogeneous freezing of water by the arrangement of hydrophobic and hydrophilic groups. While an interaction similar to polyvinyl alcohol could help explain LINA's role in higher ice nucleation temperatures, these

interactions alone may not be sufficient to explain the full effect of the LINA molecule (Mochizuki *et al.* 2017), suggesting that the LINA molecule aids primarily in heterogenous freezing of water.

From previous work, the estimated size of the LINA molecule is >100kDa (Failor *et al.* 2017). This result was also confirmed for *L. parviboronicapiens* BAM-582^{T} (data not shown). However, the predicted structure for the molecule shows a largely hydrophobic region. Consequently, it is possible that the LINA molecule is binding weakly to the membrane surface of the 100kDa filter, preventing an accurate size estimate for this molecule. Furthermore, the large hydrophobic tail presented in the predicted model of the molecule may cause the polyketide to form micelles, simple bilayers, or larger aggregates that prevent it from passing through the 100kDa membrane during the filtrations that were performed.

The predicted variability of the polyketide tail length may, in part, explain the variation in INA seen between strains. Changes in tail length may allow for greater or lesser association with surrounding water molecules, thereby affecting the efficiency of ice nuclei formation. Additional variability may also arise from minor differences within the T1PKS/NRPS gene cluster. We have shown that mutations within this cluster drastically alter INA strength, to the point of a complete knockout of activity.

While we predict the polyketide produced by the T1PKS/NRPS gene cluster to have INA, it is possible that the molecule serves as a scaffold or precursor molecule which, after further modifications by other enzymes becomes the final active molecule. Additionally, the polyketide may, after secretion from the cell, associate with other molecules in the extracellular matrix in such a way that these associated molecules may become ice+ and the polyketide may only serve to modify these unknown molecules. However, based on the current limitations to understanding the LINA molecule, such claims are purely speculation.

During the characterization of the LINA molecule, it was found that it has properties shared with INA molecules of certain fungi and pollen. While some fungi, like members of the genus *Fusarium*, do produce proteinaceous, moderately heat-sensitive (stable to 45-60°C), ice nucleating particles that can pass through a 0.22μm membrane and are more resistant to pH changes than the proteins produced by gammaproteobacteria (Humphreys *et al.* 2001, Lagzian *et al.* 2014, Pouleur *et al.* 1992) , one clade of *Mortierella alpina* ATT234 utilizes a relatively heat stable (ice+ at 60°C), proteinase-resistant molecule active as warm as -5.5°C (Frohlich-Nowoisky *et al.* 2015). Another class of ice+ fungi, the rust fungi, demonstrate activity from the urediospores warmer than -10°C and are thought to be due to a polysaccharide based solely on the decrease in activity after exposure to lysozyme (Morris *et al.* 2013).

Ice nucleation active molecules of pollen species, like the activity of rust fungi, are thought to be derived from carbohydrates and larger polysaccharide molecules (Dreischmeier *et al.* 2017, O'Sullivan *et al.* 2015, Pummer *et al.* 2012). Upon further inspection of the characteristics of birch pollen ice nucleating particles, numerous similarities can be observed to the LINA molecule, namely that both are nanometer sized, secreted, heat-stable, and proteinase resistant molecule (O'Sullivan *et al.* 2015, Pummer *et al.* 2012). While these commonalities are neither evidence that pollen possess a polyketide ice+ molecule, nor that the LINA molecule is a polysaccharide, there may be structural similarities between both classes of ice+ molecule that may aid in the characterization of both.

Furthermore, one of the current assumptions regarding bacterial ice nucleation particles states that one ice+ bacterial cell produces one ice nucleus in order to be relevant for the production of precipitation (Failor *et al.* 2017). However, if each bacterial cell was able to produce molecules capable of being separated from the whole cell and remain ice+, these bacteria may contribute a

larger number of ice nuclei per cell into the environment than what current calculations currently account for.

In order to identify the role that polyketides play in the formation of precipitation, the overall abundance of *L. parviboronicapiens*, the LINA molecule, and any other polyketides and polyketide-producing organisms must be better understood. Also, by better understanding the ecological niches inhabited by these organisms and better understanding the environmental requirements for production of INA molecules will help to determine if these organisms are actively contributing to the formation of precipitation.

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Table 1. Genome information for all strains sequenced as part of this study.

	Type Strain	Size (Mb)	Contigs
L. acetophenoni JC23	Yes	3.9	87
L. chugkukjangi 2RL3-2	Yes	4.2	269
L. fluoroglycofenilyticus cmg86	Yes	3.5	71
L. jejuensis N2-5	Yes	2.8	262
L. mangiferahumi M-GX18	Yes	5.2	153
L. meyeri WS4626	Yes	4.3	406
L. parviboronicapiens BAM-582	Yes	4.6	132
L. parviboronicapiens VT1065	No	4.6	73
L. parviboronicapiens VT1066	No	4.6	75
L. tabacifolii K3514	Yes	4.4	51

Table 2: List of mutations present in UV mutated L. parviboronicapiens VT1065.

Gene #	Gene Function	Position	Original	Mutation
Pooled UV Mutants (Lysinibacillus parviboronicapiens 1065-UV1, 11, 14, 32, and 35 (BAV5236, 5276, 5288, 5335, 5338))				
77	Large Subunit Ribosomal RNA; lsuRNA; LSU rRNA	136	T	C
80	Small Subunit Ribosomal RNA; ssuRNA; SSU rRNA	463	C	T
162	Phosphoribosylformylglycinamidine synthase, synthetase subunit	2233	T	A
210	Serine protein kinase (prkA protein), P-loop containing	3	G	GG
409	Potassium uptake protein, integral membrane component, KtrB	631	A	T
1303	Hypothetical protein	130	G	A
		187	CA	A
2179	Mobile element protein	403	T	G
		404	G	T
2586	ATP-dependent DNA helicase RecG	19	C	T
2955	Transposase	23	A	T
3841	Nitrite-sensitive transcriptional repressor NsrR	256	G	A
3894	Uncharacterized iron compound ABC uptake transporter, substrate-binding protein	851	C	T
4406	Stage 0 sporulation two-component response regulator (Spo0A)	489	T	TA
		632	C	T
4486	Glyoxalase family protein	935	A	AT
4636	Transcriptional regulator, HxlR family	368	A	AT
Lysinibacillus parviboronicapiens 1065-UV1 (BAV5236)				
1440	**Malonyl CoA-acyl carrier protein transacylase**	**406**	**T**	**TA**
Lysinibacillus parviboronicapiens 1065-UV11 (BAV5276)				
1440	**Malonyl CoA-acyl carrier protein transacylase**	**746**	**G**	**A**
Lysinibacillus parviboronicapiens 1065-UV14 (BAV5288)				
1440	**Malonyl CoA-acyl carrier protein transacylase**	**1826**	**T**	**A**
		2479	**C**	**T**
Lysinibacillus parviboronicapiens 1065-UV35 (BAV5338)				
1440	**Malonyl CoA-acyl carrier protein transacylase**	**1271**	**G**	**A**
Lysinibacillus parviboronicapiens 1065-UV131 (BAV5484)				
987	Putrescine transport ATP-binding protein PotA	749	A	T
1439	**Non-ribosomal peptide synthase/polyketide synthase**	**3474**	**T**	**A**
2561	Helicase PriA essential for oriC/DnaA-independent DNA replication	1405	C	T
3266	Spore germination protein xc. bacillus	635	CA	C
4580	Hbl B protein	286	A	T
Lysinibacillus parviboronicapiens 1065-UV158 (BAV5512)				
192	Rhs family protein (phage)	296	C	A
900	Sporulation initiation phosphotransferase B (Spo0B)	215	G	A
2453	ABC transporter substrate-binding protein	190	C	T
4060	Oligopeptide transport ATP-binding protein OppF	451	C	T
4642	Flagellar hook-length control protein FliK	1160	G	A
Lysinibacillus parviboronicapiens 1065-UV168 (BAV5522)				
1317	Pyruvate dehydrogenase (acetyl-transferring) E1 component subunit alpha	462	TG	T
Lysinibacillus parviboronicapiens 1065-UV187 (BAV5550)				
1389	Sporulation kinase A	741	C	A
3901	Sensor histidine kinase	2290	G	A

Figure 1. Ice nucleation spectra of Lysinibacillus type strains grown on R2A for 48hr at 28°C. For each temperature, average values were calculated from at least three independent cultures and bars correspond to standard errors. *Lysinibacillus spp.* curves were obtained using the species type strains. The minimum detection threshold obtained for *Lysinibacillus spp.* was $10^{-10.05}$ IN per bacterium. Each strain ▲ was compared to *Lysinibacillus parviboronicapiens* VT1065 ▲ and a pure water control ●. (a) Ice nucleation spectra of *Lysinibacillus acetophenone* JC23 and *L. boronitolerans* 10a (b) *L. chungkukjamgi* 2RL3-2, *L. fusiformis* 19A1, *L. jejuensis* N2-5, *L. meyeri* WS 46226, *L. odyssey* 34hs-1, and *L. tabacifolii* K3514 (c) *L. composti* NCCP-36 and *L. massiliensis* 4400831 (d) *L. fluoroglycofenilyticus* cmg86 (e) *L. mangiferahumi* M-GX18 (f) *L. pakistanensis* NCCP-54 (g) *L. parviboronicapiens* BAM-582 (h) *L. sinduriensis*, and (i) *L. sphaericus* DSM 28, *L. varians* GY32, and *L. xylanilyticus* XDB9.

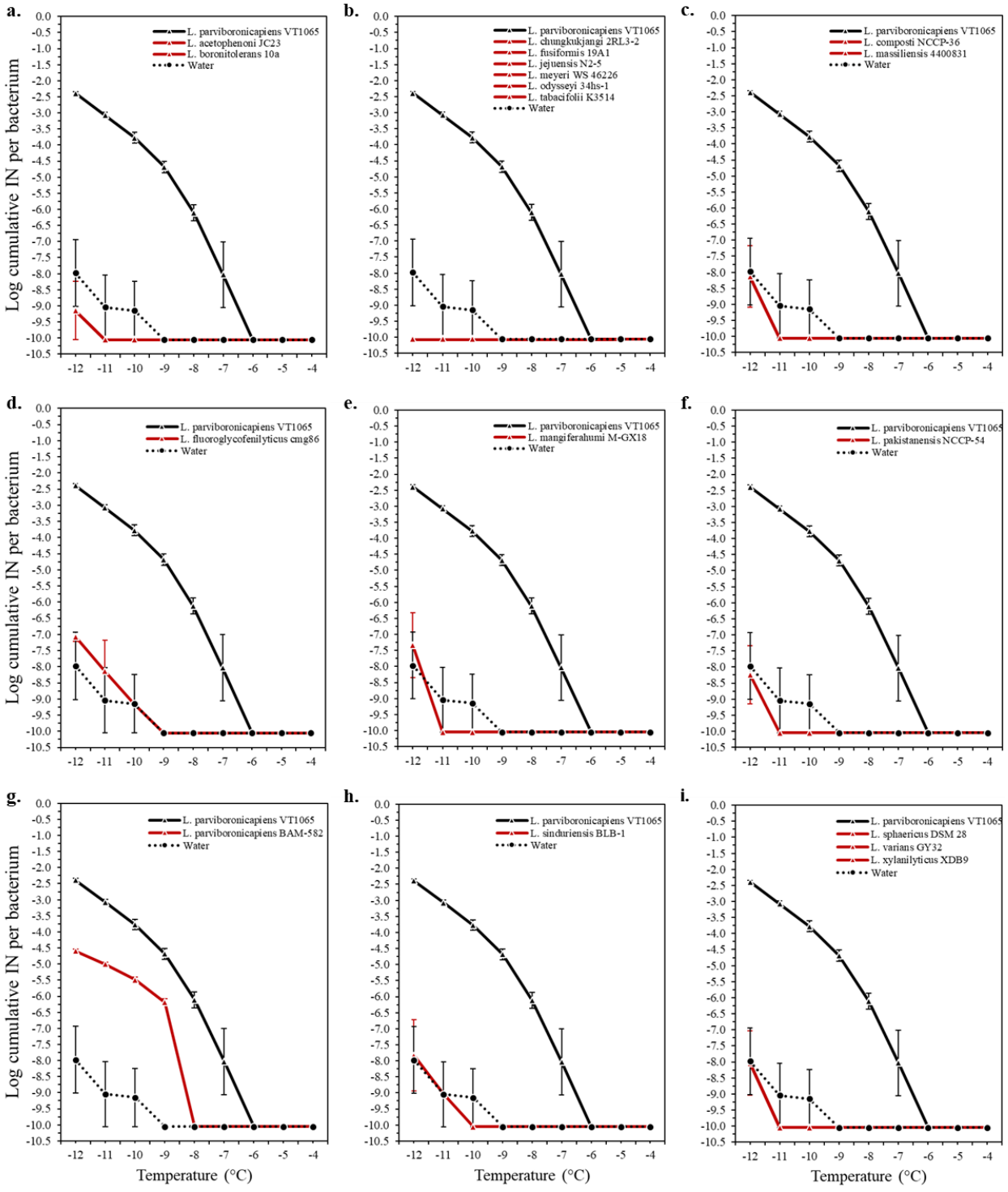

a.
b.
c.
d.
e.
f.
g.
h.
i.
Log cumulative IN per bacterium
Temperature (°C)
L. parviboronicapiens VT1065
L. acetophenoni JC23
L. boronitolerans 10a
L. chungkukjangi 2RL3-2
L. fusiformis 19A1
L. jejuensis N2-5
L. meyeri WS 46226
L. odysseyi 34hs-1
L. tabacifolii K3514
L. composti NCCP-36
L. massiliensis 4400831
L. fluoroglycofenilyticus cmg86
L. mangiferahumi M-GX18
L. pakistanensis NCCP-54
L. parviboronicapiens BAM-582
L. sinduriensis BLB-1
L. sphaericus DSM 28
L. varians GY32
L. xylanilyticus XDB9
Water

Figure 2. Ice nucleation spectra of Lysinibacillus parviboronicapiens grown on R2A for 48hr at 28°C that had been incubated with Proteinase K, lysozyme, filtered through a 0.22 µm membrane or heated at 99 °C for 1 h. For each temperature, average values were calculated from at least three independent cultures and bars correspond to standard errors. *Lysinibacillus spp.* curves were obtained using the species type strains. The minimum detection threshold obtained for *Lysinibacillus parviboronicapiens* VT1065 was $10^{-10.05}$ IN per bacterium. *Lysinibacillus parviboronicapiens* BAM-582 shows a unique shift in curves which may be due to a number of nucleotide variations present in the putative ice nucleation gene cluster compared to VT1065.

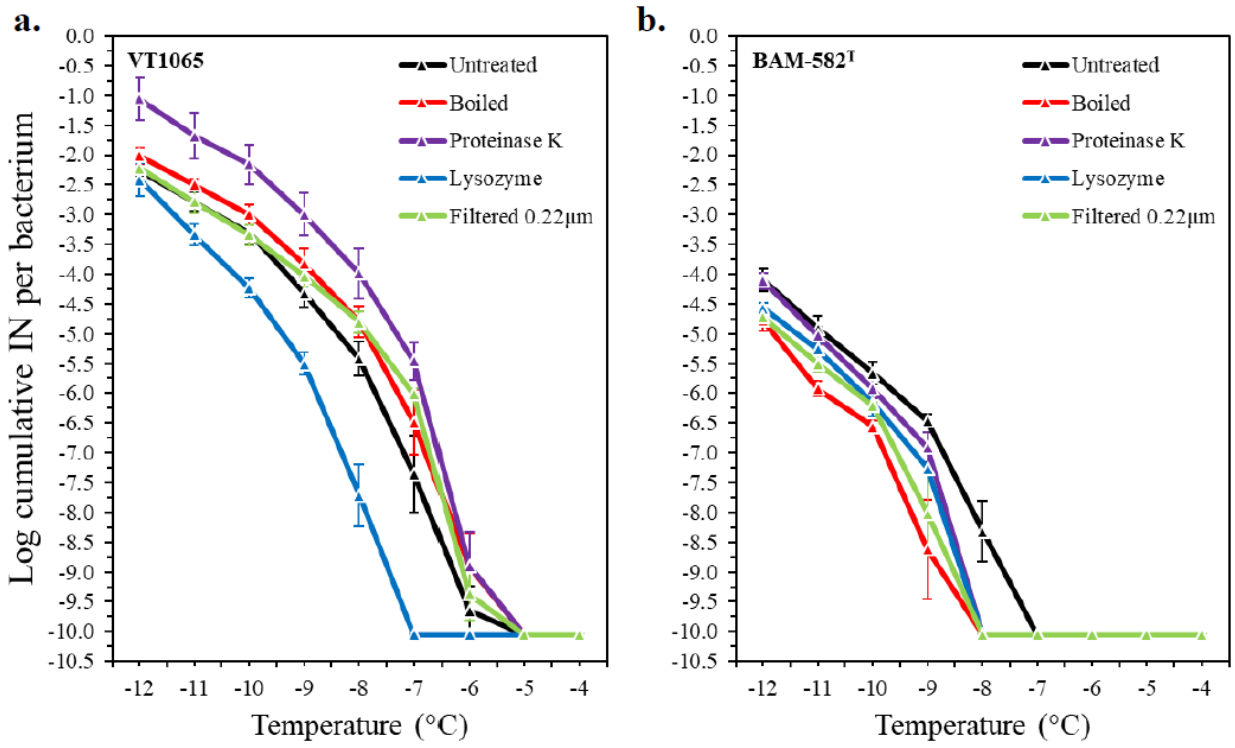


Figure 3. Ice nucleation spectra of Lysinibacillus parviboronicapiens VT1065 UV-treated mutants grown on R2A for 48hr at 28°C. For each temperature, average values were calculated from at least three independent cultures and bars correspond to standard errors. *Lysinibacillus spp.* curves were obtained using the species type strains. The minimum detection threshold obtained for *Lysinibacillus parviboronicapiens* VT1065 was $10^{-10.05}$ IN per bacterium. Each strain ▲ was compared to *Lysinibacillus parviboronicapiens* VT1065 ▲ and a pure water control ●. (a) Ice nucleation spectra of *Lysinibacillus parviboronicapiens* VT1065-UV1 (b) VT1065-UV11 (c) VT1065-UV14, VT1065-UV35, and VT1065-UV38 (d) VT1065-UV131 and 158 (e) VT1065-UV168 and (f) VT1065-UV187.

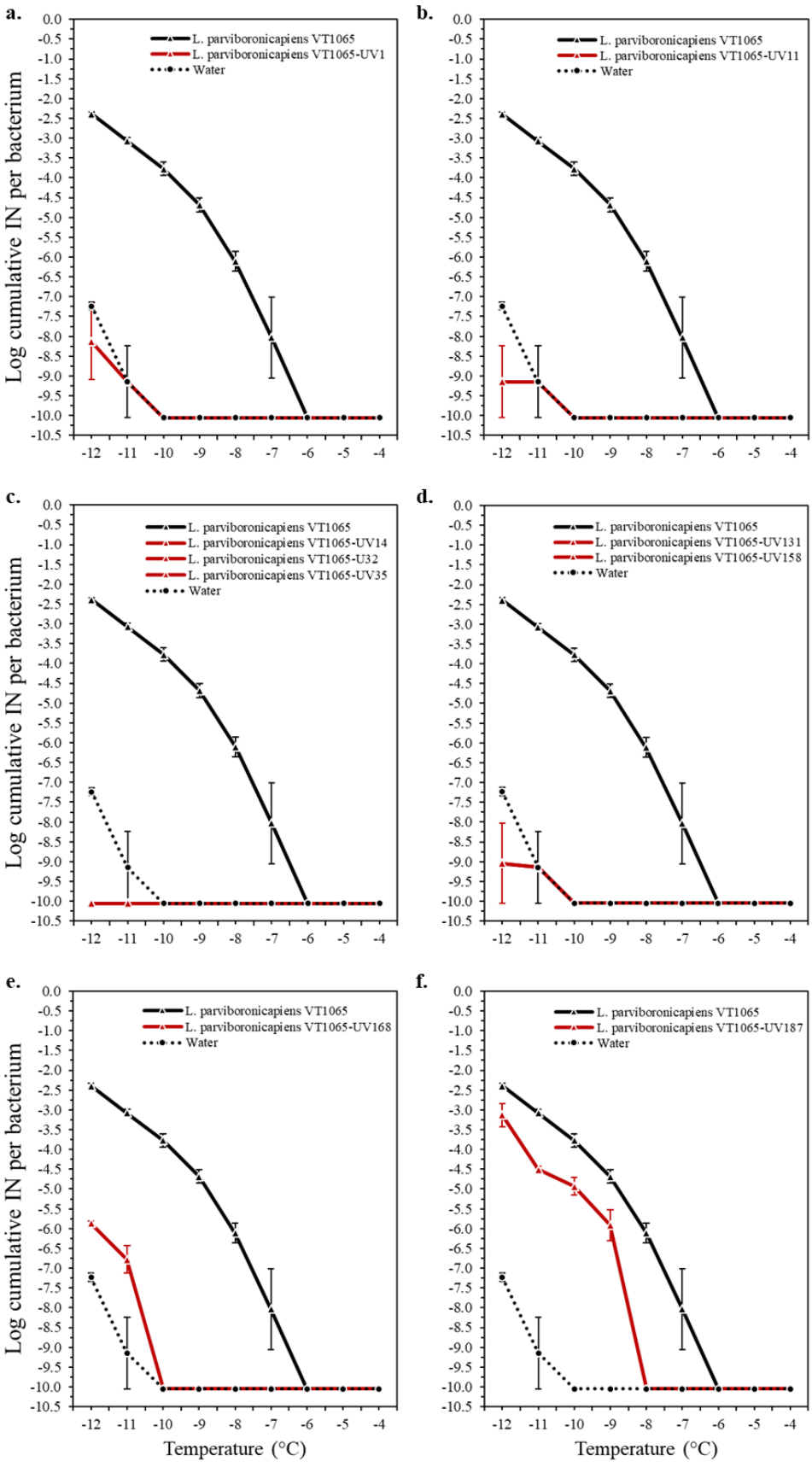

a.
b.
c.
d.
e.
f.
Log cumulative IN per bacterium
Temperature (°C)
L. parviboronicapiens VT1065
L. parviboronicapiens VT1065-UV1
L. parviboronicapiens VT1065-UV11
L. parviboronicapiens VT1065-UV14
L. parviboronicapiens VT1065-U32
L. parviboronicapiens VT1065-UV35
L. parviboronicapiens VT1065-UV131
L. parviboronicapiens VT1065-UV158
L. parviboronicapiens VT1065-UV168
L. parviboronicapiens VT1065-UV187
Water

Figure 4. Distribution of putative LINA genes identified by comparative genomics (genes exclusively present in ice+ L. parviboronicapiens genomes), transcriptomics (genes expressed in ice+ L. parviboronicapiens VT1065 under LINA-inducing conditions), and UV mutagenesis (genes mutated in ice- UV mutant strains). Each circle denotes the number of genes identified by each approach. Relative sizes of circles corresponds with the number of genes present within each circle but the areas of the circles are not to scale. Overlap between circles denotes shared genes between two or more approaches. The intersect of all three circles identifies the genes with the highest probability to be at the base of LINA molecule biosynthesis and correspond to genes 1439 and 1440 in Figure 5.

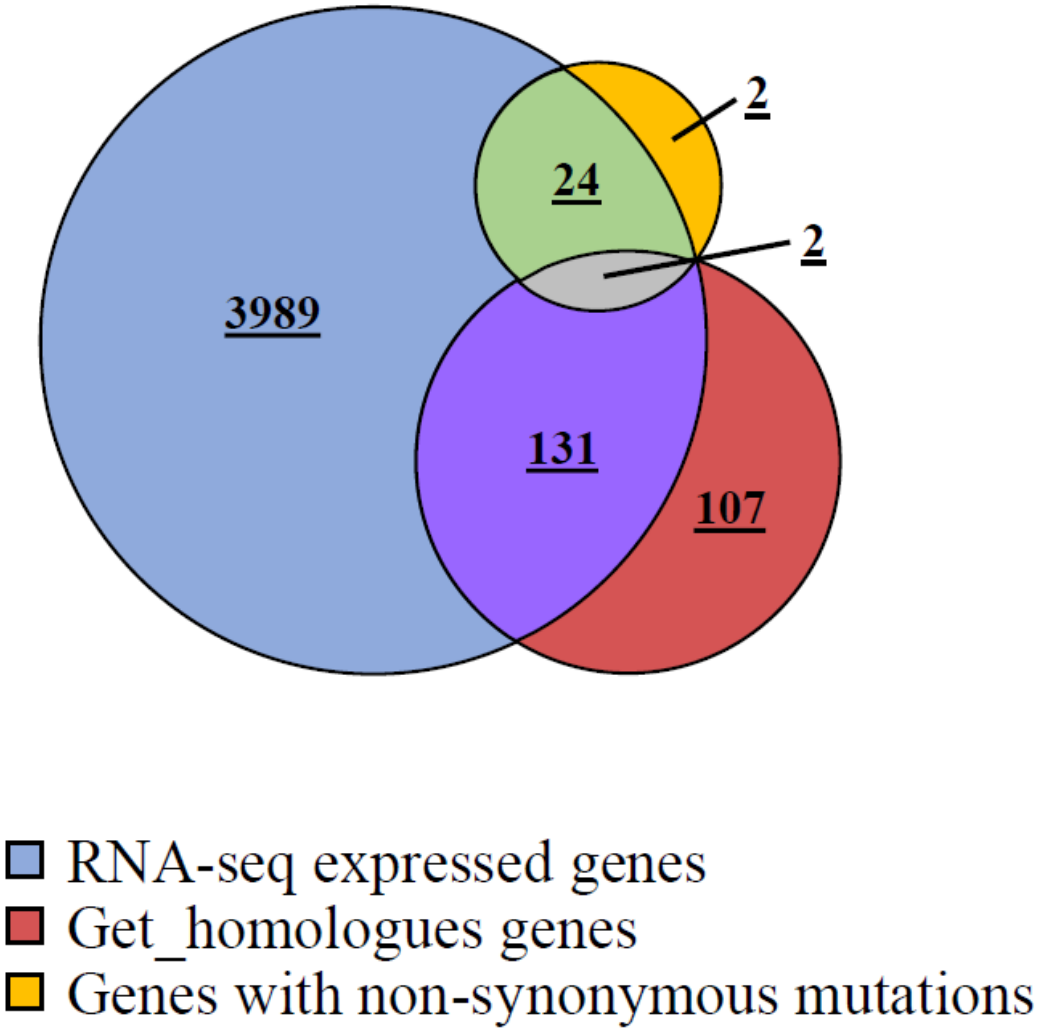


Figure 5. Type 1 polyketide synthase/non-ribosomal peptide synthase gene cluster identified by antiSMASH secondary metabolite biosynthetic pathway prediction tool. The type 1 polyketide synthase/non-ribosomal peptide synthase gene cluster was the only biosynthetic pathway identified by antiSMASH among genes with non-synonymous mutations generated by UV mutagenesis. Arrows denote the direction of the gene. Genes in red correspond to core biosynthetic genes, while those in orange are any additional biosynthetic genes. Genes in blue are transport-related genes, and all additional genes in grey are unrelated. Asterisks mark the relative location of the mutations present across the five UV mutant strains with mutations in this cluster. Gene (a) corresponds to gene 1438, a thioesterase, (b) is gene 1439, a non-ribosomal peptide synthase, and (c) is gene 1440, a polyketide synthase.

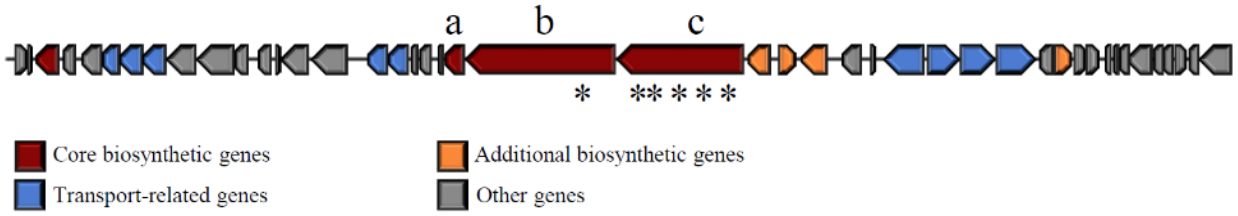


Figure 6. Proposed preliminary structure of the polyketide produced by the Type 1 polyketide synthase/non-ribosomal peptide synthase gene cluster shown in Figure 5. (a) Predicted structure of the polyketide after the initial polyketide synthase gene activity, and (b) predicted structure of the polyketide after action by the non-ribosomal peptide synthase. This also includes the formation of the thiazoline ring after a dehydration reaction.

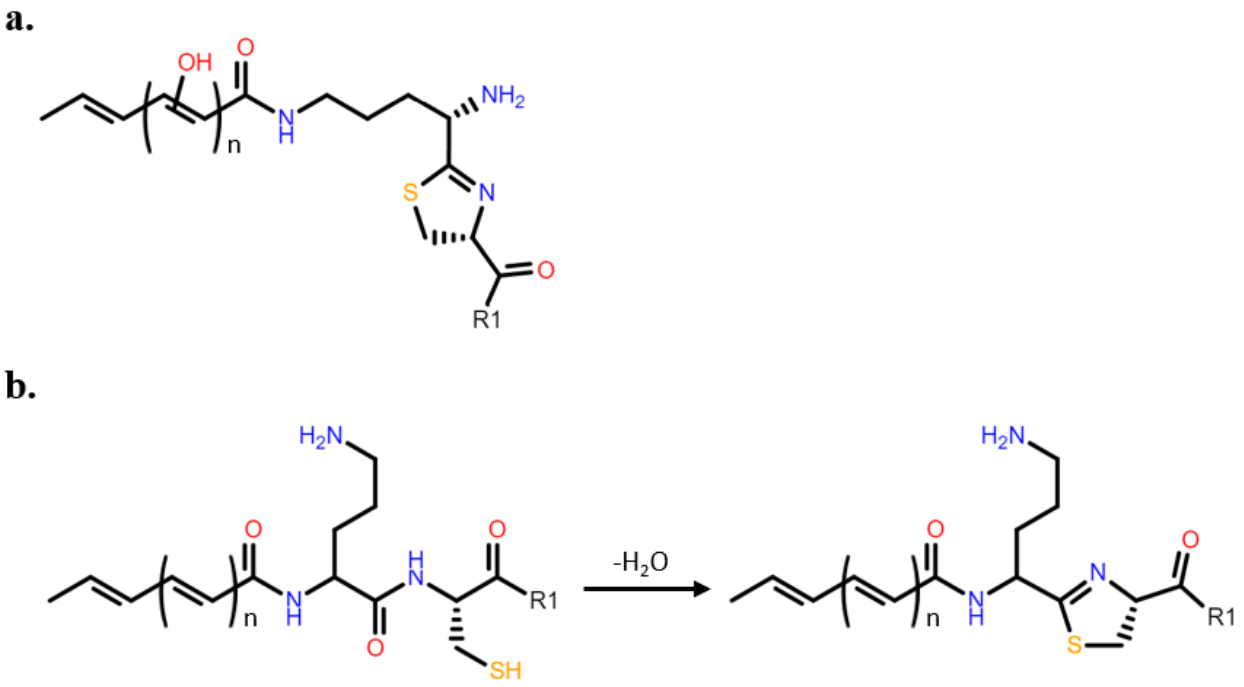
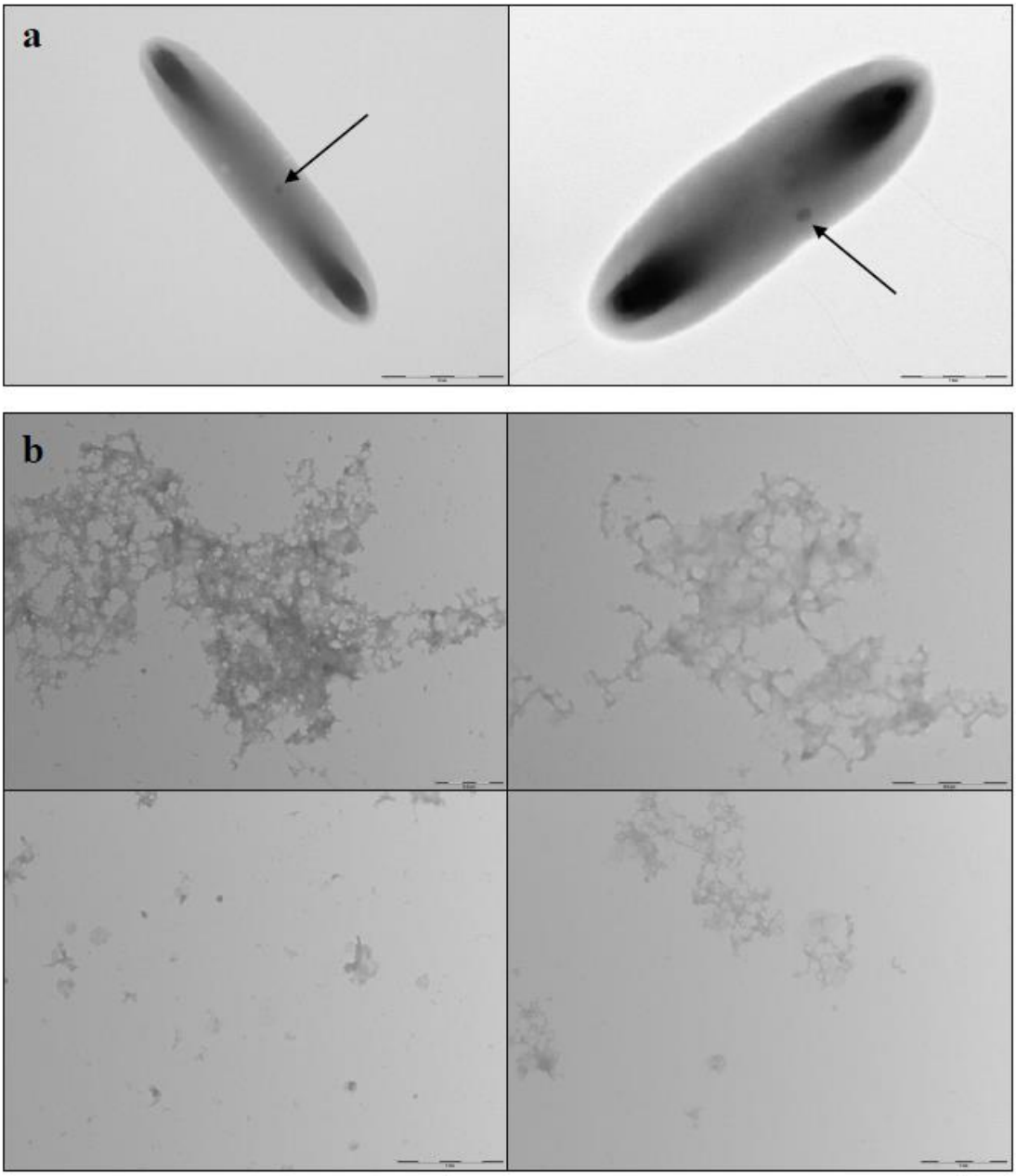


Figure 7. Scanning Electron Microscopy images of Lysinibacillus. (a) Comparison of *L. parviboronicapiens* VT1065 (left) and VT1065-UV14 (right). Arrows indicate the presence of a small inclusion body in both strains. (b) Comparison of *L. parviboronicapiens* VT1065 (left) and VT1065-UV131 (right) LINA preparations. Upper panels depict regions with abundance of amorphous materials of unknown identity while lower panels depict regions with low abundance of similar material.



Chapter Four: Bergey's Manual of Systematics of Archaea and Bacteria: Lysinibacillus

Failor, K.C., Tian, L., Monteil, C.L., Vinatzer, B.A.

Lysinibacillus

Ahmed 2007, 1117^{VP}

Ly.si'ni.ba.cil'lus. N.L. n. *lysinum* lysine; *Lysinibacillus* masc. n. *bacillus* a small staff or rod; N.L. masc. n. *Lysinibacillus* lysine bacillus, referring to the presence of the Lys–Asp type of peptidoglycan in the cell wall.

Rod-shaped, 0.3-1.5 x 0.5-14.0μm with **parallel sides and blunt ends** and may form long filaments. **Endospores are formed**, no more than one per cell, and are typically terminally located and bulging. Most species stain Gram-positive, with one species staining Gram-variable and two species staining Gram-negative. All species except two demonstrate motility, typically with peritrichous flagella. Bacteria in the genus are **aerobic** with only one facultative anaerobic species. All species have a **L-Lys-D-Asp (A4α) cell wall composition** and typically have iso-$C_{15:0}$ as the primary fatty acid, though two species have anteiso$_{15:0}$ primary fatty acid. Additional important fatty acids include iso-$C_{16:0}$, $C_{16:1}\omega 7c$ alcohol, anteiso-$C_{17:0}$, and iso-$C_{17:1}\omega$10c. All species utilize **MK-7 major menaquinone**, though several have an additional MK-6 or MK-7 (H_2) major menaquinone. All species have **diphosphatidylglycerol (DPG) and phosphatidylglycerol (PG) polar lipids** and typically also have phosphatidethanolamine (phosphatidethanolamine) as a primary polar lipid. All species are **catalase positive**, **cannot reduce nitrate, and are unable to produce indole**. **All species are incapable of utilizing 2-ketogluconate, capric acid, D-arabinose, gentiobiose, indole, *myo*-inositol, stachyose, starch, β-gentiobiose, and β-**

methylxyloside. Strains are typically **susceptible to Penicillin G.** Species can be commonly isolated from soil and water and are globally distributed, though some species have phenotypes that allow them to live in more unusual and extreme environments. **Four primer sets were designed based on Lysinibacillus genes to aid in identification of Lysinibacillus isolates: iron-sulfur oxidoreductase (FeSO-f: TCTTAACAGTCGTGGTTGTGC, FeSO-r: CACTGRTCTTCRCAGTTACG), glutamine synthetase (GS1-f: ACAAAATGATGTTCGATGGCTC, GS1-r: TGGCGCTTCATAACCWGGWA), lipoteichoic acid synthase Type IIIa (LtAS-f: GATACTAGCAGTYRTAGCAACT, LtAS-r: ACTGAAATAATACGTGTCTKKAT), and topoisomerase IV subunit A (T4SA-f: ATTATTCAAGACCGCGCGTT, T4SA-r: GCTTCTGTATAACGCATCSCT).**

DNA G+C content (mol%): 35-43.3.

Type species: **Lysinibacillus boronitolerans** Ahmed, Yokota, Yamazoe, Fujiwara 2007, 1117^{VP}

Number of validated species: 25

Further descriptive information

Phylogenetic treatment

Lysinibacillus corresponds to a sub-group of bacteria originally assigned to the *Bacillus* rRNA group 2 based on 16S rRNA sequencing and characterized by containing L-lysine or D-ornithine in the peptide subunit (Ash *et al.* 1991).

A phylogenetc tree of *Lysinibacillus* type strains based on the alignment of partial 16S rDNA sequences obtained from NCBI is shown in Figure 1. The maximum likelihood tree was built from the 1228bp-long sequence of 27 type strains downloaded from NCBI using the GTR model (Nei and Kumar 2000) and 100 bootstrap replicates (Felsenstein 1985). Gaps are considered

as missing data and are partially deleted with a site coverage cut-off of 95%. The strain *Bacillus subtilis* ATCC 6051^{T} is used as root. NCBI names are comprised of the genus and species name and the associated strain code. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.* 2016).

An additional maximum likelihood tree (Figure 2) of 52 *Lysinibacillus* strains rooted with *Bacillus subtilis subsp. subtilis* strain 168 was generated based on the alignment of 72 core genes (33,047 polymorphic sites). The tree is drawn to scale and branch length represents the number of base substitutions per site. Nodes annotated with a circle are supported by bootstrap values above 70%. The tree was constructed from whole genome and draft genome sequences of 41 *Lysinibacillus* type strains downloaded from NCBI (January 2017) and genome sequences of 11 additional *Lysinibacillus* strains (see Table 1 for NCBI accession numbers of all 52 genomes). 3474 predicted coding sequences of the closed and annotated genome of *Lysinibacillus sphaericus* LMG 22257 were used as reference to which all other genome sequences were aligned gene-by-gene with MAFFT (Katoh and Toh 2008) using BIGSdb open source software (Jolley and Maiden 2010). A gene was defined as core when detected in all genomes using a BLAST word size of 15 pb, a minimum sequence identity of 70% and alignment length of 50%. The resulting concatenated aligned sequences was used to build a maximum likelihood tree with RAxML 8.2.6 (Stamatakis 2014) under the GAMMA model of rate heterogeneity using empirical nucleotide frequencies and the GTR nucleotide substitution model. The total number of bootstrap replicates was automatically determined by the MRE-based bootstrapping criterion and were conducted under the rapid bootstrapping algorithm, among which 100 were sampled to generate proportional support values.

Colony characteristics

Lysinibacillus typically produces circular colonies whose margins are highly variable and may be entire, jagged, or undulate. Colonies are typically flat or convex, but can rarely produce umbonate colonies. Colonies are typically glossy and smooth and are typically opaque. Colony color ranges from dark brown to white-greyish, with most species having an ivory to yellow pigmentation. Colonies are typically larger than 0.75mm in diameter and, with the exception of *Lysinibacillus macroides*, *Lysinibacillus parviboronicapiens*, and *Lysinibacillus saudimassiliensis*, are smaller than 5mm in diameter (Coorevits *et al.* 2012, Miwa *et al.* 2009, Papadioti *et al.* 2016). Under lower nutrient conditions, colonies take on a smaller, more translucent and greyish appearance then when grown on rich media.

Lysinibacillus macroides and *Lysinibacillus varians* can produce filamentous colonies when grown in liquid culture. *Lysinibacillus macroides* can produce long filaments of 10-100μm when grown in liquid broth (Coorevits *et al.* 2012). *Lysinibacillus varians* exhibits a filament-to-rod cell cycle in enrichment media. These filaments can be as long as 466.1μm in length as early as 90 minutes after spore germination (Zhu *et al.* 2014).

Nutrition and metabolism

Most *Lysinibacillus* can be grown on general media, including Columbia, LB, NA, Reasoner's 2A (R2A), and TSA at a neutral pH between 6.0 and 8.0 with most species growing optimally at 7.0. Most species have a low NaCl tolerance, with the exception of *Lysinibacillus halotolerans* and *Lysinibacillus telephonicus* (Kong *et al.* 2014, Rahi *et al.* 2017). *Lysinibacillus halotolerans* has an optimum NaCl concentration of 3% (w/v) and several other species have increased optimal salt concentrations, including *Lysinibacillus mangiferahumi* (0-2% (w/v)),

Lysinibacillus meyeri and *Lysinibacillus saudimassiliensis* (0.5% (w/v)) (Papadioti *et al.* 2016, Seiler *et al.* 2013, Yang *et al.* 2012).

Lysinibacillus can form spores on sporulation salt media, 2xSG media, or after being subject to heat treatment for an extended period of time.

Carbon utilization is highly variable between species, with little commonality between species. Three species (*Lysinibacillus boronitolerans* 10a, *Lysinibacillus cresolivorans* 2RL3-2, and *Lysinibacillus xylanilyticus* XDB9) are capable of degrading *m*-cresol (Ren *et al.* 2015).

Insect and nematode pathogenicity

Lysinibacillus sphaericus Neide was first identified as a mosquito pathogen in 1965 after its isolation from moribund larvae of various Californian mosquitoes. This strain infects the mosquito larvae via the alimentary canal and produces fatal septicemia in the affected species. Colonies of *Lysinibacillus sphaericus* Neide are cultured on brain-heart-infusion agar slants and suspended in sterile water prior to introduction to the larvae. Larvae lost normal turgor and motility three days post exposure to the bacterium prior to death of the larvae (Kellen *et al.* 1965). Further examination of the strains and toxins produced by these bacteria showed toxicity against the larval stage of the nematode *Trichostrongylus colubriformis* (Bone and Tinelli 1987), grass shrimp *Palaemonetes pugio* (Key and Scott 1992), and *Chironomus riparus* (Partridge and Berry 2002) in addition to the previously described mosquito populations. *Lysinibacillus sphaericus* toxic strains produce several toxins including sphaericolysin, Mtx1, Mtx2 family toxins, Bin toxins, and Cry48/Cry49 toxins. *Lysinibacillus sphaericus* is an excellent biocontrol agent against *Culex spp.* and *Anopheles spp.* mosquitoes. The species also controls *Aedes vexans* and several *Ochlerotatus spp.*, but has little to no effect on *Aedes aegypti* (Berry 2012).

L. *mangiferahumi* was initially identified in the mango rhizosphere soil and has nematocidal activity against the root-knot nematode *Meloidogyne incognita*. The activity is due to several volatile compounds produced by the bacterium. These volatiles have been collected by solid phase microextraction (SPME) and identified using gas chromatography. It was found that all juvenile nematodes are killed after a 24hr incubation with *Lysinibacillus mangiferahumi* M-GX18. Twenty-nine volatile compounds were identified, eight of which have confirmed nematocidal activity. The volatile compounds responsible for the death of the nematodes include: included 2-octanol (LC90 = 0.09 mmol L-1), cyclohexene (0.09 mmol L-1), 3-chloro-4-fluorobenzaldehyde (0.15 mmol L-1), dibutyl phthalate (0.11 mmol L-1), 2-nitro-2-chloropropane (0.12 mmol L-1), dimethachlore (1.34 mmol L-1), weedar (r) (0.15 mmol L-1) and dimethyl disulfide (0.17 mmol L-1) (Yang *et al.* 2012).

Ecology

The majority of *Lysinibacillus* type strains have been isolated in East Asia; however, several species have been isolated outside of this region, including the Middle East (Ahmed *et al.* 2014), Europe (Kampfer *et al.* 2013), or the Americas (Failor *et al.* 2017, Gomez-Garzon *et al.* 2016, La Duc *et al.* 2004, Pena-Montenegro and Dussan 2013), suggesting that the genus is cosmopolitan.

Lysinibacillus strains have been isolated from a wide variety of sources, suggesting that there is not one single ecological niche the genus occupies. Several strains, including, *Lysinibacillus alkaliphilus* OMN17 (Zhao *et al.* 2015), *Lysinibacillus alkalisoli* YA20 (Sun *et al.* 2017), *Lysinibacillus boronitolerans* 10a (Ahmed *et al.* 2007), *Lysinibacillus fluoroglycofenilyticus* cmg86 (Cheng *et al.* 2015), *Lysinibacillus fusiformis* ATCC 7055 (Priest *et*

al. 1988), *Lysinibacillus halotolerans* LAM612 (Kong *et al.* 2014), *Lysinibacillus manganicus* Mn1-7 (Liu *et al.* 2013), *Lysinibacillus mangiferahumi* M-GX18 (Yang *et al.* 2012), *Lysinibacillus pakistanensis* NCCP-54 (Ahmed *et al.* 2014), and *Lysinibacillus parviboronicapiens* BAM-582 (Miwa *et al.* 2009) were isolated from soil samples. Two of these species (*Lysinibacillus mangiferahumi* M-GX18 (Yang *et al.* 2012) and *Lysinibacillus pakistanensis* NCCP-54 (Ahmed *et al.* 2014)) along with *Lysinibacillus endophyticus* C9 (Yu *et al.* 2016)and *Lysinibacillus tabacifolii* K3514 (Duan *et al.* 2013) all associate closely with plants, either on the plant surface or within the rhizosphere. These strains may provide benefits to the plant, as evident by the production of nematocidal compounds by *Lysinibacillus mangiferahumi*. At least three species (*Lysinibacillus meyeri* WS4626, *Lysinibacillus parviboronicapiens* VT1065, and *Lysinibacillus saudimassiliensis* 13S34_air) have been found associated with the atmosphere and were either cultured from air samples (Papadioti *et al.* 2016, Seiler *et al.* 2013) or precipitation (Failor *et al.* 2017), suggesting that these bacteria can survive and be transported through the atmosphere.

Ice nucleation

Ice nucleation activity (INA) in Lysinibacillus was discovered in a rain-isolated strain in 2014. Unlike other ice nucleation active (ice+) bacteria, *Lysinibacillus* does not utilize a membrane bound, heat sensitive protein. The strain, *Lysinibacillus parviboronicapiens* VT1065 appears to utilize a secreted, heat-stable, proteinase- and lysozyme-resistant, nanometer sized molecule based on the study's findings (Failor *et al.* 2017). At the time of writing of this chapter, only *Lysinibacillus parviboronicapiens* strains have been found to exhibit INA.

Genome information

Lysinibacillus species have an average genome size of 4.515Mb, based on 52 genomes (20 type strains and 32 additional strains). Based on these strains, there are a total of 644 genes in the *Lysinibacillus* core genome, and a total of 19,547 genes present in the pangenome, as determined by comparative genomics using the software *get_homologues* (Conteras-Moreira and Vinuesa 2013). The rarefaction curve (Figure 3) indicates an open pangenome since there is a lack of a plateau. The core genome begins leveling off at eight genomes, suggesting that the core genome size is not much below the 644 genes identified to be the core genome of the 20 type strains.

Enrichment/isolation

A selective growth medium was formulated to test the growth of *Lysinibacillus sphaericus* 2363 (Russell *et al.* 1989) on specific carbon sources. This medium contains (per liter): Na_2HPO_4, 5.57g; KH_2PO_4, 2.4g; $(NH_4)_2SO_4$, 2.0g; $MgSO_4 \cdot 7H_2O$, 50mg; $MnCl_2 \cdot 4H_2O$, 4.0mg; $FeSO_4 \cdot 7H_2O$, 810µg; $CaCl_2 \cdot 2H_2O$, 1.5mg; H_2SO_4, 0.3µl; sodium acetate, 321.8mg; thiamine 200mg; biotin, 20µg. The biotin, thiamine, and a carbon source at a concentration of 40mM are prepared in a separate, filter-sterilized stock solution. The Mg^{2+}, Mn^{2+}, Fe^{2+}. and Ca^{2+} salts are prepared in a single, acidified (0.03% H_2SO_4 [vol/vol]), filter sterilized stock solution. Both stock solutions are added to the remaining autoclaved solutions after cooling.

Based on Russell *et al,*. a modified defined medium was created to aid in the culturing of *Lysinibacillus* from the environment and in the purification of the ice+ *Lysinibacillus parviboronicapiens* strain VT1065. This medium contains (per liter): Na_2HPO_4, 5.57g; KH_2PO_4, 2.4g; $(NH_4)_2SO_4$, 2.0g; $MgSO_4 \cdot 7H_2O$, 50mg; $MnCl_2 \cdot 4H_2O$, 4.0mg; $FeSO_4 \cdot 7H_2O$, 810µg; $CaCl_2 \cdot 2H_2O$, 1.5mg; H_2SO_4, 0.3µl; sodium acetate, 321.8mg; thiamine 25.4mg; biotin, 20µg. The

biotin, thiamine, and sodium acetate are prepared in a separate, filter-sterilized stock solution. The Mg^{2+}, Mn^{2+}, Fe^{2+}. and Ca^{2+} salts are prepared in a single, acidified (0.03% H_2SO_4 [vol/vol]), filter sterilized stock solution. Both stock solutions were added to the remaining autoclaved solutions after cooling.

Maintenance procedures

Lysinibacillus strains can be preserved in 25% (v/v) glycerol stocks kept at -80°C for long term storage. For even longer-term preservation, lyophilization and liquid nitrogen may be utilized provided a cryoprotectant is added.

Procedures for testing special characters

Mosquito-larvicidal activity

Mosquito larvae are exposed to suspensions of *Lysinibacillus sphaericus*. Dosage-mortality values and relative susceptibility of mosquito strains are determined using dried *Lysinibacillus sphaericus* spores (Kellen *et al.* 1965). Susceptibility of nematode eggs was determined by exposing the eggs to *Lysinibacillus sphaericus* spore extracts and examining the larvicidal activity via dose-response analysis (Bone and Tinelli 1987).

Nematocidal activity

Volatiles are collected using SPME following the protocol outlined by Diaz *et al.* (Diaz *et al.* 2004). All volatile compounds are screened utilizing a three-component Petri dish method (Gu *et al.* 2007) using the pure, commercially available, volatile compound rather than the candidate (*Lysinibacillus spp.* isolated) volatile. LC_{90} values of the volatiles are calculated using the lethal

rates of the chemical at various concentrations using the same three-compartment Petri dish method. LC_{90} values are estimated based on a regression value of the lethal rate transformed using a logit transformation, ln[y/1 – y].

Ice nucleation

Procedures for testing ice nucleation activity are described in the 2017 article identifying ice nucleation activity in *Lysinibacillus* (Failor *et al.* 2017).

Strains are tested for their ability to freeze sterile water from −2°C to −12°C or until a sterile water control begins to freeze using a droplet-freezing assay (Vali 1971). Strains to be tested are grown on R2A agar overnight at 28°C from frozen stock. Strains are then sub-cultured at a cellular concentration of approx. 3.5-4.5x10^5 CFU/L and 100µL are plated onto fresh R2A plates and grown for 72hr at 28°C. Thirty droplets of 20µL of the 72hr-old culture at a cellular concentration of approx. 3.5-4.5x10^8 CFU/L and thirty droplets of six series of 10−fold dilutions are tested. The cumulative nucleus concentration K at −8°C is determined using the equation $K(\theta) = [\ln N_0 - \ln N(\theta)]/V$, where N_0 is the number of droplets tested, $N(\theta)$ is the number of unfrozen droplets at temperature θ, and V is the droplet volume. The cumulative IN concentrations per cell of *Lysinibacillus* sp. is determined at every temperature from −2°C to −12°C. The average number of cells per drop (A) is determined by dilution-plating. The cumulative IN concentrations per cell is determined using the equation given above replacing the component V of the equation with A. Strains presenting a statistically significant difference in ice nuclei production compared to pure water can be considered ice nucleation active.

Differentiation from other closely related genera

The primary diagnostic characteristic for the differentiation of *Lysinibacillus* from other RNA group 2 genera by the composition of the peptidoglycan cell wall. *Lysinibacillus* species are characterized by the presence of the L-Lys-D-Asp (A4α) cell wall composition (Ahmed *et al.* 2007).

Unique regions of four *Lysinibacillus* housekeeping genes can also be exploited for the identification of *Lysinibacillus* species. Conserved regions within the *Lysinibacillus* genomes were selected for generation of diagnostic PCR amplification. A sequence from 29-1154 of the iron-sulfur oxidoreductase gene (5'-TCTTAACAGTCGTGGTTGTGC-3', 3'-CACTGRTCTTCRCAGTTACG-5'), a sequence from 143-918 of the glutamine synthetase gene (5'-ACAAAATGATGTTCGATGGCTC-3', 3'-TGGCGCTTCATAACCWGGWA-5'), a sequence from 36-861 of the lipoteichoic acid synthase Type IIIa gene (5'-GATACTAGCAGTYRTAGCAACT-3', 3'- ACTGAAATAATACGTGTCTKKAT-5'), and a sequence from 79-371 of the topoisomerase IV subunit A gene (5'-ATTATTCAAGACCGCGCGTT-3', 3'- GCTTCTGTATAACGCATCSCT-5') were used to create oligonucleotide probes (*Lysinibacillus parviboronicapiens* VT1065 numberings for all probes) for the identification of *Lysinibacillus* via PCR amplification. Primers were examined for specificity for *Lysinibacillus* using 18 *Lysinibacillus* type strains and ten strains that do not belong to the genus. PCR amplification of these four genes have been performed for 18 of the 28 described species utilizing the following PCR amplification parameters: 95°C for ten minutes followed by 35 cycles of: 95°C for 30 seconds, 58°C for 30 seconds, 72°C for two minutes, followed by 72°C for ten minutes.

Taxonomic comments

Based on average nucleotide identity (ANI) (Goris *et al.* 2007, Richter and Rossello-Mora 2009)calculations between 21 type strains, four strains share an ANI value greater than 96%, suggesting that these strains should not be classified as separate species. These strains include *Lysinibacillus sphaericus* DSM26, *Lysinibacillus mangiferahumi* M-GX18, *Lysinibacillus tabacifolii* K3514, and *Lysinibacillus varians* GY32. Therefore, these four species may, in fact, be members of the same species. However, no formal taxonomic revision of these species has been published or proposed to date.

In a 2016 report, Gomez-Garzon *et al.* proposed that the *Lysinibacillus sphaericus* species comprises at least two distinct lineages that, based on ANI (<96%) and phenotypic differences in toxin production, should be treated as two separate species. The mosquito-larvicidal lineage, which has been widely studied due to its potential for biocontrol, shows a high degree of conservation of this trait and could be easily utilized to differentiate these strains from the non-toxic strains. The non-toxic strains include the current species type strain and all other Lysinibacillus sphaericus strains (Gomez-Garzon *et al.* 2016). While Gomez-Garzon *et al.* made this informal proposal, no formal taxonomic revision of the species has been validly published.

List of species

Lysinibacillus boronitolerans Ahmed, Yokota, Yamazoe, Fujiwara 2007, 1117VP

bo.ro’ni.to’le.rans. N.L. n. boron -onis boron; Lysinibacillus part. adj. tolerans tolerating; N.L. part. adj. boronitolerans boron-tolerating.

Cells are Gram-positive, aerobic, motile, spore forming rods (0.8-1.5μm x 3.0-5.0μm). Round or oval spores are formed in terminally located, swollen sporangia. Colonies grown on Nutrient Agar media are circular with entire margins, flat or umbonate in elevation, opaque in transparency, butyrous in texture, and usually 2-3mm in diameter after 48 hours of growth at 37°C at pH 7. Growth occurs at 16-45°C with an optimal growth temperature of 35-37°C. Growth can occur at a pH of 5.5-9.5 with an optimal pH of 7-8. Growth can occur up to 5% NaCl (w/v). Growth cannot occur under anaerobic conditions. Can tolerate up to 150mM boron. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, and ninhydrin-positive phosphoglycolipids are the primary polar lipids. The three major fatty acids are iso-C15:0 (32.0%), anteiso-C16:0 (21.0%), and iso-C16:0 (11.0%). Lysinibacillus boronitolerans shows variable activity for nitrate reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, esterase, esterase lipase, naphthol-AS-BI-phosphohydrolase, oxidase, urease, valine arylamidase. Weak activity for α-chemotrypsin, and β-glucosidase. No activity observed for gelatinase, trypsin, tryptophanase, and β-galactosidase (ONPG). Tests positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce dihydroxyacetone or hydrogen sulfide. Can degrade up to 100mg/L of m-cresol. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to ampicillin, cephalothin, colistin, fusinic acid, lincomycin, metronidazole, novobiocin, oxolinic acid, polymixin B, and sulfamethizol, but does not show any additional resistance as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above.

Source: Soil sample taken from an experimental field of the University of Tokyo (Yayoi campus), Japan.

DNA G+C content (mol%): 36.5 (HPLC)

Type strain: 10a, ATCC BAA-1146, CIP 109652, DSM 17140, IAM 15262, JCM 21713, KACC 15323, NBRC 103108.

EMBL/GenBank accession number (16S rRNA) of the type strain: AB199591 (10a).

GenBank accession number (genome) of the type strain: GCA_000772935, GCA_002200915 (NBRC 103108); GCA_001312025 (JCM 21713).

Lysinibacillus acetophenoni Azmatunnisa, Rahul, Lakshmi, Sasikala, Ramana 2015, 1741^{VP}

a.ce.to.phe.no'ni. N.L. n. *acetophenonum* acetophenone; N.L. gen. n. *acetophenoni* of/from acetophenone.

Cells are Gram-positive, aerobic, motile rods (0.3-0.5μm x 2.0-10.0μm). Colonies grown on Nutrient Agar media are irregular, convex in elevation, brownish-white in color and usually 1-3mm in diameter. Growth occurs at 25-40°C with an optimal growth temperature of 35°C. Growth can occur at a pH of 6-9 with an optimal pH of 7. Growth can occur up to 2% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinones are MK-6 and MK-7. Diphosphatidylglycerol, phosphatidylglycerol, β-geniobiosyldiacylglycerol, two unknown glycolipids, two unknown lipids, and two unknown phospholipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (23.4%), anteiso-$C_{17:0}$ (16.7%), and anteiso-$C_{15:0}$ (15.6%). *Lysinibacillus acetophenoni* is negative for both nitrate and nitrite reduction. Positive activity for catalase and oxidase. No activity observed for gelatinase, tryptophanase, urease, and β-glucosidase. Tests negative for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. . Viable and non-viable carbon sources can be found in Table 4. Capable of utilizing ammonium chloride, L-aspartate, L-glutamate, and L-glutamine as nitrogen sources. Shows resistance to acetophenone

and nalidixic acid, but does not show any additional resistance as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above.

Source: Organic solvent acetophenone.

DNA G+C content (mol%): 41.0 (HPLC).

Type strain: JC23, CCUG 57911, DSM 23394, KACC 18506, KCTC 13605, NBRC 105754.

EMBL/GenBank accession number (16S rRNA) of the type strain: FN179488

Lysinibacillus alkaliphilus Zhao, Feng, Chen, Zhang, Lin 2015, 2426^{VP}

al.ka.li'phi.lus. N.L. n. alkali (from Arabic article al the; Arabic n. qaliy ashes of saltwort) alkali; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. masc. adj. *alkaliphilus* liking alkaline environments.

Cells are Gram-positive, aerobic, spore forming rods (0.5-2.0μm). Motile by peritrichous flagella. Oval spores are formed in sub-terminally located, flat sporangia. Colonies grown on TSA media are circular with jagged or undulate margins, flat in elevation, pale brown in color, with a granular surface and usually 0.5-2.0mm in diameter after 48 hours of growth at 30°C at pH 7. Growth occurs at 25-45°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 5.5-12.0 with an optimal pH of 8-10. Growth can occur up to 2% NaCl (w/v) with an optimal salt concentration of 1-2% (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK -7. Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (53.4%), anteiso-$C_{15:0}$ (15.4%), and iso-$C_{14:0}$ (6.2%). %). *Lysinibacillus alkaliphilus* is for nitrate reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, esterase, naphthol-AS-BI-phosphohydrolase. Weak activity for esterase lipase. Variable

activity for gelatinase, trypsin, α-chemotrypsin, β-galactosidase (ONPG), and β-glucosidase. No activity observed for oxidase, tryptophanase, urease, valine arylamidase. Tests positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4.

Source: Sandy loam soil at the State Experimental Station for Agro-Ecology, Fengqiu country, Henan province, China.

DNA G+C content (mol%): 38.1 (HPLC).

Type strain: OMN17, CCTCC AB 2014073, DSM 28019.

EMBL/GenBank accession number (16S rRNA) of the type strain: KF771256 (OMN17).

Lysinibacillus alkalisoli Sun, Xu, Wu 2017, 67^{VP}

al.ka.li.so'li. N.L. n. *alkali* (from Arabic al-qaliy), alkali; *Lysinibacillus* n. *solum* soil; N.L. gen. n. *alkalisoli* of alkaline soil, referring to the fact that the type strain was isolated from alkaline–saline soil.

Cells are Gram-positive, aerobic, spore forming rods (0.6-1.0μm x 1.3-2.6μm). Motile by peritrichous flagella. Oval spores are formed in terminally located, swollen sporangia. Colonies grown on LB media are circular with jagged margins, flat in elevation, ivory in color, with a rough surface and usually 2-3mm in diameter after 48 hours of growth at 30°C. Growth occurs at 10-45°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 6-10 with an optimal pH of 8-9. Growth can occur up to 8% NaCl (w/v). Growth cannot occur under anaerobic conditions. The primary menaquinone is MK-6, MK-7 and MK-7(H_2). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, two unknown aminophospholipids, and three unknown phospholipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$

(39.3%), anteiso-$C_{16:0}$ (12.2%), and iso-$C_{17:0}$ (11.1%). %). *Lysinibacillus alkalisoli* is negative for nitrate reduction. Positive activity for acid phosphatase, catalase, esterase, esterase lipase, naphthol-AS-BI-phosphohydrolase, urease, valine arylamidase, α-chemotrypsin. No activity observed for gelatinase, oxidase, trypsin, tryptophanase, β-galactosidase (ONPG), and β-glucosidase. Tests negative for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to bacitracin, carbenicillin, and rifiampicin, but does not show any additional resistance as seen in Table 5.

Source: Saline-alkaline soil from farmland in Hangjin Banner, Inner Mongolia, China.

DNA G+C content (mol%): 39.0 (T_m).

Type strain: Y2A20, CGMCC 1.15760, KCTC 33825.

EMBL/GenBank accession number (16S rRNA) of the type strain: KX258757 (Y2A20).

Lysinibacillus chungkukjangi Kim, Jang, Hamada, Ahn, Weon, Suzuki, Whang, Kwon 2013, 3931^{VP} (Effective Publication: Kim, Jang, Hamada, Ahn, Weon, Suzuki, Whang, Kwon 2013 400) chung.kuk.jan'gi. N.L. gen. n. *chungkukjangi*, of chungkukjang, a traditional Korean fermented food.

Cells are Gram-positive, aerobic, motile, spore forming rods (1.0μm x 2.0-4.5μm). Round spores are formed in terminally located, swollen sporangia. Colonies grown on TSA media are circular, flat in elevation, opaque in transparency, dark brown in color. Growth occurs at 10-45°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 6-9 with an optimal pH of 7. Growth can occur up to 3% NaCl (w/v) with an optimal salt concentration of 0% (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the

primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, two unknown lipids, and one unknown phospholipids are the primary polar lipids. ___sugars___. The three major fatty acids are anteiso-$C_{15:0}$ (47.3%), anteiso-$C_{16:0}$ (16.3%), and anteiso-$C_{17:0}$ (11.3%). %). *Lysinibacillus chugkukjangi* is negative for both nitrate and nitrite reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, gelatinase, oxidase. Variable activity observed for esterase, naphthol-AS-BI-phosphohydrolase, valine arylamidase, α-chemotrypsin, and β-glucosidase. No activity observed for esterase lipase, trypsin, tryptophanase, urease, and β-galactosidase (ONPG). Tests weakly positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce indole-3-acetic acid or hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to rifamycin SV, but does not show any additional resistance as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above.

Source: One gram of fermented soybean (chungkukjang).

DNA G+C content (mol%): 41.8 (HPLC).

Type strain: 2RL3-2, KACC 16626, NBRC 108948.

EMBL/GenBank accession number (16S rRNA) of the type strain: JX217747 (2RL3-2).

Lysinibacillus composti Hayat, Ahmed, Paek, Sin, Ehsan, Iqbal, Yokota, Chang 2014, 3603^{VP} (Effective Publication: Hayat, Ahmed, Paek, Sin, Ehsan, Iqbal, Yokota, Chang 2014, 1081)

com.pos'ti. N.L. gen. n. *composti*, of compost, from which the organism was isolated.

Cells are Gram-positive, aerobic, motile, spore forming rods (1.0-2.0μm). Colonies grown on TSA media are circular with entire margins, light yellow in color, with a glossy and smooth surface after 24 hours of growth at 28°C. Growth occurs at 10-45°C with an optimal growth

temperature of 28°C. Growth can occur at a pH of 6.5-8.5 with an optimal pH of 7. Growth can occur up to 5% NaCl (w/v). Growth cannot occur under anaerobic conditions. Can tolerate up to 50mM boron. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, and one unknown phospholipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (45.0%), anteiso-$C_{15:0}$ (11.5%), and iso-$C_{16:0}$ (7.4%). *Lysinibacillus composti* is negative for nitrate reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, Weak activity for esterase lipase, naphthol-AS-BI-phosphohydrolase. No activity observed for esterase, gelatinase, oxidase, trypsin, tryptophanase, urease, valine arylamidase, α-chemotrypsin, β-galactosidase (ONPG), and β-glucosidase. Tests positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4. Shows no ice nucleation activity when tested under the protocol described above.

Source: Fruit and vegetable compost.

DNA G+C content (mol%): 37.0 (HPLC).

Type strain: NCCP-36, DSM 24785, JCM 18777, KCTC 13796.

*EMBL/GenBank accession number (16S rRNA) of the type strain:*AB547124 (NCCP-36).

Lysinibacillus contaminans Kämpfer, Martin, Glaeser 2013, 3148^{VP}

con.ta'mi.nans. *Lysinibacillus* part. adj. *contaminans* contaminating, polluting, isolated as a contaminant from an agar plate.

Cells are Gram-positive or Gram-variable, aerobic, motile or non-motile, spore forming rods (0.8-1.0μm x 2.0-3.0μm). Round spores are formed in terminally located sporangia, though

sporulation is less common than in other species. Colonies grown on TSA media are circular, convex in elevation, beige in color, with a glossy surface and usually 2-3mm in diameter after 48 hours of growth. Growth occurs at 15-45°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 6.5-10.5 with an optimal pH of 7-8. Growth can occur up to 8% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-6, and MK-7d. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, one unknown aminolipid, one unknown aminophospholipid, and four unknown phospholipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (37.5%), anteiso-$C_{15:0}$ (21.7%), and $C_{16:1}\omega7c$ alcohol (7.5%). %). *Lysinibacillus contaminans* is negative for nitrate reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, esterase, esterase lipase, gelatinase, naphthol-AS-BI-phosphohydrolase, trypsin, valine arylamidase, α-chemotrypsin. Weak activity for oxidase, urease. No activity observed for tryptophanase, β-galactosidase (ONPG), and β-glucosidase. Tests positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4.

Source: R2A plate of *Enterobacter spp.* in Giessen, Germany.

*Type strain:*FSt3A, CCM 8383, CCUG 62186, CIP 110362, DSM 25560.

EMBL/GenBank accession number (16S rRNA) of the type strain: KC254732 (FSt3A).

GenBank accession number (genome) of the type strain: NZ_LGRV01000003, NZ_LGRV00000000 (DSM 25560).

Lysinibacillus cresolivorans Ren, Chen, Yao, Deng 2015, 4250^{VP}

cre.so.li.vo'rans. N.L. n. *cresololis* cresol; *Lysinibacillus* part. adj. *vorans* devouring; N.L. part. adj. *cresolivorans* cresol-degrading.

Cells are Gram-positive, aerobic, motile, spore forming rods (1.1-1.3μm x 3.5-5.0μm). Oval spores are formed in terminally located, swollen sporangia. Colonies grown on NA media are irregular with undulate margins, opaque in transparency, cream in color, with a dull and rough surface and usually 0.8-2mm in diameter after 48 hours of growth at 30°C at pH 7. Growth occurs at 25-40°C with an optimal growth temperature of 35°C. Growth can occur at a pH of 5-9 with an optimal pH of 7. Growth can occur up to 5% NaCl (w/v). Growth can occur weakly under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (59.5%), iso-$C_{16:0}$ (13.5%), and anteiso-$C_{15:0}$ (8.6%). %). *Lysinibacillus cresolivorans* is positive for nitrate reduction and negative for nitrite reduction. Positive activity for catalase and urease. No activity observed for gelatinase, oxidase, tryptophanase, and β-glucosidase. Tests negative for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Can degrade up to 100mg/L of *m*-cresol. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to chloromycetin, rifampicin, and tetracycline, but does not show any additional resistance as seen in Table 5.

Source: Aerobic sludge of a coking wastewater treatment plant in Guangdong Province, China.

DNA G+C content (mol%): 41.2 (HPLC).

Type strain: SC03, CCTCC M 208210, NRRL B-59352.

EMBL/GenBank accession number (16S rRNA) of the type strain: EU043375 (SC03).

Lysinibacillus endophyticus Yu, Guan, Liu, Xiang, Yu, Liu, Wang 2017, 1^{VP} (Effective publication: Yu, Guan, Liu, Xiang, Yu, Liu, Wang 2017, 1337 erratum; Yu, Guan, Liu, Xiang, Yu, Liu, Wang 2017, 173

en.do.phy'ti.cus. Gr. pref. *endo* within; Gr. n. *phyton* plant. N.L. masc. adj. *endophyticus* within plant, phosphatidethanolaminertaining to the isolation of the type strain from plant tissues.

Cells are Gram-positive, aerobic, spore forming rods (0.5-0.8μm x 1.1-1.4μm). Motile by peritrichous flagella. Colonies grown on TSA media are circular, convex in elevation, opaque in transparency, light yellow in color after 48 hours of growth. Growth occurs at 20-50°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 5-8 with an optimal pH of 7. Growth can occur up to 4.5% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, one unknown lipids, and two unknown phospholipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (34.7%), anteiso-$C_{15:0}$ (33.6%), and anteiso-$C_{17:0}$ (10.6%). *Lysinibacillus endophyticus* is positive for nitrite reduction and negative for nitrate reduction. Positive activity for acid phosphatase, catalase, esterase, esterase lipase, gelatinase, naphthol-AS-BI-phosphohydrolase, oxidase, and α-chemotrypsin. No activity observed for alkaline phosphatase, trypsin, urease, valine arylamidase, β-galactosidase (ONPG), and β-glucosidase. Tests negative for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Produces indole-3-acetic acid and hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4.

Source: Corn root nodule promoting growth of bacteria producing indole acetic acid.

DNA G+C content (mol%): 37.9 ± 0.7 (T_m).

Type strain: C9, CGMCC 1.15291, DSM 100506.

EMBL/GenBank accession number (16S rRNA) of the type strain: KP334990 (C9).

Lysinibacillus fusiformis (Priest, Goodfellow, Todd 1988) Ahmed, Yokota, Yamazoe, Fujiwara 2007, 1117^{VP} (Priest, Goodfellow, Todd 1988, 1847)

fu.si.for'mis. *Lysinibacillus* n. *fusus* spindle; *Lysinibacillus* n. *forma* shape, form; N.L. adj. *fusiformis* spindle-shaped.

Cells are Gram-positive, aerobic, motile, spore forming rods. Round spores are formed in central or terminally located, swollen sporangia. Colonies are flat in elevation and opaque in. Growth occurs at 17-40°C. Growth can occur at a pH of 6-9. Growth can occur up to 7% NaCl (w/v). Growth cannot occur under anaerobic conditions. Can tolerate up to 60mM boron. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, and ninhydrin-positive phosphoglycolipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (23.9%), anteiso-$C_{15:0}$ (17.9%), and iso-$C_{16:0}$ (15.7%). %). *Lysinibacillus fusiformis* is negative for nitrate reduction. Positive activity for catalase, gelatinase, urease, α-chemotrypsin. Weak activity for naphthol-AS-BI-phosphohydrolase. No activity observed for acid phosphatase, trypsin, tryptophanase, β-galactosidase (ONPG), and β-glucosidase. Variable results for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Cannot degrade *m*-cresol. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to cephalothin, chloramphenicol, erythromycin, lincomycin, polymixin B, and streptomycin, but does not show any additional resistance as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above.

DNA G+C content (mol%):

Type strain: DSM 2898, ATCC 7055, CCUG 28888, JCM 12229, KACC 10903, LMG 9816, NBRC 15717, NRRL NRS-350.

*EMBL/GenBank accession number (16S rRNA) of the type strain:*AJ310084 (DSM 2898).

Additional remark: this species was initially classified as *Bacillus fusiformis*.

Lysinibacillus halotolerans Kong, Wang, Zhao, Li, Song, Zhai, Zhang, Wang, Chen, Zhao, Ruan 2014, 2593^{VP}

ha.lo.to'le.rans. Gr. n. *hals*, *halos* salt; *Lysinibacillus* part. adj. *tolerans* tolerating; N.L. part. adj. *halotolerans* salt-tolerating, referring to the organism's ability to tolerate high salt concentrations.

Cells are Gram-positive, aerobic, motile, spore forming rods (0.3-0.7μm x 1.0-2.5μm. Growth occurs at 15-55°C with an optimal growth temperature of 35°C. Growth can occur at a pH of 5-10 with an optimal pH of 6. Growth can occur up to 10% NaCl (w/v) with an optimal salt concentration of 3% (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (40.8%), iso-$C_{16:0}$ (15.2%), and iso-$C_{14:0}$ (5.3%). %). *Lysinibacillus halotolerans* is negative for nitrate reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, esterase, esterase lipase, gelatinase, naphthol-AS-BI-phosphohydrolase, oxidase, α-chemotrypsin,. Weak activity for valine arylamidase. Variable activity for gelatinase. No activity observed for trypsin, tryptophanase, urease, β-galactosidase (ONPG), and β-glucosidase. Tests negative for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4. Shows no resistance to tested compounds as seen in Table 5.

Source: Saline-alkaline soil samples from Lingxian Country, Shandong Province, China.

DNA G+C content (mol%): 36.4 (T_m).

Type strain: LAM612, ACCC 00718, JCM 19611.

EMBL/GenBank accession number (16S rRNA) of the type strain: KF443809 (LAM612).

Lysinibacillus louembi Ouoba, Vouidibio, Thorsen, Anyogu, Nielsen, Kobawila, Sutherland 2015, 4256^{VP}

lou.em'be.i. N.L. gen. masc. n. *louembei* Louembe) named in honour of Professor Delphin Louembe from the Republic of the Congo for his substantial contribution to a better understanding of the microbial diversity of Congolese traditional fermented foods.

Cells are Gram-positive, aerobic, motile, spore forming rods (0.7-0.8μm x 4.0-14.0μm). Round spores are formed in terminally located, swollen sporangia. Colonies grown on NA media are circular with entire margins, convex in elevation, cream in color, with a glossy and smooth surface and usually 1-2mm in diameter after 48 hours of growth at 37°C. Growth occurs at 10-48°C with an optimal growth temperature of 37°C. Growth can occur at a pH of 6-9.5 with an optimal pH of 7-9. Growth can occur up to 7% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-6, and MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, one unknown aminolipid, one unknown aminophospholipid, two unknown lipids, and two unknown phospholipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (24.0%), anteiso-$C_{17:0}$ (19.1%), and anteiso-$C_{17:0}$ (17.8%). %). *Lysinibacillus louembi* is negative for nitrate reduction. Positive activity for acid catalase, and gelatinase. Weak activity for β-galactosidase (ONPG), and β-glucosidase. No activity observed for tryptophanase, and urease. Tests positive

for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4.

Source: Ntoba Mbodi, the alkaline fermentation of cassava leaves in the Republic of the Congo.

DNA G+C content (mol%): 38.0 (HPLC).

Type strain: NM73, DSM 25583, LMG 26837.

EMBL/GenBank accession number (16S rRNA) of the type strain: HG937791 (NM73).

Lysinibacillus macroides Coorevits, Dinsdale, Heyrman, Schumann, Van Landschoot, Logan, De Vos 2012, 1121^{VP}

ma.cro.i'des. Gr. adj. *makros* large or long; Gr. suff. *-eides* (from Gr. n. *eidos* form or shape) resembling, similar; N.L. masc. adj. *macroides* long in form, describing the elongated appearance of the rods.

Two strains of *Lysinibacillus macroides* were initially characterized in 1947 before the strain lost standing in bacterial nomenclature. Cells are Gram-variable, aerobic, motile, spore forming rods (0.9μm x 3.0-5.0μm). 100μm long filaments can be formed in liquid culture. Oval spores are formed in terminally located, swollen sporangia. Colonies grown on TSA media are circular with entire margins, moist and loose in texture, cream in color, and usually 0.5-5mm in diameter after 24 hours of growth at 30°C. Growth occurs at 10-45°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 7-9 with an optimal pH of -8. Growth can occur up to 4% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (45.3%), iso-$C_{16:0}$ (12.8%), and $C_{16:1}\omega7c$ alcohol (12.3%). *Lysinibacillus*

macroides shows variable activity for nitrate reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, esterase, esterase lipase, naphthol-AS-BI-phosphohydrolase, and α-chemotrypsin. Weak activity for gelatinase, oxidase, trypsin, tryptophanase, urease, valine arylamidase, α-chemotrypsin, β-galactosidase (ONPG), and β-glucosidase. No activity observed for acid phosphatase, alkaline phosphatase, catalase, esterase, esterase lipase, gelatinase, naphthol-AS-BI-phosphohydrolase, oxidase, trypsin, tryptophanase, urease, β-galactosidase (ONPG), and β-glucosidase. Tests positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Cannot degrade *m*-cresol. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to gentamycin, novobiocin, streptomycin, and tetracycline, but does not show any additional resistance as seen in Table 5.

Source: Cow dung.

DNA G+C content (mol%): 38.2 (HPLC).

Type strain: LMG 18474, ATCC 12905, DSM 54, IAM 15292, JCM 21742, KACC 16627, NCIB 8796.

EMBL/GenBank accession number (16S rRNA) of the type strain: AJ628749 (LMG 18474).

GenBank accession number (genome) of the type strain: NZ_LGCI01000005, NZ_LGCI00000000 (DSM 54).

Additional note: this species was initially classified as *Bacillus macroides*, but lost standing in bacteriological nomenclature.

Lysinibacillus manganicus Liu, Song, Chen, Zheng, Wang 2013, 3568^{VP}

man.ga'ni.cus. N.L. n. *manganum* manganese; *Lysinibacillus* suff. -*icus* suffix used with the sense of pertaining to; N.L. masc. adj. *manganicus* pertaining to manganese, referring to the isolation of the type strain from a manganese mining soil.

Cells are Gram-positive, aerobic, spore forming rods (0.3μm x 1.7-2.5μm). Motile by lateral flagella. Round spores are formed in terminally located sporangia. Colonies are circular opaque or translucent in transparency, yellow in color, with a smooth surface and usually 1-2mm in diameter. Growth occurs at 15-45°C with an optimal growth temperature of 37°C. Growth can occur at a pH of 5-10 with an optimal pH of 78. Growth can occur up to 5.5% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol are phosphatidylglycerol are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (33.9%), iso-$C_{16:0}$ (23.0%), and iso-$C_{14:0}$ (12.8%). Fatty acid iso$C_{16:1}$ is present. *Lysinibacillus manganicus* is positive for both nitrate and nitrite reduction. *Lysinibacillus manganicus* is negative for nitrate reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, esterase, esterase lipase, gelatinase, naphthol-AS-BI-phosphohydrolase, oxidase, and trypsin. Weak activity for α-chemotrypsin. No activity observed for tryptophanase, urease, valine arylamidase, β-galactosidase (ONPG), and β-glucosidase. Tests negative for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4.

Source: Mining soil sample in Tianjin, China.

DNA G+C content (mol%): 38.4 (HPLC).

Type strain: Mn1-7, CCTCC AB 2012916, DSM 26584.

EMBL/GenBank accession number (16S rRNA) of the type strain: JX993821 (Mn1-7).

GenBank accession number (genome) of the type strain: GCA_000772945, GCA_002200865 (DSM 26584).

Lysinibacillus mangiferahumi Yang, Huang, Liu, Ma, Mo, Li, Yang 2012, 2045^{VP} (Effective Publication: Yang, Huang, Liu, Ma, Mo, Li, Yang 2012, 53)

Man'gi.fer'a.hu.mi N.L. n. *mangifera*, scientific name of mango; *Lysinibacillus* n. *humus*, soil; N.L. gen. n. *mangiferahumi*, of soil of a mango field.

Cells are Gram-positive, aerobic, motile, spore forming rods (0.6μm x 2.0-5.0μm). Colonies grown on LB media are circular with entire margins, pale yellow in color, and usually 0.4-0.8mm in diameter after 24 hours of growth at 28°C. Growth occurs at 10-45°C with an optimal growth temperature of 28-37°C. Growth can occur at a pH of 6-9 with an optimal pH of 7-8. Growth can occur up to 5% NaCl (w/v) with an optimum salt concentration of 0-2% (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, ninhydrin-positive phosphoglycolipids, and three unknown phospholipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (56.0%), anteiso-$C_{15:0}$ (16.8%), and iso-$C_{16:0}$ (7.8%). *Lysinibacillus mangiferahumi* is negative for nitrate reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, esterase, esterase lipase, gelatinase, naphthol-AS-BI-phosphohydrolase, and oxidase. Variable activity for α-chemotrypsin. No activity observed for trypsin, tryptophanase, urease, valine arylamidase, β-galactosidase (ONPG), and β-glucosidase. Tests positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4.

Shows no resistance to tested compounds as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above.

L. *mangiferahumi* has nematocidal activity against the root-knot nematode *Meloidogyne incognita*. Eight volatile compounds produced by the bacterium have confirmed nematocidal activity. The volatile compounds responsible for the death of nematode larvae include: 2-octanol, cyclohexene, 3-chloro-4-fluorobenzaldehyde, dibutyl phthalate, 2-nitro-2-chloropropane, dimethachlore, weedar (r) and dimethyl disulfide (Yang *et al.* 2012).

Source: Rhizosphere soil of mangos from Guangzi province, China.

DNA G+C content (mol%): 37.8 ± 0.3 (HPLC).

Type strain: M-GX18, CCTCC AB 2010389, DSM 24076, KACC 17178.

EMBL/GenBank accession number (16S rRNA) of the type strain: JF731238 (M-GX18).

Lysinibacillus massiliensis (Glazunova, Raoult, Roux 2008) Jung, Kim, Paek, Styrak, Park, Sin, Paek, Park, Kim, Kim, Chang 2012, 2347^{VP} (Bacillus massiliensis Glazunova, Raoult, Roux 2008, 1485)

mas.si.li.en'sis. *Lysinibacillus* masc. adj. *massiliensis* of *Massilia*, the ancient Greek and Roman name for Marseille, France, where the type strain was isolated.

Cells are Gram-negative, aerobic, spore forming rods (0.3-0.5μm x 1.5-4.0μm). Motile by peritrichous flagella. Colonies grown on sheep-blood agar media are circular, white-greyish in color, with a glossy and smooth surface and usually 1-2mm in diameter after 24 hours of growth. Growth occurs at 25-45°C with an optimal growth temperature of 30-37°C. Growth can occur up to 5% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol,

two unknown glycolipids, and three unknown phospholipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (48.0%), anteiso-$C_{15:0}$ (15.3%), and iso-$C_{16:0}$ (13.5%). *Lysinibacillus massiliensis* is negative for nitrate reduction. Positive activity for catalase, oxidase, and urease. Weak activity for alkaline phosphatase and esterase lipase. No activity observed for acid phosphatase, esterase, gelatinase, naphthol-AS-BI-phosphohydrolase, trypsin, tryptophanase, valine arylamidase, α-chemotrypsin, β-galactosidase (ONPG), and β-glucosidase. Tests positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce dihydroxyacetone or hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to azetronam, ceftrazidime, fosfomycin, and very weak resistance to cefepime and cefpirome, but does not show any additional resistance as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above.

Source: 54-year-old male's cerebrospinal fluid who was diagnosed with Whipple's disease.

DNA G+C content (mol%): 36.3 (qPCR).

Type strain: 4400831, CCUG 49529, CIP 108446, KACC 14317, KCTC 13178.

EMBL/GenBank accession number (16S rRNA) of the type strain: NR_043092 (4400831).

GenBank accession number (genome) of the type strain: GCA_000772965, GCA_002200855 (4400831).

Additional remark: this species was initially classified as *Bacillus massiliensis.*

Lysinibacillus meyeri Seiler, Scherer, Wenning 2013, 1512^{VP}

me'yer.i. N.L. gen. masc. n. *meyeri* of Meyer, named in honor of Arthur Meyer, who, together with Ernst Neide in 1904, described the species *B. sphaericus*, now *Lysinibacillus sphaericus.*

Cells are Gram-positive, aerobic, motile, spore forming rods (0.8-1.0μm x 4.0-9.0μm). Round spores are formed in terminally located, swollen sporangia. Colonies grown on TSA media have undulate margins, are flat or convex in elevation, translucent in transparency, and usually 2-4mm in diameter after 72 hours of growth at 30°C. Growth occurs at 10-42°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 6.5-8.5 with an optimal pH of 7-8. Growth can occur up to 5.5% NaCl (w/v) with an optimal salt concentration of 0.5% (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-6 and MK-7. Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (34.1%), anteiso-$C_{15:0}$ (13.9%), and iso-$C_{17:1}$ω10c (13.0%). *Lysinibacillus meyeri* is negative for nitrate reduction. Positive activity for esterase lipase and oxidase. Variable activity for alkaline phosphatase, catalase, and gelatinas. No activity observed for acid phosphatase, esterase, naphthol-AS-BI-phosphohydrolase, trypsin, tryptophanase, urease, valine arylamidase, α-chemotrypsin, β-galactosidase (ONPG), and β-glucosidase. Variable reactions for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce dihydroxyacetone or hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4. Shows no ice nucleation activity when tested under the protocol described above.

Source: Air sample taken from a medical practice in Bavaria.

DNA G+C content (mol%): 37.1 (HPLC).

Type strain: WS 4626, DSM 25057, KACC 17179, LMG 26643.

EMBL/GenBank accession number (16S rRNA) of the type strain: HE577173 (WS 4626)

Lysinibacillus odysseyi (La Duc, Satomi, Venkateswaran 2004) Jung, Kim, Paek, Styrak, Park, Sin, Paek, Park, Kim, Kim, Chang 2012, 2347^{VP} (La Duc, Satomi, Venkateswaran 2004, 195) o.dys.se'yi. *Lysinibacillus* n. *Odyssea* the Odyssey; N.L. gen. n. *odysseyi* pertaining to the Mars Odyssey spacecraft, from which the organism was isolated.

Cells are Gram-positive, aerobic, motile, spore forming rods (1.0μm x 4.5-5.0μm). Round spores are formed in terminally located, swollen sporangia. Colonies grown on TSA media are circular with entire margins, flat in elevation, beige in color, with a smooth surface. Growth occurs at 25-42°C with an optimal growth temperature of 30-35°C. Growth can occur at a pH of 5-10 with an optimal pH of 7. Growth can occur up to 5% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, and four unknown phospholipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (50.4%), $C_{16:1}\omega7c$ alcohol (12.7%), and iso-$C_{16:0}$ (11.3%). *Lysinibacillus odysseyi* is negative for both nitrate and nitrite reduction. Positive activity for catalase, esterase, esterase lipase, oxidase, and α-chemotrypsin. Weak activity for trypsin. No activity observed for acid phosphatase, alkaline phosphatase, gelatinase, naphthol-AS-BI-phosphohydrolase, tryptophanase, urease, valine arylamidase, β-galactosidase (ONPG), and β-glucosidase. Tests positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce dihydroxyacetone or hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4. Spores exhibit resistance to UV (254 nm), gamma radiation, 5% liquid H2O2 and desiccation conditions. Shows no ice nucleation activity when tested under the protocol described above.

Source: One strains of *Lysinibacillus odysseyi* was isolated from the surface of the Mars Odyssey spacecraft, United States.

DNA G+C content (mol%): 35.6 (qPCR).

Type strain: 34hs-1, ATCC PTA-4993, CIP 108263, DSM 18869, KCTC 3961, LMG 24110, NBRC 100172, NRRL B-30641.

EMBL/GenBank accession number (16S rRNA) of the type strain: AF526913 (34hs-1).

GenBank accession number (genome) of the type strain: GCA_000773015 (34hs-1).

Additional remark: this species was initially classified as *Bacillus odyssey*.

Lysinibacillus pakistanensis Ahmed, Sin, Paek, Whsan, Hayat, Iqbal, Chang 2014, 2184^{VP} (Effective publication: Ahmed, Sin, Paek, Whsan, Hayat, Iqbal, Chang 2014, 447).

pa.kis.tan.en'sis. N.L. masc. adj. *pakistanensis* from Pakistan, where the organism was isolated.

Cells are Gram-positive, aerobic, motile, spore forming rods (1.0-4.0μm). Round spores are formed in terminally located, swollen sporangia. Colonies are punctiform with undulate margins, flat in elevation, transparent, butyrous in texture, off-white to yellow in color, with a dull surface and usually 1.0-4.0mm in diameter. Growth occurs at 10-45°C with an optimal growth temperature of 28°C. Growth can occur at a pH of 6-9 with an optimal pH of 7. Growth can occur up to 6% NaCl (w/v). Growth cannot occur under anaerobic conditions. Can tolerate up to 150mM boron. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-6, MK-7 and MK-7(H_2). Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$, anteiso-$C_{17:0}$, and iso-$C_{17:1}$ω10c. Fatty acid iso$C_{16:1}$ is present. *Lysinibacillus pakistanensis* is positive for nitrate reduction. Positive activity for acid phosphatase, catalase, esterase lipase. Weak activity for alkaline phosphatase and α-chemotrypsin. No activity observed for esterase, esterase lipase, naphthol-AS-BI-phosphohydrolase, oxidase, trypsin, valine arylamidase, β-galactosidase (ONPG), and β-

glucosidase. Additional enzymatic activity can be found in Table 3. Viable and non-viable carbon sources can be found in Table 4. Shows no ice nucleation activity when tested under the protocol described above.

Source: Rhizosphere of *Glycine max* from the experimental area of Soil Science Department located at the Research Farm of Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

DNA G+C content (mol%): 37.0

Type strain: NCCP-54, DSM 24784, JCM 18776, KCTC 13795.

EMBL/GenBank accession number (16S rRNA) of the type strain: AB558495 (NCCP-54).

GenBank accession number (genome) of the type strain: BBDJ01000001, BBDJ01000000 (JCM 18776).

Lysinibacillus parviboronicapiens Miwa, Ahmed, Yokota, Fujiwara 2009, 1427^{VP}

par'vi.bo'ro.ni.ca'pi.ens. *Lysinibacillus* adj. *parvus* little, low; N.L. n. *boron* boron; *Lysinibacillus* part. adj. *capiens* containing; N.L. part. adj. *parviboronicapiens* containing little boron.

Cells are Gram-positive, aerobic, motile, spore forming rods (0.5-1.0μm x 2.0-5.0μm). Round spores are formed in terminally located, swollen sporangia. Colonies grown on TSA media are circular with entire margins, opaque in transparency, butyrous in texture, and usually 2-5mm in diameter after 72 hours of growth at 30°C at pH 7. Growth occurs at 10-37°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 6-8 with an optimal pH of 7. Growth can occur up to 6% NaCl (w/v). Growth cannot occur under anaerobic conditions. Can tolerate up to 50mM boron. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, and ninhydrin-positive

phosphoglycolipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (37.4%), anteiso-$C_{15:0}$ (19.0%), and anteiso-$C_{17:0}$ (9.6%). Fatty acid iso$C_{16:1}$ is present. *Lysinibacillus parviboronicapiens* is negative for nitrate reduction. Positive activity for catalase, esterase lipase, urease. Variable activity for α-chemotrypsin and β-glucosidase. No activity observed for acid phosphatase, alkaline phosphatase, esterase, gelatinase, naphthol-AS-BI-phosphohydrolase, oxidase, trypsin, tryptophanase, valine arylamidase, and β-galactosidase (ONPG). Tests positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to ampicillin, bacitracin, cephalothin, chloramphenicol, colistin, enrofloxacin, flumequine, fusidic acid, lincomycin, metronidazole, oxolinic acid, polymixin B, streptomycin, but does not show any additional resistance as seen in Table 5. Ice nucleation activity was first identified in a rain isolated strain. The type strain exhibits ice nucleation activity starting at -8°C when tested under the protocol described above.

Sources: Soil of the Hisarcik area in Kutahya province, Turkey. Freezing rain sample in Blacksburg, Virginia, United States.

DNA G+C content (mol%): 38.7 (HPLC).

Type strain: BAM-582, KCTC 13154, NBRC 103144.

EMBL/GenBank accession number (16S rRNA) of the type strain: AAB300598 (BAM-582).

GenBank accession number (genome) of the type strain: GCA_001311625 (JCM 18861).

Lysinibacillus sinduriensis Jung, Kim, Paek, Styrak, Park, Sin, Paek, Park, Kim, Kim, Chang 2012, 2347^{VP}

sin.du.ri.en'sis. N.L. masc. adj. *sinduriensis* pertaining to the Sinduri, Republic of Korea, geographical origin of the type strain of the species.

Cells are Gram-positive, aerobic, spore forming rods (0.5-1.2μm x 0.7-3.5μm). Motile by peritrichous flagella. Oval spores are formed in terminally located, swollen sporangia. Colonies grown on TSA media are circular with undulate margins, convex in elevation, and white-greyish in color. Growth occurs at 15-45°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 5-9 with an optimal pH of 7. Growth can occur up to 5% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-6, MK-7 and MK-7(H_2). Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine, and three unknown phospholipids are the primary polar lipids. The three major fatty acids are anteiso-$C_{15:0}$ (35.6%), iso-$C_{16:0}$ (25.6%), and anteiso-$C_{16:0}$ (16.5%). *Lysinibacillus sinduriensis* is negative for nitrate reduction. Positive activity for acid phosphatase, catalase, gelatinase, naphthol-AS-BI-phosphohydrolase, oxidase, and trypsin. Weak activity for alkaline phosphatase. Variable activity for esterase and α-chemotrypsin. No activity observed for esterase lipase, tryptophanase, urease, valine arylamidase, β-galactosidase (ONPG), and β-glucosidase. Tests negative for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce dihydroxyacetone, indole-3-acetic acid or hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4. Shows no resistance to tested compounds as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above. *Source:* Tidal flat sediment collected at a depth of 20cm from the Shinduri sand dunes at Tae-An on the Yellow Sea coast, Republic of Korea.

DNA G+C content (mol%): 35.9 (qPCR).

Type strain: BLB-1, JCM 15800, KACC 15207, KCTC 13296, NBRC 103144.

EMBL/GenBank accession number (16S rRNA) of the type strain: FJ169465 (BLB-1).

GenBank accession number (genome) of the type strain: GCA_002200845, GCA_000772955 (BLB-1).

Lysinibacillus sphaericus (Cohn 1872) Ahmed, Yokota, Yamazoe, Fujiwara 2007, 1117VP (Cohn 1872, 174)

sphae'ri.cus. *Lysinibacillus* masc. adj. *sphaericus* spherical'

Cells are Gram-positive, aerobic, motile, spore forming rods (0.6-1.0μm x 1.5-5.0μm). Round spores are formed in terminally located, swollen sporangia. Colonies are circular with undulate margins, with a glossy and smooth surface. Growth occurs at 10-40°C. Growth can occur at a pH of 6-9.5 with an optimal pH of 6-9.5. Growth can occur up to 7% NaCl (w/v). Growth cannot occur under anaerobic conditions. Can tolerate up to 75mM boron. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, ninhydrin-positive phosphoglycolipids, one unknown lipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (41.9%), $C_{16:1}\omega7c$ alcohol (18.3%), and iso-$C_{17:1}\omega10c$ (8.3%). *Lysinibacillus sphaericus* is negative for nitrate reduction. Positive activity for alkaline phosphatase, catalase, esterase lipase, gelatinase, naphthol-AS-BI-phosphohydrolase, oxidase, and α-chemotrypsin. Weak activity for trypsin and valine arylamidase. No activity observed for acid phosphatase, tryptophanase, urease, β-galactosidase (ONPG), and β-glucosidase. Variable results for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce dihydroxyacetone or hydrogen sulfide. Cannot degrade *m*-cresol. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to ampicillin, cephalothin, gentamycin, lincomycin, polymixin B, and

streptomycin. Shows weak resistance to chloramphenicol, erythromycin, and rifampicin. Shows variable resistance to tetracycline, but does not show any additional resistance as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above.

Members of the *Lysinibacillus sphaericus* OT4b.49 clade reported by Gomez-Garzon *et al.* (Gomez-Garzon *et al.* 2016) show pathogenicity in mosquito larvae. This lineage contains toxin-encoding genes, including *binA, B,* and *mtx1, 2,* and *3,* which are absent from the species type strain and all other non-toxic strains. This lineage demonstrates pathogenicity to *Anopheles spp.*, *Culex spp., Ochlerotatus spp.*and *Aedes vexans*. These strains may also demonstrate pathogenicity against *Trichostrongylus colubriformis* (Bone and Tinelli 1987), *Palaemonetes pugio* (Key and Scott 1992), and *Chironomus riparius* (Partridge and Berry 2002).

Sources: Soil and aquatic environments (Gomez-Garzon *et al.* 2016); mosquito breeding sites, China (Hu *et al.* 2008); The strain was isolated from a moribund fourth-instar larva of the mosquito *Culiseta incidens* (Kellen *et al.* 1965).

DNA G+C content (mol%): 37.3

Type strain: DSM 28, ATCC 14577, BCRC 12825, CCM 2120, CCUG 7428, CIP 65.30, IAM 13420, JCM 2502, KACC 10554, KCTC 3346, LMG 7134, NBRC 15095, NCIB 9370, NCTC 10338, NRRL B-23268.

EMBL/GenBank accession number (16S rRNA) of the type strain: AJ310084 (DSM 28).

GenBank accession number (genome) of the type strain: GCA_000427235 (KCTC 3346).

Additional remark: this species was initially classified as *Bacillus sphaericus*.

Lysinibacillus tabacifolii Duan, He, Li, Wang, Wang, Zhe, Cao, Mo, Zhai, Li 2014, 693^{VP} (Effective Publication: Duan, He, Li, Wang, Wang, Zhe, Cao, Mo, Zhai, Li 2013, 289)

to.bac'i.fo.li.i N.L. n. *tabaci,* scientific name of *Nicotiana tabacum*, the tobacco plant; *Lysinibacillus* n. *folii,* gen. *folium* meaning from a leaf of the tobacco plant.

Cells are Gram-positive, aerobic, non-motile, spore forming rods (0.4-1.5μm x 5.0-10.0μm). Oval spores are formed in terminally located, swollen sporangia. Colonies grown on KMB media are circular with entire margins, white in color, and usually 0.6-1mm in diameter after 72 hours of growth at 32°C at pH 7. Growth occurs at 8-40°C with an optimal growth temperature of 28-32°C. Growth can occur at a pH of 5-10 with an optimal pH of 6-8. Growth can occur up to 7% NaCl (w/v), with an optimal salt concentration of 0% (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7 and MK-7(H_2). Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, phosphatidylinositol, and two unknown phospholipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (32.6%), anteiso-$C_{15:0}$ (19.0%), and iso-$C_{16:0}$ (8.9%). *Lysinibacillus tabacifolii* is negative for nitrate reduction. Positive activity for acid phosphatase, catalase, esterase lipase, gelatinase, naphthol-AS-BI-phosphohydrolase, oxidase, urease. Variable activity for trypsin and α-chemotrypsin. No activity observed for alkaline phosphatase, esterase, tryptophanase, valine arylamidase, β-galactosidase (ONPG), and β-glucosidase. Tests negative for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4. . Shows no resistance to tested compounds as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above.

Source: Surface-sterilized leaves of *Nicotiana tabacum* in the Yunnan province, China.

DNA G+C content (mol%): 36.5 (HPLC).

Type strain: K3514, CCTCC AB 2012050, KCTC 33042.

*EMBL/GenBank accession number (16S rRNA) of the type strain:*JQ754706 (K3514).

Lysinibacillus telephonicus Rahi, Kurli, Khairnar, Jagtap, Pansare, Dastager, Shouche, 2017, 2289^{VP}

te.le.pho'ni.cus. N.L. n. *telephonum*, telephone; *Lysinibacillus* suff. *-icus*, suffix used with the sense of pertaining to; N.L. masc. adj. *telephonicus*, phosphatidethanolaminertaining to the telephone, the type strain was isolated from a cellular phone.

Cells are Gram-positive, aerobic, motile, spore forming rods (2.0-4.0μm). Round spores are formed in terminally located, swollen sporangia. Colonies grown on TSA media are circular with undulate margins, convex in elevation, and pale white in color. Growth occurs at 10-55°C with an optimal growth temperature of 37°C. Growth can occur at a pH of 5-9 with an optimal pH of 7. Growth can occur up to 10% NaCl (w/v) with an optimum salt concentration of 0% (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidethanolamine, and four unknown lipids are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (32.0%), anteiso-$C_{16:0}$ (21.0%), and iso-$C_{16:0}$ (11.0). Positive activity for acid phosphatase, alkaline phosphatase, catalase, esterase, esterase lipase, naphthol-AS-BI-phosphohydrolase, oxidase. No activity observed for trypsin, valine arylamidase, α-chemotrypsin, β-galactosidase (ONPG), and β-glucosidase. Additional enzymatic activity can be found in Table 3. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to 1% sodium lactate, azetronam, guanidine HCl, lithium chloride, and nalidixic acid, but does not show any additional resistance as seen in Table 5.

Source: Cellular phone screen from Prune, India.

DNA G+C content (mol%): 39.8 (HPLC).

Type strain: S5H2222, KACC 18714, LMG 29294, MCC 3065.

EMBL/GenBank accession number (16S rRNA) of the type strain: KT735049 (S5H2222).

Lysinibacillus varians Zhu, Sun, Chen, Guo, Xu 2014, 3644^{VP}

va'ri.ans. *Lysinibacillus* part. adj. *varians* varying, referring to the change of cell shape.

Cells are Gram-positive, aerobic, motile, spore forming rods (0.8-1.3μm x 5.0-10.0μm). Round or oval spores are formed in terminally located, swollen sporangia. Colonies grown on LB media are circular with jagged margins, opaque in transparency, yellow in color, with a glossy surface after 24 hours of growth at 30°C at pH 7. Colonies can form filaments >450μm in length. Growth occurs at 15-45°C. Growth can occur at a pH of 6-9. Growth can occur up to 4% NaCl (w/v). Growth cannot occur under anaerobic conditions. Can tolerate up to 150mM boron. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$, iso-$C_{16:0}$, and iso-$C_{16:1}$ω10c. *Lysinibacillus varians* is positive for nitrate reduction. Positive activity for catalase and oxidase. No activity observed for gelatinase. Tests negative for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Viable and non-viable carbon sources can be found in Table 4. Can degrade decabrominated diphenyl ether (BDE-209). Shows no ice nucleation activity when tested under the protocol described above.

Source: Electronic-waste-recycling site sediment in Guiyu, Guangdong, China.

DNA G+C content (mol%): 43.2 (HPLC).

Type strain: GY32, CCTCC M 2011307, CGMCC 1.12212, NBRC 109424.

EMBL/GenBank accession number (16S rRNA) of the type strain: JN860068 (GY32).

GenBank accession number (genome) of the type strain: CP006837 (GY32).

Lysinibacillus xylanilyticus Lee, Jung, Park, Oh, Yoon 2010, 281^{VP}

xy.la.ni.ly'ti.cus. N.L. n. *xylanum* xylan; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lutikos*) able to loosen, able to dissolve; N.L. masc. adj. *xylanilyticus* xylan-dissolving.

Cells are Gram-positive, aerobic, motile, spore forming rods (0.8-1.0μm x 3.0-5.0μm). Round spores are formed in terminally located, swollen sporangia. Colonies grown on TSA media are circular with entire margins, opaque in transparency, and dark yellow in color. Growth occurs at 10-45°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 5-9.5 with an optimal pH of 7-8. Growth can occur up to 5% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cannot tolerate boron. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (50.7%), $C_{16:1}\omega7c$ alcohol (10.4%), and iso-$C_{11:0}$ (8.8%). *Lysinibacillus xylanilyticus* shows variable activity for nitrate and nitrite reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, gelatinase, naphthol-AS-BI-phosphohydrolase, and oxidase. Variable activity for esterase and β-glucosidase. No activity observed for esterase lipase, trypsin, tryptophanase, urease, valine arylamidase, α-chemotrypsin, and β-galactosidase (ONPG). Variable results for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce dihydroxyacetone or hydrogen sulfide. Can degrade up to 50mg/L of *m*-cresol. Capable of degrading xylan, a polymer of β-1,4-Dd-xylopyranosyl units. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to ampicillin, cephalothin, erythromycin, gentamycin, lincomycin, novobiocin, polymixin B, streptomycin, and tetracycline, but does not

show any additional resistance as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above.

Source: Forest humus in Gyeryong Mountain, Taejon, Republic of Korea.

DNA G+C content (mol%): 37.2 (HPLC).

Type strain: XDB9, CCUG 57438, DSM 24393, KCTC 13423.

*EMBL/GenBank accession number (16S rRNA) of the type strain:*FJ477040 (XDB9).

GenBank accession number (genome) of the type strain: LFXJ00000000 (DSM 24393).

Other organisms

"Lysinibacillus fluoroglycofenilyticus" Cheng, Zhang, Zhang, Hu, Zhang, He, Huang 2015, 157 flu.o.ro.gly.co.fe.ni.ly'ti.cus. N.L. neut. n. *fluoroglycofenum* fluoroglycofen; N.L. masc. adj. *lyticus* (from Gr. masc. adj. *lutikos*) able to dissolve; N.L. masc. adj. *fluoroglycofenilyticus* fluoroglycofen degrading.

Cells are Gram-positive, aerobic, motile, spore forming rods (0.4-0.7μm x 2.0-4.0μm). Colonies grown on LB media have entire margins, are opaque in transparency, yellow in color, with a smooth surface. Growth occurs at 25-45°C. Growth can occur at a pH of 6-8. Growth can occur up to 5.5% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cannot tolerate boron. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-6 and MK-. Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (25.6%), anteiso-$C_{15:0}$ (19.2%), and anteiso-$C_{17:0}$ (13.9%). *Lysinibacillus fluoroglycofenilyticus* is negative for nitrate reduction. Positive activity for acid phosphatase, catalase, gelatinase, trypsin, and valine arylamidase. No activity observed for oxidase, urease, and β-galactosidase (ONPG). Tests positive for Voges-Proskauer. Additional

enzymatic activity can be found in Table 3. Capable of degrading fluoroglycofen. Viable and non-viable carbon sources can be found in Table 4. Shows no ice nucleation activity when tested under the protocol described above.

Source: Herbicide-polluted farmland soil in Tongjing, Nanjing Province, China.

DNA G+C content (mol%): 37.6 (HPLC).

*Type strain:*cmg86, CCTCC AB 2013247, KCTC 33183.

EMBL/GenBank accession number (16S rRNA) of the type strain: JX524514 (cmg86).

"Lysinibacillus jejuensis" Kim, Park, Oh, Kim 2013, 872

je.ju.en'sis. N.L. fem. adj. *jejuensis* referring to Jeju Island in the Republic of Korea, where the type strain was isolated.

Cells are Gram-positive, aerobic, motile, spore forming rods (0.7-1.0μm x 2.5-6.0μm. Colonies grown on TSA media are circular or ovoid, convex in elevation, ivory in color, with a smooth surface after growth at 30°C at pH 7. Growth occurs at 10-37°C with an optimal growth temperature of 30°C. Growth can occur at a pH of 6-10 with an optimal pH of 7. Growth can occur up to 3% NaCl (w/v). Growth cannot occur under anaerobic conditions. Cell wall type is L-Lys-D-Asp (A4α) and the primary menaquinone is MK-7. Diphosphatidylglycerol, phosphatidylglycerol, and phosphatidethanolamine are the primary polar lipids. The three major fatty acids are iso-$C_{15:0}$ (54.9%), iso-$C_{17:1}\omega10c$ (12.1%), $C_{16:1}\omega7c$ alcohol (11.9%). *Lysinibacillus jejuensis* is negative for nitrate reduction. Positive activity for acid phosphatase, alkaline phosphatase, catalase, esterase, gelatinase, and oxidase. Variable activity for acid phosphatase, esterase lipase, naphthol-AS-BI-phosphohydrolase, urease, α-chemotrypsin, and β-glucosidase. No activity observed for trypsin, tryptophanase, valine arylamidase, and β-galactosidase (ONPG).

Tests positive for Voges-Proskauer. Additional enzymatic activity can be found in Table 3. Does not produce hydrogen sulfide. Viable and non-viable carbon sources can be found in Table 4. Shows resistance to erythromycin, gentamycin, novobiocin, streptomycin, and tetracycline, but does not show any additional resistance as seen in Table 5. Shows no ice nucleation activity when tested under the protocol described above.

Source: Swinery waste collected in Jeju, Republic of Korea.

DNA G+C content (mol%): 43.3 (HPLC).

*Type strain:*N2-5, DSM 28310, KCTC 13837.

EMBL/GenBank accession number (16S rRNA) of the type strain: HQ392513 (N2-5).

"Lysinibacillus saudimassiliensis" Papadioti, Azhar, Bibi, Jiman-Fatani, Aboushoushah, Yasir, Raoult, Angelakis 2016, 25

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Cells are Gram-positive, aerobic, motile, spore forming rods. Colonies grown on Columbia agar media are circular, opaque in transparency, and usually 4-6mm in diameter. Growth can occur up to 5% NaCl (w/v) with an optimal salt concentration of 0.5% (w/v). Growth cannot occur under anaerobic conditions. The primary menaquinone is MK-7. Positive activity for catalase. No activity observed for oxidase. Viable and non-viable carbon sources can be found in Table 4.

Source: Two air samples in the urban environment of Makkah, Saudi Arabia.

Type strain: 13S34_air, CSUR P1222.

EMBL/GenBank accession number (16S rRNA) of the type strain: HG931343 (13S34_air).

GenBank accession number (genome) of the type strain: GCA_000935965.1 (13S34_air).

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Tables

Table 1: Genome information for all available Lysinibacillus genomes.

	Accession Number	Type strain	Genome size (Mb)	Number of Contigs	Number of genes
1. L. acetophenoni JC23	GCA_900220965	Yes	3.9	43	3,908
4a. L. boronitolerans 10a	GCA_000772935	Yes	4.4	81	4,604
4b. L. boronitolerans F1182	GCA_000286375	No	4.5	309	
5. L. chungkukjangi 2RL32	TBA	Yes	4.2	269	4,163
7. L. contaminans Fst3A	GCA_001278945	Yes	4.0	9	
10. L. fluoroglycofenilyticus cmg86	TBA	Yes	3.5	71	3,405
11a. L. fusiformis ATCC 7055	TBA	Yes	4.7	72	4,821
11b. L. fusiformis H1k	GCA_000633275	No	4.9	60	
11c. L. fusiformis M5	GCA_001726065	No	5.0	8	
11d. L. fusiformis RB21	GCA_000724775	No	4.9	1	
11e. L. fusiformis SW-B9	GCA_000755455	No	4.8	22	
11f. L. fusiformis ZB2	GCA_000313955	No	4.6	58	
11g. L. fusiformis ZC1	GCA_000178135	No	4.7	113	
13. L. jejuensis N25	TBA	Yes	2.8	262	2,760
15. L. macroides LMG 18474	GCA_001281525	Yes	4.7	15	4,779
16. L. manganicus Mn1-7	GCA_000772945	Yes	3.9	81	3,910
17. L. mangiferahumi M-GX18	TBA	Yes	5.2	153	5,523
18. L. massiliensis 4400831	GCA_000772965	Yes	3.9	156	3,899
19. L. meyeri WS4626	TBA	Yes	4.3	406	4,315
20. L. odysseyi 34hs-1	GCA_000773015	Yes	4.4	34	4,649
21. L. pakistanensis NCCP-54	GCA_001312325	Yes	4.8	73	8,505
22a. L. parviboronicapiens BAM-582	TBA	Yes	4.6	132	4,743
22b. L. parviboronicapiens VT1065	TBA	No	4.6	73	4,750
22c. L. parviboronicapiens VT1066	TBA	No	4.6	75	4,725
24. L. saudimassiliensis 13S34_air	GCA_000935965	Yes	2.5	11	
23. L. sinduriensis BLB-1	GCA_000772955	Yes	3.9	55	4,006
25a. L. sphaericus DSM 28	GCA_000427235	Yes	4.4	83	4,500
25b. L. sphaericus 1987	GCA_000712415	No	5.0	70	
25c. L. sphaericus 2297	GCA_000733505	No	4.6	221	
25d. L. sphaericus 2362	GCA_001629735	No	4.8	1	
25e. L. sphaericus B1-CDA	GCA_001306735	No	4.6	84	
25f. L. sphaericus C3-41	GCA_000017965	No	4.9	1	
25g. L. sphaericus CBAM5	GCA_000568835	No	5.2	93	
25h. L. sphaericus FSL M8-0037	GCA_001728805	No	4.6	64	
25i. L. sphaericus III7	GCA_001598075	No	4.9	1	
25j. L. sphaericus LMG-22257	GCA_001753205	No	3.5	1	
25k. L. sphaericus LP1-G	GCA_000733575	No	4.6	175	
25l. L. sphaericus NRS1693	GCA_000733585	No	4.7	448	
25m L. sphaericus OT4b.25	GCA_001581875	No	4.9	1	
25n. L. sphaericus OT4b.31	GCA_000392615	No	4.9	72	
25o. L. sphaericus OT4b.49	GCA_001623495	No	4.7	3	
25p. L. sphaericus SSII-1	GCA_000733525	No	4.7	188	
26. L. tabacifolii K3514	TBA	Yes	4.4	51	4,400
28. L. varians GY32	GCA_000600105	Yes	4.5	1	4,599
29a. L. xylanilyticus XDB9	GCA_001183605	Yes	5.0	13	5,129
29b. L. xylanilyticus SR-86	GCA_001708185	No	4.8	49	
30a. L. sp. A1	GCA_000801965	No	4.8	57	
30b. L. sp. AR18-8	GCA_001705465	No	4.7	55	
30c. L. sp. F5	GCA_001482485	No	4.6	117	
30d. L. sp. FJAT-14745	GCA_001275675	No	5.2	173	
30e. L. sp. LK3	GCA_001043675	No	4.6	59	
30f. L. sp. ZYM-1	GCA_001308875	No	4.9	84	

Table 2:Morphological characteristics for Lysinibacillus type strains. Strain information was taken from the following sources: 1. *Lysinibacillus acetophenone* (Azmatunnisa *et al.* 2015), 2. *Lysinibacillus alkaliphilus* (Zhao *et al.* 2015), 3. *Lysinibacillus alkalisoli* (Sun *et al.* 2017), 4. *Lysinibacillus boronitolerans* (Ahmed *et al.* 2007), 5. *Lysinibacillus chungkukjangi* (Kim *et al.* 2013), 6. *Lysinibacillus composti* (Hayat *et al.* 2014), 7. *Lysinibacillus contaminans* (Kampfer *et al.* 2013), 8. *Lysinibacillus cresolivorans* (Ren *et al.* 2015), 9. *Lysinibacillus endophyticus* (Yu *et al.* 2016), 10. *Lysinibacillus fluoroglycofenilyticus* (Cheng *et al.* 2015), 11. *Lysinibacillus fusiformis (Ahmed et al. 2007, Priest et al. 1988),* 12. *Lysinibacillus halotolerans* (Kong *et al.* 2014), 13. *Lysinibacillus jejuensis* (Kim *et al.* 2013), 14. *Lysinibacillus louembei* (Ouoba *et al.* 2015), 15. *Lysinibacillus macroides* (Coorevits *et al.* 2012), 16. *Lysinibacillus manganicus* (Liu *et al.* 2013), 17. *Lysinibacillus mangiferahumi* (Yang *et al.* 2012), 18. *Lysinibacillus massiliensis* (Glazunova *et al.* 2006, Jung *et al.* 2012), 19. *Lysinibacillus meyeri* (Seiler *et al.* 2013), 20. *Lysinibacillus odysseyi* (Jung *et al.* 2012, La Duc *et al.* 2004), 21. *Lysinibacillus pakistanensis* (Ahmed *et al.* 2014), 22. *Lysinibacillus parviboronicapiens* (Miwa *et al.* 2009), 23. *Lysinibacillus sinduriensis* (Jung *et al.* 2012), 25. *Lysinibacillus sphaericus* (Ahmed *et al.* 2007, Logan and De Vos 2009), 26. *Lysinibacillus tabacifolii* (Duan *et al.* 2013), 27. *Lysinibacillus telephonicus* (Rahi *et al.* 2017), 28. *Lysinibacillus varians* (Zhu *et al.* 2014), and 29. *Lysinibacillus xylanilyticus* (Lee *et al.* 2010).

Characteristic	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. borontolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macrotides*	16. *L. mangamticus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*	21. *L. pakistanensis*	22. *L. pararibotonticapiens*	23. *L. sinduriensis*	24. *L. saudimassiliensis*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	28. *L. variens*	29. *L. xylanilyticus*
Colony Morphology																													
Colony diameter																													
≥0.75	+	-	+	+		-	+	+																+					
≥1.0	+	-	+	+		+	+	-																+					
≥2.0	+	+	+	+		+	+	+																+					
≥3.0	+	-	+	-		-	-	-																+					
≥4.0	-	-	-	-		-	-	-																+					
≥5.0	-	-	-	-		-	-	-																+					
≥6.0	-	-	-	-		-	-	-																+					
Gram	+	+	+	+	+	-	+, V	+	+	+	+	+	+	+	V	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
Cell Morphology																													
Cell length																													
≥1.0	+	-	+	+	-	-	+	+	+	-		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
≥2.0	+	-	-	+	+	-	+	+	+	-		-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
≥3.0	+	-	-	+	+	-	+	+	+	-		-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
≥5.0	+	-	-	+	+	-	+	+	+	-		-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
≥10.0	+	-	-	+	+	-	+	+	+	-		-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+
Cell width																													
≥0.5	-	-	+	+	+	-	+	+	-	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
≥0.7	-	-	+	+	+	-	+	+	-	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
≥0.75	-	-	+	+	+	-	+	+	-	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
≥1.0	-	-	+	+	+	-	+	+	-	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
≥1.3	-	-	+	+	+	-	+	+	-	+	-	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
Spore Morphology																													
Endospore Formation	+	+	+	+	+	+	+/w	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Swell sporangia		-	+	+	+		+	+					+	+	+														
Round		-	-	+	+		-	-					+	+	-	+													
(Slightly) Oval	+	+	+	+	-	-	-	+			-			-	+	-													
Spore Position																													
Terminal	-		+	+	+		+	+			V			+	+	+			+	+	+	+	+					
Motility																													
Motile	+	+	+	+	+	+	V	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxygen Requirement																													
Aerobic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anaerobic	-	-	-	-	-	-	-	+/w	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Characteristic	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macroides*	16. *L. manganicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*	21. *L. pakistanensis*	22. *L. parviboronicapiens*	23. *L. sinduriensis*	24. *L. saudimassiliensis*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	28. *L. varians*	29. *L. xylanilyticus*
Cell Wall Composition																													
Cell Wall Type																													
L-Lys-D-Asp (A4α)	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Primary Major Fatty Acid																													
iso-$C_{15:0}$	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-		+	+	+	+	+
Secondary Major Fatty Acid																													
anteiso-$C_{15:0}$	-	+	-	+	-	+	+	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-		-	+	+	-	-
iso-$C_{16:0}$	-	-	+	-	+	-	-	+	-	-	-	+	-	-	+	+	-	-	-	-	-	-	-		-	-	-	+	-
Presence of iso-$C_{16:1}$	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-		-	-	-	-	-
Polar lipids																													
Diphosphatidylglycerol (DPG)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Phosphatidylglycerol (PG)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
Phosphatidethanolamine (PE)	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+		+	+	+	+	+
Ninhydrin-Positive Phosphoglycolipid	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-		+	-	-	-	-
Phosphotidylinositol (PI)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	+	-	-	-
β-Geniobiosyldiacylglycerol (GL3)	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-
Major Menaquinone																													
MK-7	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MK-7 (H_2)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
MK-6	+	-	-	-	-	-	+	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Growth Conditions																													
Boron Tolerance																													
50 mM				+		-				-	+										+	+			+			+	-
75 mM				+		-				-	-										+	-			+			+	-
150 mM				+		-				-	-										+	-			-			+	-
Growth Temperature																													
<10°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	+	-	-	-
10°C	-	-	+	-	+	+	-	-	-	-	-	-	+	+	+	-	+	-	+	-	+	+	-		+	+	+	-	+
25°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
40°C	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+		+	+	+	+	+
48°C	-	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-		-	-	+	-	-
55°C	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-		-	-	+	-	-

Characteristic	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macrotides*	16. *L. mangamicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*	21. *L. pakistanensis*	22. *L. paryiboronicapiens*	23. *L. sinduriensis*	24. *L. saudimassiliensis*	25. *L. sphaericus*	26. *L. iabacifolii*	27. *L. telephonicus*	28. *L. varians*	29. *L. xylanilyticus*
pH																													
5.0	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+
6.0	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+
9.0	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+
10.0	-	-	-	-	-	-	+	-	-	+	-	+	+	-	-	+	+	-	-	+	-	-	+	+	+	+	+	+	+
12.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NaCl Tolerance																													
3 (w/v)	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5 (w/v)	-	-	-	+	-	-	+	+	-	+	-	+	-	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+
7 (w/v)	-	-	-	-	-	-	-	+	-	-	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	+	+	+	-
10 (w/v)	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+

Table 3: Enzymatic activity for Lysinibacillus *type strains.* Strain information was taken from the following sources: 1. *Lysinibacillus acetophenone* (Azmatunnisa *et al.* 2015), 2. *Lysinibacillus alkaliphilus* (Zhao *et al.* 2015), 3. *Lysinibacillus alkalisoli* (Sun *et al.* 2017), 4. *Lysinibacillus boronitolerans* (Ahmed *et al.* 2007), 5. *Lysinibacillus chungkukjangi* (Kim *et al.* 2013), 6. *Lysinibacillus composti* (Hayat *et al.* 2014), 7. *Lysinibacillus contaminans* (Kampfer *et al.* 2013), 8. *Lysinibacillus cresolivorans* (Ren *et al.* 2015), 9. *Lysinibacillus endophyticus* (Yu *et al.* 2016), 10. *Lysinibacillus fluoroglycofenilyticus* (Cheng *et al.* 2015), 11. *Lysinibacillus fusiformis (Ahmed et al. 2007, Priest et al. 1988)*, 12. *Lysinibacillus halotolerans* (Kong *et al.* 2014), 13. *Lysinibacillus jejuensis* (Kim *et al.* 2013), 14. *Lysinibacillus louembei* (Ouoba *et al.* 2015), 15. *Lysinibacillus macroides* (Coorevits *et al.* 2012), 16. *Lysinibacillus manganicus* (Liu *et al.* 2013), 17. *Lysinibacillus mangiferahumi* (Yang *et al.* 2012), 18. *Lysinibacillus massiliensis* (Glazunova *et al.* 2006, Jung *et al.* 2012), 19. *Lysinibacillus meyeri* (Seiler *et al.* 2013), 20. *Lysinibacillus odysseyi* (Jung *et al.* 2012, La Duc *et al.* 2004), 21. *Lysinibacillus pakistanensis* (Ahmed *et al.* 2014), 22. *Lysinibacillus parviboronicapiens* (Miwa *et al.* 2009), 23. *Lysinibacillus sinduriensis* (Jung *et al.* 2012), 24. *Lysinibacillus saudimassiliensis* (Papadioti *et al.* 2016), 25. *Lysinibacillus sphaericus* (Ahmed *et al.* 2007, Logan and De Vos 2009), 26. *Lysinibacillus tabacifolii* (Duan *et al.* 2013), 27. *Lysinibacillus telephonicus* (Rahi *et al.* 2017), 28. *Lysinibacillus varians* (Zhu *et al.* 2014), and 29. *Lysinibacillus xylanilyticus* (Lee *et al.* 2010).

Characteristic	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*	11. *L. fusiformis*
Enzymatic Activity											
Acid Phosphatase		+	+	+	+	+	+		+	+	-
Alginase											
Alkaline Phosphatase		+		+	+	+	+		-		
Aminopeptidase				+							
Amylase											
Arginie Dihydrolase			+		-						
Catalase	+	+	+	+	+	+	+	+	+	+	+
Chemotrypsin		+					+		-		
Cysteine Arylamidase		-	-	+	-	-	+		-		
Cytochrome Oxidase											
Esterase (C4)		+	+	+	V	-	+		+		
Esterase Lipase (C8)		+/w	+	+	-	+/w	+		+		
Gelatinase (Gelatin Hydrolysis)	-	V	-	-	+	-	+	-	+	+	+
L-Arginine Dihydrolase	-	-	+	+	-	-	-	-			
Lecithinase								-			
Leucine Arylamidase		+/w	+	+	-	-			-		
Lipase C14		+	-	+	-	-	+		-		
L-Lysine Decarboxylase	-	-		-	-	-	-	-			-
L-Ornithine Decarboxylase		-		-	-		-				-
Methyl Red	-	-						-			
N-Acetyl-β-Glucosaminidase			-	-	-	-					
Naphthol-AS-BI-Phosphohydrolase		+	+	+	V	+/w	+		+		+/w
Nitrate Reduction	-	-	-	V	-	-	-	+	-	-	-
Nitrite Reduction	-							-	+		
Oxidase	+	-	-	+	+	-	+/w	-	+	-	+
Phenylalanine Deaminase	-							-			
Phosphoamidase		+					+		-		
Protease		+									
Pyrazinamidase				+							
Pyroglutamate Arylamidase				+							
Trypsin		V	-	-	-	-	+		-	+	-
Tryptophan Deaminase		-		+	-	+/w	-				
Tryptophanase (Indole Production)	-	-	-	-	-	-	-	-			-
Urease (Urea Hydrolysis)	-	-	+	+	-	-	+/w	+	-	-	+
Valine arylamidase		-	+	+	V	-	+		-	+	
Voges-Proskauer test (Acetoin Production)	-	+	-	+	+/w	+	+	-	-	+	V

Characteristic	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. lowembei*	15. *L. macroides*	16. *L. manganicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*	21. *L. pakistanensis*	22. *L. paviboronicapiens*
Enzymatic Activity											
Acid Phosphatase	+	+		+	+	+	-	-	-	+	-
Alginase									-		
Alkaline Phosphatase	+	+		+	+	+	+/w	V	-	+/w	-
Aminopeptidase							-	-	-		
Amylase									-		
Arginie Dihydrolase	-	+					+	-			
Catalase	+	+	+	+	+	+	+	V	+	+	+
Chemotrypsin					-	-		-		-	+
Cysteine Arylamidase		-			-	+	-	-	-	-	-
Cytochrome Oxidase	-							-			
Esterase (C4)	+	+		+	+	+	-	-	+	-	-
Esterase Lipase (C8)	+	V		+	+	+	+/w	+	+	+	+
Gelatinase (Gelatin Hydrolysis)	V	+	+	-	+	+	-	V	-		-
L-Arginine Dihydrolase	-	+	-	-	-	-	-	-	-		-
Lecithinase											
Leucine Arylamidase	+	-		+/w	-	+	-	-	+/w	-	-
Lipase C14		V			-	-	-	-	-	-	-
L-Lysine Decarboxylase	-		-	-		-	V	-	V		-
L-Ornithine Decarboxylase	-		-	-		-	+	-	-		-
Methyl Red											
N-Acetyl-β-Glucosaminidase		-			-	+	-	-	-		
Naphthol-AS-BI-Phosphohydrolase	+	V		+	+	+	-	-	-	-	-
Nitrate Reduction	-	-	-	V	-	-	-	-	-	+	-
Nitrite Reduction									-		
Oxidase	+	+		-	+	+	+	+	+	-	-
Phenylalanine Deaminase								-			
Phosphoamidase					-	-		-		-	-
Protease			-					-			
Pyrazinamidase							+	+	+		
Pyroglutamate Arylamidase							+	+	+		
Trypsin	-	-		-	+	-	-	-	+/w	-	-
Tryptophan Deaminase	-		+	-		-	+	-	-		+/w
Tryptophanase (Indole Production)	-	-	-	-	-	-	-	-	-		-
Urease (Urea Hydrolysis)	-	V	-	-	-	-	+	-	-		+
Valine arylamidase	+/w	-			-	-	-	-	-	-	-
Voges-Proskauer test (Acetoin Production)	-	+	+	+	-	+	+	V	+		+

Characteristic	23. *L. sinduriensis*	24. *L. saudimassiliensis*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	28. *L. varians*	29. *L. xylanilyticus*
Enzymatic Activity							
Acid Phosphatase	+		-	+	+		+
Alginase							
Alkaline Phosphatase	+/w		+	-	+		+
Aminopeptidase	-		+				-
Amylase							
Arginie Dihydrolase				+			
Catalase	+	+	+	+	+	+	+
Chemotrypsin				-	-		-
Cysteine Arylamidase	-			-	-		-
Cytochrome Oxidase							
Esterase (C4)	V		+	-	+		V
Esterase Lipase (C8)	-			+	+		-
Gelatinase (Gelatin Hydrolysis)	+		+	+		-	+
L-Arginine Dihydrolase	-			+			-
Lecithinase							
Leucine Arylamidase	V		+	-	-		-
Lipase C14	-		-	-	-		-
L-Lysine Decarboxylase	-		-	+			-
L-Ornithine Decarboxylase	-		-	+			-
Methyl Red							
N-Acetyl-β-Glucosaminidase	-		-				-
Naphthol-AS-BI-Phosphohydrolase	+		+	+	+		+
Nitrate Reduction	-		-	-		+	V
Nitrite Reduction							
Oxidase	+	-	+	+	+	+	+
Phenylalanine Deaminase							
Phosphoamidase				-	-		-
Protease							
Pyrazinamidase	+		+				+
Pyroglutamate Arylamidase	+		+				+
Trypsin	+		+/w	V	-		-
Tryptophan Deaminase	-		-	-			-
Tryptophanase (Indole Production)	-		-	-			-
Urease (Urea Hydrolysis)	-		-	+			-
Valine arylamidase	-		+/w	-	-		-
Voges-Proskauer test (Acetoin Production)	-		V	-		-	V

Characteristic	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. borontolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenihyticus*	11. *L. fusiformis*
Enzymatic Activity											
α-Chymotrypsin		V	+	+/w	V	-	+		+		+
α-Fucosidase		+		-	-	-	-		-	-	
α-Galactosidase		+	-	-	-	-	-		-	-	
α-Glucosidase		+	-	-	-	-	-		-		
α-Mannosidase		+	-	-	-	-	-		-	-	
β-Galactosidase (ONPG)		V	-	-	-	-	-		-	-	-
β-Glucosaminidase		+	-		-	-	+		-		
β-Glucosidase (Aesculin hydrolysis)	-	V	-	+/w	V	-	-	-	-		-
β-Glucuronidase		+	-	-	-	-	-		-		
Hydrolysis of:											
Casein	-			-		-	-	-	-		+
Cellulose			-		-				-		
Chitin					-						
DNA				+	+						
DNase											-
Glyceryl tributyrate				-							
Hypoxanthine				-	-						
Lethicin				-							
o-Nitrophenyl-β-D-Galactopyranoside		-									
Pectin					-						
Protease		+									
Starch	-	-	-	-	-	-	-	-	-	+	-
Testosterone											+
Tween 20				+					-	-	-
Tween 40				+	-				+	-	-
Tween 60				+	-				+	+	-
Tween 80	-	-		-	-			+	-	-	-
Tyrosine				-	-						
Xanthine				-	-						
β-D-Galactopyranoside		-									
Biodegredation of *m* -cresol											
50mg/L				+				+	-		-
100mg/L				+				+	-		-

Characteristic	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macroides*	16. *L. manganicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*	21. *L. pakistanensis*	22. *L. parviboronicapiens*
Enzymatic Activity											
α-Chymotrypsin	+	V		+	+/w	V	-	-	+	+/w	V
α-Fucosidase		-			-	-	-	-	-	-	-
α-Galactosidase		-			-	-	-	-	-	-	-
α-Glucosidase		-			-	-	-	-	-	-	-
α-Mannosidase		-			-	-	-	-	-	-	-
β-Galactosidase (ONPG)	-	-	+/w	-	-	-	-	-	-	-	-
β-Glucosaminidase		-			-	-		-		-	-
β-Glucosidase (Aesculin hydrolysis)	-	V	+/w	-	-	-	-	-	-	-	V
β-Glucuronidase		-			-	-	-	-	-	-	-
Hydrolysis of:											
Casein				-	-			+	-		
Cellulose											
Chitin											
DNA							-	+	+		
DNase											
Glyceryl tributyrate							-	-	-		
Hypoxanthine							-	-	-		
Lethicin							-	-	-		
o-Nitrophenyl-β-D-Galactopyranoside											
Pectin			+								
Protease											
Starch			+	-	-		-	-	-		
Testosterone											
Tween 20							+	+	+		
Tween 40							+	+	+		
Tween 60							+	+	+		
Tween 80							-	-	+		
Tyrosine							-	-	-		
Xanthine							-	-	-		
β-D-Galactopyranoside											
Biodegredation of *m* -cresol											
50mg/L				-							
100mg/L				-							

Characteristic	23. *L. sinduriensis*	24. *L. saudimassiliensis*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	28. *L. varians*	29. *L. xylanilyticus*
Enzymatic Activity							
α-Chymotrypsin	V		+	V	-		-
α-Fucosidase	-		-	-	-		-
α-Galactosidase	-		-	-	-		-
α-Glucosidase	-		-	-	-		-
α-Mannosidase	-		-	-	-		-
β-Galactosidase (ONPG)	-		-	-	-		-
β-Glucosaminidase				-	-		-
β-Glucosidase (Aesculin hydrolysis)	-		-	-	-		V
β-Glucuronidase	-		-	-	-		-
Hydrolysis of:							
Casein	-		+				+
Cellulose							
Chitin							
DNA	-		+				+
DNase							
Glyceryl tributyrate	-		-				-
Hypoxanthine	-		-				-
Lethicin	-		-				-
o-Nitrophenyl-β-D-Galactopyranoside							
Pectin							
Protease							
Starch	-		-			-	-
Testosterone							
Tween 20	+		+				+
Tween 40	V		+				+
Tween 60	V		+				+
Tween 80	-		-				-
Tyrosine	-		-				-
Xanthine	-		-				-
β-D-Galactopyranoside							
Biodegredation of *m* -cresol							
50mg/L			-				+
100mg/L			-				-

Characteristic	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*	11. *L. fusiformis*
Production of:											
Dihydroxyacetone				-							
Iole-3-acetic acid					-				+		
Hydrogen sulfide	-	-		-	-	-	-	-	+		-

Characteristic	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macroides*	16. *L. manganicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*	21. *L. pakistanensis*	22. *L. parviboronicapiens*
Production of:											
Dihydroxyacetone							-	-	-		
Iole-3-acetic acid											
Hydrogen sulfide	-	-	-	-	-	-	-	-	-		-

Characteristic	23. *L. sinduriensis*	24. *L. saudimassiliensis*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	28. *L. varians*	29. *L. xylanilyticus*
Production of:							
Dihydroxyacetone	-		-				-
Iole-3-acetic acid	-						
Hydrogen sulfide	-		-	-			-

Table 4. Carbon utilization of Lysinibacilllus *type strains.* Strain information was taken from the following sources: 1. *Lysinibacillus acetophenone* (Azmatunnisa *et al.* 2015), 2. *Lysinibacillus alkaliphilus* (Zhao *et al.* 2015), 3. *Lysinibacillus alkalisoli* (Sun *et al.* 2017), 4. *Lysinibacillus boronitolerans* (Ahmed *et al.* 2007), 5. *Lysinibacillus chungkukjangi* (Kim *et al.* 2013), 6. *Lysinibacillus composti* (Hayat *et al.* 2014), 7. *Lysinibacillus contaminans* (Kampfer *et al.* 2013), 8. *Lysinibacillus cresolivorans* (Ren *et al.* 2015), 9. *Lysinibacillus endophyticus* (Yu *et al.* 2016), 10. *Lysinibacillus fluoroglycofenilyticus* (Cheng *et al.* 2015), 11. *Lysinibacillus fusiformis (Ahmed et al. 2007, Priest et al. 1988)*, 12. *Lysinibacillus halotolerans* (Kong *et al.* 2014), 13. *Lysinibacillus jejuensis* (Kim *et al.* 2013), 14. *Lysinibacillus louembei* (Ouoba *et al.* 2015), 15. *Lysinibacillus macroides* (Coorevits *et al.* 2012), 16. *Lysinibacillus manganicus* (Liu *et al.* 2013), 17. *Lysinibacillus mangiferahumi* (Yang *et al.* 2012), 18. *Lysinibacillus massiliensis* (Glazunova *et al.* 2006, Jung *et al.* 2012), 19. *Lysinibacillus meyeri* (Seiler *et al.* 2013), 20. *Lysinibacillus odysseyi* (Jung *et al.* 2012, La Duc *et al.* 2004), 21. *Lysinibacillus pakistanensis* (Ahmed *et al.* 2014), 22. *Lysinibacillus parviboronicapiens* (Miwa *et al.* 2009), 23. *Lysinibacillus sinduriensis* (Jung *et al.* 2012), 25. *Lysinibacillus sphaericus* (Ahmed *et al.* 2007, Logan and De Vos 2009), 26. *Lysinibacillus tabacifolii* (Duan *et al.* 2013), 27. *Lysinibacillus telephonicus* (Rahi *et al.* 2017), 28. *Lysinibacillus varians* (Zhu *et al.* 2014), and 29. *Lysinibacillus xylanilyticus* (Lee *et al.* 2010).

Carbon source	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*
2,3-Butanediol	-	-						+		
2-Aminoethanol				+						
2'-Deoxyadenosine	+	-		+	+			+	-	
2-Ketogluconate		-		-	-	-			-	-
2-Oxoglutarate							-			
3-Hydroxybenzoic acid										-
3-Hydroxybutyric acid										-
3-Methyl-D-glucose		-								
4-Aminobutyrate							-			-
4-Hydroxybenzoic acid										-
5-Ketogluconate		+/w		-		-			-	-
6-Ketogluconate										-
Acetic acid	-	+		V		+	-	+		-
Acetoacetic acid										
Adenosine	+	-		+		-		+		
Adenosine-5'-monophosphate		-		+						
Adipic acid			-	-	-	-	-			
Aesculin		-		+/w		-			-	-
Alaninamide				+						
Amygdalin		-		-		-			-	-
Arbutin		+/w		-		-	-		-	-
Azelate							-			
Bromosuccinic acid				+						
Capric acid		-	-	-	-	-				-
cis-Acoitic acid				+						
cis-Aconitate							-			
Citric acid	-	+	+	V	+	-	-	-	-	-
Creatine			+							
Cyclohexanhexol										-
Cyclohexanol										-
D-Adonitol		+/w		-		-	-		-	-
D-Alanine		-		+						
D-Arabinose		-		-		-			-	-
D-Arabitol		+/w		-		-	-		-	-
D-Aspartic acid										
D-Cellobiose	-	-		V		-	-		-	-
D-Fructose	+	+/w	+	V		-	-		-	+

Carbon source	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macroides*	16. *L. manganicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*
2,3-Butanediol										
2-Aminoethanol										
2'-Deoxyadenosine	+	-			+	-		-		+
2-Ketogluconate		-		-		-	-	-	-	-
2-Oxoglutarate										
3-Hydroxybenzoic acid						-				
3-Hydroxybutyric acid						+				
3-Methyl-D-glucose							-			
4-Aminobutyrate										
4-Hydroxybenzoic acid						-				
5-Ketogluconate		-		-		-	-	-	+	-
6-Ketogluconate						-				
Acetic acid	V	+				-	-	+		+
Acetoacetic acid							-			
Adenosine	+					-		-		+
Adenosine-5'-monophosphate	+							-		V
Adipic acid		-	-			-		-		-
Aesculin		+		-		-	-	-	-	-
Alaninamide										
Amygdalin		-		-		-	-	-	-	-
Arbutin		-		-		-	+	-	-	-
Azelate										
Bromosuccinic acid							-			
Capric acid		-	-			-		-		-
cis-Acoitic acid										
cis-Aconitate										
Citric acid	V	-	-	+	V	-	V	+	-	-
Creatine										
Cyclohexanhexol										
Cyclohexanol						-				
D-Adonitol		-		-		-	-	-	-	-
D-Alanine										
D-Arabinose	-	-		-		-	-	-	-	V
D-Arabitol		-		-		-	-	-	-	-
D-Aspartic acid							-			
D-Cellobiose	+	-		-	-	-	V	-	-	-
D-Fructose	+	-	-	+	-	V	-	-	+	-

Carbon source	21. *L. pakistanensis*	22. *L. parviboronicapiens*	23. *L. sinduriensis*	25. *L. sphaericus*	26. *L. tabæifolii*	27. *L. telephonicus*	28. *L. varians*	29. *L. xylanilyticus*
2,3-Butanediol		-		-			-	
2-Aminoethanol								
2'-Deoxyadenosine		-	+	+			+	+
2-Ketogluconate	-		-		-			-
2-Oxoglutarate								
3-Hydroxybenzoic acid								
3-Hydroxybutyric acid								
3-Methyl-D-glucose		-		-	-	-	-	
4-Aminobutyrate								
4-Hydroxybenzoic acid								
5-Ketogluconate	-	+	-		-			
6-Ketogluconate								
Acetic acid	+	V	-	+	-	+	+	
Acetoacetic acid					-	+		
Adenosine	-	-	-	-			V	-
Adenosine-5'-monophosphate		-	-	V			-	
Adipic acid			-					-
Aesculin	-	+	-		-			
Alaninamide								
Amygdalin	-	-	-	-	-		-	
Arbutin	-	-	-	-	-		-	
Azelate								
Bromosuccinic acid					-	-		
Capric acid			-					-
cis-Acoitic acid								
cis-Aconitate								
Citric acid		-	+/w	V	-	-		V
Creatine								
Cyclohexanhexol								
Cyclohexanol								
D-Adonitol	-		-		-			
D-Alanine	-	-		-			-	
D-Arabinose	-	-	-		-			
D-Arabitol	-	-	-	-	-	-	-	
D-Aspartic acid					-	-		
D-Cellobiose	-	-	-	+	-	-	-	+
D-Fructose	-	-	-	-	-	-	+/w	V

Carbon source	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*
D-Fructose-6-phosphate		-		+						
D-Fucose		-		-	-	-			-	-
D-Galactose	-	-		V	-	-			-	-
D-Galacturonic acid		-								
D-Gluconic acid		-		+						
D-Glucose	-	-	-	V	-	-	-	-	-	-
D-Glucose-1-phosphate		-								
D-Glucose-6-phosphate		-			+/w					
D-Glucuronic acid										
D-Lactic acid methyl ester		-				-				
D-Lyxose		+/w		-	-	-			-	-
D-Malic acid		-			-					
D-Maltitol							-			
D-Maltose		-	-	-	-	-	-			
D-Mannitol	V	+/w	+	-	-	-	-	-	-	+
D-Mannose	V	-	+	-	-	-	-		-	-
D-Melezitose	-	-		-	-	-			-	-
D-Melibiose	+	-		V	-	-	-		-	-
D-Psicose	-	-								
D-Raffinose	-	-		V	-	-	-		-	-
D-Ribose		+		V	-	-	-	+	-	-
D-Saccharic acid										
D-Salicin	-	-		-		-	-		-	-
D-Serine										
D-Sorbitol	+	+/w		V		-	-		-	-
D-Tagatose		+/w		V		-			-	-
D-Trehelose	-	-		-		-	-		-	-
D-Turanose		-		-	-	-			-	-
D-Xylose	+	V		V	-	-	-	-	-	-
D,L-Lactic acid				+						-
D,L-α-Glycerol phosphate		+								
Dextrin	-	-		+		+				
Dulcitol		+/w		-		-	-		-	-
Erythritol		-		-	-	-	-		-	-
Ferric citrate										
Formic acid				V						
Fumarate	+			+			-			

Carbon source	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macroides*	16. *L. manganicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*
D-Fructose-6-phosphate	+				-					
D-Fucose		-		-		-	-	-	-	-
D-Galactose	-	-	-	-	-	-	-	-	-	-
D-Galacturonic acid										
D-Gluconic acid	+				+					
D-Glucose	+	-	-	-	-	-	-	-	-	-
D-Glucose-1-phosphate										
D-Glucose-6-phosphate		-					-			
D-Glucuronic acid							-			
D-Lactic acid methyl ester							+			
D-Lyxose		-		-		-	-	-	-	-
D-Malic acid		+				+	-			
D-Maltitol										
D-Maltose	-	-	-		-	-	+	-	-	-
D-Mannitol		-	-	+/w		-	-	-	V	-
D-Mannose	V	-	-	-	+	-	V	-	-	-
D-Melezitose		-		-		-	-	-	-	-
D-Melibiose	-	-		-	-	-	V	-	-	-
D-Psicose										
D-Raffinose	V	-		-	+	-	V	-	-	-
D-Ribose	+	V	+	-	V	-	+	V	-	-
D-Saccharic acid							-			
D-Salicin		-		-		-	V	-	-	-
D-Serine							-			
D-Sorbitol	-	-		-	-	-	-	-	-	-
D-Tagatose		-		-		-	-	-	-	-
D-Trehelose		-		-		-	-	-	-	-
D-Turanose		-		-		-	-	-	-	-
D-Xylose	-	V	+	-	V	-	-	V	-	-
D,L-Lactic acid					+/w	-				
D,L-α-Glycerol phosphate										
Dextrin	-						+	+		+
Dulcitol		-		-		-	-	-	-	-
Erythritol		-		-		-	-	-	-	-
Ferric citrate										
Formic acid	-				-		-			
Fumarate	-					-		+		

Carbon source	*L. pakistanensis* 21.	*L. parviboronicapiens* 22.	*L. sinduriensis* 23.	*L. sphaericus* 25.	*L. tabæifolii* 26.	*L. telephonicus* 27.	*L. varians* 28.	*L. xylanilyticus* 29.
D-Fructose-6-phosphate		-		-		+	+/w	-
D-Fucose	-		-		-	-		-
D-Galactose	-	-	-	V	-	+/w	-	-
D-Galacturonic acid		-		-		-	+/w	
D-Gluconic acid		-		-		+	-	-
D-Glucose	-		-	V	+		-	V
D-Glucose-1-phosphate		-		-			-	
D-Glucose-6-phosphate		-	-	-	-	-	-	
D-Glucuronic acid					-	+		
D-Lactic acid methyl ester	-	-		-		-	-	
D-Lyxose	-		-		-			
D-Malic acid		-	-	-	-	-	-	
D-Maltitol								
D-Maltose		-	-	-	-	-	-	V
D-Mannitol	-	-	-	-	-	-	-	V
D-Mannose	-	-	-	V	V	-	-	-
D-Melezitose	-	-	-	-	-		-	-
D-Melibiose	-	-	-	-	V	-	-	-
D-Psicose		-		-			+/w	
D-Raffinose	-	-	-	-	-	-	-	-
D-Ribose	-	-	V	+	-		+	V
D-Saccharic acid					-	-		
D-Salicin	-	-	-	-	-	-	-	
D-Serine					-	-		
D-Sorbitol	-	-	-	-	V	-	-	-
D-Tagatose	-	-	-	+	-		+	
D-Trehelose	-	-	-	-	-	-	-	
D-Turanose	-	-	-	-	-	-	-	-
D-Xylose	-	-	-	V	-		+	V
D,L-Lactic acid								
D,L-α-Glycerol phosphate		-		-			-	
Dextrin	+	-	-	-	+	-	-	+
Dulcitol	-		-		-			
Erythritol	-		-		-			-
Ferric citrate		+						
Formic acid				+	-	-		+
Fumarate		+		-				+

Carbon source	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*
Gelatin										
Gentioibiose		-			-					
Gluconate		+/w	-	V	+	-	-		-	-
Glucuronamide										
Glycerol	-	-		-	-	-			-	-
Glycogen		-		V	-	-			-	V
Glycyl-L-aspartic acid				+						
Glycyl-L-glutamic acid	-	-		+						
Glycyl-L-proline										
Histidine				-						+
i-Erythitol				+						
Indole				-	-					
Inosine	-	-		+	+/w			+		
Inositol		-		-		-			-	-
Inulin		-		-	-	-			-	-
Itaconic acid										-
Lactamide		-		+						
Lactose	-	-	+	-	-	-	-		-	-
Lactulose	-	-								
L-Alaninamide		-				-		+		
L-Alanine	+	+		+	-	-		+		+
L-Alanyl-glycine		-								
L-Arabinose	-	-	-	-	-	-	-		-	-
L-Arabitol		+/w		-		-		-	-	-
L-Arginine										
L-Asparagine		+		+						
L-Aspartic acid				+	-					
L-Fucose		-		-	-	-			-	-
L-Galactonic acid lactone										
L-Glutamic acid		+		+	-	-			-	
L-Histidine				+	+					
L-Lactic acid	-	-		+	-					
L-Leucine				+						
L-Lylose		-		-		-			-	-
L-Malic acid		+	+	+	V					
L-Ornithine				+						
L-Proline			+	+						+

Carbon source	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macroides*	16. *L. manganicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*
Gelatin							-			
Gentioibiose		-					-			
Gluconate		-	-	-		-	-	V	+	-
Glucuronamide							-			
Glycerol	-	V		+	-	-	-	-	+	-
Glycogen		-		-		V	-	-	-	-
Glycyl-L-aspartic acid										
Glycyl-L-glutamic acid	+					-		-		+
Glycyl-L-proline							-			
Histidine						-			+	-
i-Erythitol										
Indole								-	-	-
Inosine	+	-					-	-		+
Inositol	-	+		-		-	+	-	-	-
Inulin		-		-		-	-	-	-	-
Itaconic acid						-				
Lactamide										
Lactose		-		-		-	+	-	-	-
Lactulose										
L-Alaninamide										
L-Alanine	+	-			+	-	-	-	+	V
L-Alanyl-glycine										
L-Arabinose	-	V	-	-	+	-	+	V	V	-
L-Arabitol		-		-		-	-	-	-	-
L-Arginine							-			
L-Asparagine										
L-Aspartic acid		+					-			
L-Fucose		-		-		-	-	-	-	-
L-Galactonic acid lactone							+			
L-Glutamic acid		-			+	-	-	-		
L-Histidine		+/w			+/w	-	-			
L-Lactic acid		-					-			
L-Leucine										
L-Lylose		-		-		-	-	-	-	-
L-Malic acid		V			-	-	-	+		+
L-Ornithine										
L-Proline					+/w	-				

Carbon source	21. *L. pakistanensis*	22. *L. parviboronicapiens*	23. *L. sinduriensis*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	28. *L. varians*	29. *L. xylanilyticus*
Gelatin					-	-		
Gentioibiose		-	-	-	-	-	-	-
Gluconate	-		V		-			+
Glucuronamide					-	+		
Glycerol	-	-	V	V	-	-	-	V
Glycogen	-	-	-	-	-		-	-
Glycyl-L-aspartic acid								
Glycyl-L-glutamic acid		-		V			-	
Glycyl-L-proline					-	+		
Histidine								
i-Erythitol								
Indole			-	-				-
Inosine		-	-	-	-	+/w	+/w	
Inositol	-	-	-		-			
Inulin	-	-	-	-	-		-	-
Itaconic acid								
Lactamide		-		-			-	
Lactose	-	+	-		-			-
Lactulose		-		-			+/w	
L-Alaninamide	+	-		-			-	
L-Alanine		-	-	+	-	+	-	V
L-Alanyl-glycine		-		-			-	
L-Arabinose	-	-	-	+	-		+	V
L-Arabitol	-		-		-			
L-Arginine					-			
L-Asparagine		-		+		-	+	
L-Aspartic acid			-		-	-		
L-Fucose	-	-	-	-	-	-	-	-
L-Galactonic acid lactone						+		
L-Glutamic acid	-	-	V	+	-	+	+	V
L-Histidine			-		-	+/w		
L-Lactic acid		-	-	+	-	+/w	+	
L-Leucine								
L-Lylose	-		-		-			
L-Malic acid		-	V	+	-	-	+	+
L-Ornithine								
L-Proline								

Carbon source	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*
L-Pyroglutamic acid		-								
L-Rhamnose	-	-	+	-	-	-	-		-	-
L-Serine		-	+	+	+			+	-	+
L-Sorbose		+/w		-		-			-	-
L-Threonine				+						
L-Xylose	+	+/w								
Malate			+	-	+	-	-			
Malonic acid	+									-
Maltose	-	-		-	-	-	-		-	-
Maltotriose		-								
Mannan		-								
Mesaconate							-			
Methy- β-D-xylopyranoside		-			-					
Methyl pyruvate				+	+					
Methyl-α-D-glucopyranoside		-			-					
Methyl-α-D-mannopyranoside		-			-					
Monomethyl succinate				+						
Mucic acid										
myo -inositol	-	-					-			
N-Acetyl-D-galactosamine										
N-Acetyl-D-glucosamine		+/w	-	V	-	-	-		-	+
N-Acetyl-L-glutamic acid		-								
N-Acetyl-L-lactamide glutamic acid										
N-Acetyl-neuraminic acid										
N-Acetyl-β-D-mannosamine		-								
N-Acetyl-β-D-mannoside				+						
N-Acetyl-β-glucosamine										
N-Acyl-D-glucosamine										
Nulin										
Palatinose	-	-								
Pectin										
Pentanoates				-						-
Phenylacetic acid		-	-	-	-	-				
p-Hydroxy-phenylacetic acid		-								
Propionic acid		-		V			-			+
Putrescine		-					-			
Pyruvate	+	+		+		-	-			

Carbon source	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macroides*	16. *L. manganicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*
L-Pyroglutamic acid							-			
L-Rhamnose	-	-		-		-	-	-	-	-
L-Serine		-			+	+	-	-	+	-
L-Sorbose		-		-		-	-	-	-	-
L-Threonine										
L-Xylose										
Malate		-	-			+				
Malonic acid			-			-				
Maltose	-	-	+	-	+	-	+	-	-	-
Maltotriose										
Mannan										
Mesaconate										
Methy- β-D-xylopyranoside		-								
Methyl pyruvate	+	-					-	+		+
Methyl-α-D-glucopyranoside		-								
Methyl-α-D-mannopyranoside		-								
Monomethyl succinate										
Mucic acid							-			
myo -inositol							-			
N-Acetyl-D-galactosamine							-			
N-Acetyl-D-glucosamine	+	-	-	+/w	-	-	-	-	V	-
N-Acetyl-L-glutamic acid										
N-Acetyl-L-lactamide glutamic acid										
N-Acetyl-neuraminic acid							-			
N-Acetyl-β-D-mannosamine							-			
N-Acetyl-β-D-mannoside	+				-		-			
N-Acetyl-β-glucosamine				+						
N-Acyl-D-glucosamine	-									
Nulin										
Palatinose										
Pectin							+			
Pentanoates						-			+	-
Phenylacetic acid		-	-			-		-		-
p-Hydroxy-phenylacetic acid							-			
Propionic acid	+				+	-	-		-	-
Putrescine										
Pyruvate	+	+	+		-			+		+

Carbon source	21. *L. pakistanensis*	22. *L. parviboronicapiens*	23. *L. sindhuriensis*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	28. *L. varians*	29. *L. xylanilyticus*
L-Pyroglutamic acid		-		-	-	-	-	
L-Rhamnose	-	-	V	-	-	+	-	
L-Serine		-	-	+	-	+/w	+	+
L-Sorbose	-		-		-			
L-Threonine								
L-Xylose								
Malate								
Malonic acid								
Maltose	-	+	-	-	-		-	-
Maltotriose		-		-			-	
Mannan		-		+			+	
Mesaconate								
Methy- β-D-xylopyranoside			-					-
Methyl pyruvate			-	+	-	-		
Methyl-α-D-glucopyranoside			-					-
Methyl-α-D-mannopyranoside			-					-
Monomethyl succinate								
Mucic acid					-	-		
myo -inositol		-		-	-	-	-	
N-Acetyl-D-galactosamine					-	-	-	
N-Acetyl-D-glucosamine	-	V	-	-	-		-	V
N-Acetyl-L-glutamic acid		-		-			-	
N-Acetyl-L-lactamide glutamic acid							-	
N-Acetyl-neuraminic acid					-	-		
N-Acetyl-β-D-mannosamine		-		-	-	-	-	
N-Acetyl-β-D-mannoside				+				+
N-Acetyl-β-glucosamine								
N-Acyl-D-glucosamine				-	-			-
Nulin							-	
Palatinose		-		-			+/w	
Pectin					+	-		
Pentanoates								
Phenylacetic acid			-					V
p-Hydroxy-phenylacetic acid		-		-	-	-	-	
Propionic acid		-		+	-	-	+	+
Putrescine		-		-			-	
Pyruvate	-	-	-	V			+	-

Carbon source	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*
Pyruvic acid methyl ester		-				-				
Quinic acid										
Saccharose										
Sedoheptulosan	-	-								
Stachyose	-	-								
Starch		-		-	-	-			-	-
Suberic acid										-
Succinamic acid		-								
Succinic acid		-			-					
Succinic acid mono-methyl ester		+				-		+		
Sucrose	-	-		V	-	-	-		-	-
Thymidine		-		+		-		+		
Thymidine-5'-monophosphate		-		+		-		+		
trans -Aconitate							-			
Trisodium citrate	+	+	+	V	-	-				
Tween 40		-			-			+		
Tween 80		-			-					
Uridine		-		+				+		
Uridine-5'-monophosphate		-		+				+		
Valeric acid										-
Xylitol	-	+/w		-		-			-	-
α-Cyclodextrin	-	-								
α-D-Glucose										
α-D-Glucose-1-phosphate										
α-D-Lactose		-								
α-Hydroxybutyric acid		-		+						
α-Ketobuteric acid				+						
α-Ketoglutaric acid		-			+/w					
α-Ketovaleric acid		-		+		-		+		
α-Methyl-D-galactoside		-								
α-Methyl-D-glucoside		-		-		-			-	-
α-Methyl-D-mannoside		-		-		-			-	-
β-Cyclodextrin		-		+						
β-Gentiobiose		-		-		-			-	-
β-Hydroxybutyric acid	+	-		+		-				
β-Hydroxy-D,L-butyric acid				+	+					
β-Methyl-D-galactoside		-								

Carbon source	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macroides*	16. *L. manganicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*
Pyruvic acid methyl ester		+						+		+
Quinic acid							-			
Saccharose									-	
Sedoheptulosan										
Stachyose							-			
Starch		-		-		-	-	-	-	-
Suberic acid						-				
Succinamic acid										
Succinic acid		+			-	-		-		
Succinic acid mono-methyl ester		+								
Sucrose	V	-		-	-	-	V	-	-	-
Thymidine		-				-				
Thymidine-5'-monophosphate										
trans -Aconitate										
Trisodium citrate		-	-			-		-		-
Tween 40		+			+	-	-	+		
Tween 80		+			+	-		+		
Uridine										
Uridine-5'-monophosphate	+							-		V
Valeric acid						+				
Xylitol		-		-		-	-	-	-	-
α-Cyclodextrin										
α-D-Glucose							-			
α-D-Glucose-1-phosphate										
α-D-Lactose							-			
α-Hydroxybutyric acid	-						+	-		V
α-Ketobuteric acid							-			
α-Ketoglutaric acid		V					-			
α-Ketovaleric acid		+						+		+
α-Methyl-D-galactoside										
α-Methyl-D-glucoside		-		-		-	-	-	-	-
α-Methyl-D-mannoside		-		-		-	-	-	-	-
β-Cyclodextrin										
β-Gentiobiose		-		-		-	-	-	-	-
β-Hydroxybutyric acid	-	-			+	+		V		+
β-Hydroxy-D,L-butyric acid		-					-			
β-Methyl-D-galactoside										

Carbon source	21. *L. pakistanensis*	22. *L. parviboronicapiens*	23. *L. sinduriensis*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	28. *L. varians*	29. *L. xylanilyticus*
Pyruvic acid methyl ester	-	-	+	-			-	-
Quinic acid					-	-		
Saccharose								
Sedoheptulosan		-		-			-	
Stachyose		-		-	-	-	-	
Starch	-		-		-			-
Suberic acid								
Succinamic acid		-		-			-	
Succinic acid		-	-	-			-	-
Succinic acid mono-methyl ester	-	-		-			-	
Sucrose	-	-	-	-	-	-	-	-
Thymidine	-	-		+			+	
Thymidine-5'-monophosphate	-	-		-			+/w	
trans -Aconitate								
Trisodium citrate			-					+
Tween 40		-	-	-	-	-	V	+
Tween 80		-	-	-			-	+
Uridine		-		+			+	
Uridine-5'-monophosphate		-	-	V			+/w	
Valeric acid								
Xylitol	-	-	-	-	-		-	
α-Cyclodextrin		-		-			-	
α-D-Glucose					+	-		
α-D-Glucose-1-phosphate							-	
α-D-Lactose		-		-	-	-	-	
α-Hydroxybutyric acid		-	-	V	+	-	+	
α-Ketobuteric acid					-	-		
α-Ketoglutaric acid		-	-	-	-	-	-	
α-Ketovaleric acid	+	-	+	+			+	+
α-Methyl-D-galactoside		-		-			-	
α-Methyl-D-glucoside	-	-	-	-	-		-	
α-Methyl-D-mannoside	-	-	-	-	-		-	
β-Cyclodextrin		-		-			+/w	
β-Gentiobiose	-		-		-			
β-Hydroxybutyric acid	-	-	-	-			-	-
β-Hydroxy-D,L-butyric acid			-		-	-		
β-Methyl-D-galactoside		-		-			-	

Carbon source	1. *L. acetophenoni*	2. *L. alkaliphilus*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	6. *L. composti*	7. *L. contaminans*	8. *L. cresolivorans*	9. *L. endophyticus*	10. *L. fluoroglycofenilyticus*
β-Methyl-D-glucoside		-								
β-Methylxyloside		-		-		-			-	-
γ-Aminobutyric acid										
γ-Hydroxybutyric acid		-								

Carbon source	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	14. *L. louembei*	15. *L. macroides*	16. *L. manganicus*	17. *L. mangiferahumi*	18. *L. massiliensis*	19. *L. meyeri*	20. *L. odysseyi*
β-Methyl-D-glucoside							-			
β-Methylxyloside		-		-		-	-	-	-	-
γ-Aminobutyric acid							+			
γ-Hydroxybutyric acid										

Carbon source	21. *L. pakistanensis*	22. *L. parviboronicapiens*	23. *L. sinduriensis*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	28. *L. varians*	29. *L. xylanilyticus*
β-Methyl-D-glucoside		-		-	-	-	-	
β-Methylxyloside	-		-		-			
γ-Aminobutyric acid					+	-		
γ-Hydroxybutyric acid		-		-			-	

Table 5. Chemical and antibiotic resistances of Lysinibacillus *type strains.* Strain information was taken from the following sources: 1. *Lysinibacillus acetophenone* (Azmatunnisa *et al.* 2015), 3. *Lysinibacillus alkalisoli* (Sun *et al.* 2017), 4. *Lysinibacillus boronitolerans* (Ahmed *et al.* 2007), 5. *Lysinibacillus chungkukjangi* (Kim *et al.* 2013), 8. *Lysinibacillus cresolivorans* (Ren *et al.* 2015), 11. *Lysinibacillus fusiformis (Ahmed et al. 2007, Priest et al. 1988)*, 12. *Lysinibacillus halotolerans* (Kong *et al.* 2014), 13. *Lysinibacillus jejuensis* (Kim *et al.* 2013), 15. *Lysinibacillus macroides* (Coorevits *et al.* 2012), 17. *Lysinibacillus mangiferahumi* (Yang *et al.* 2012), 18. *Lysinibacillus massiliensis* (Glazunova *et al.* 2006, Jung *et al.* 2012), 22. *Lysinibacillus parviboronicapiens* (Miwa *et al.* 2009), 25. *Lysinibacillus sphaericus* (Ahmed *et al.* 2007, Logan and De Vos 2009), 26. *Lysinibacillus tabacifolii* (Duan *et al.* 2013), 27. *Lysinibacillus telephonicus* (Rahi *et al.* 2017), and 29. *Lysinibacillus xylanilyticus* (Lee *et al.* 2010).

Characteristic	1. *L. acetophenoni*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	8. *L. cresolivorans*	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	15. *L. macroides*	17. *L. mangiferahumi*	18. *L. massiliensis*	22. *L. parviboronicapiens*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	29. *L. xylanilyticus*
1% Sodium lactate									-	-				-	+	
Acetophenone	+															
Amikacin											-					
Amoxicillin		-									-					
Ampicillin	-	-	+			-						+	+			+
Augmentin											-					
Azetronam									-	-	+			-	+	
Bacitracin		+										+				
Carbenicillin		+	-			-						-	-			-
Cefepime											-/w					
Cefpirome											-/w					
Cefradine		-														
Ceftrazidime											+					
Ceftriaxon											-					
Cephalothin			+			+						+	+			+
Chloramphenicol	-		-			+					-	+	+/w			
Chloromycetin		-			+											
Ciprofloxacin											-					
Claventin											-					
Colistin			+								-	+				
Enrofloxacin												+				
Erythromycin		-	-			+		+	-			-	+/w			+
Flumequine												+				
Fosfomycin											+					
Fusidic Acid									-	-		+		-	-	
Fusinic Acid			+													
Gentamycin	-	-	+			-		+	+			-	+			+
Guanidine HCl				-			-		-	-				-	+	
Imipinem											-					
Isepamycin											-					
Kanamycin	-	-	-			-						-	-			-
Lincomycin			+			+			-	-		+	+	-	-	+
Lithium chloride									-	-				-	+	
Metronidazole			+									+				
Minocycline									-	-				-	-	
Nalidixic Acid	+								-	-				-	+	
Neomycin			-			-						-	-			-
Niaproof 4									-	-				-	-	
Novobiocin		-	+			-		+	+			-	-			+

Characteristic	1. *L. acetophenoni*	3. *L. alkalisoli*	4. *L. boronitolerans*	5. *L. chungkukjangi*	8. *L. cresolivorans*	11. *L. fusiformis*	12. *L. halotolerans*	13. *L. jejuensis*	15. *L. macroides*	17. *L. mangiferahumi*	18. *L. massiliensis*	22. *L. parviboronicapiens*	25. *L. sphaericus*	26. *L. tabacifolii*	27. *L. telephonicus*	29. *L. xylanilyticus*
Ofloxacin											-					
Oleandomycin			-			-						-	-			-
Oxolinic Acid			+									+				
Penicillin G	-	-	-		-	-						-	-			-
Piperacillin-Tozabactam											-					
Polymixin B			+			+						+	+			+
Rifampicin		+	-		+	-					-	-	+/w			
Rifamycin SV			-	+/w			-		-	-				-	-	
Roxithromycin		-														
Sodium bromate									-	-				-	-	
Spectinomycin		-														
Streptomycin	-		-		-	+		+	+		-	+	+			+
Sulfamethizol			+													
Tetracycline	-	-	-		+	-		+	+		-	-	V			+
Ticarcillin											-					
Tobramycin											-					
Trimethoprim		-														
Trimethoprim-Sulfamethoxazole											-					
Troleandomycin									-	-				-	-	
Vancomycin	-	-							-	-				-	-	

Figure 1. Evolutionary relationships between Lysinibacillus *type strains based on the alignment of partial 16S rDNA sequences*. The maximum likelihood tree was built from the 1228bp-long sequence of 27 type strains downloaded from NCBI using the GTR model (Nei and Kumar 2000) and 100 bootstrap replicates (Felsenstein 1985). Gaps are considered as missing data and are partially deleted with a site coverage cut-off of 95%. The strain *Bacillus subtilis* ATCC 6051^{T} is used as root. NCBI names are comprised of the genus and species name and the associated strain code. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.* 2016).

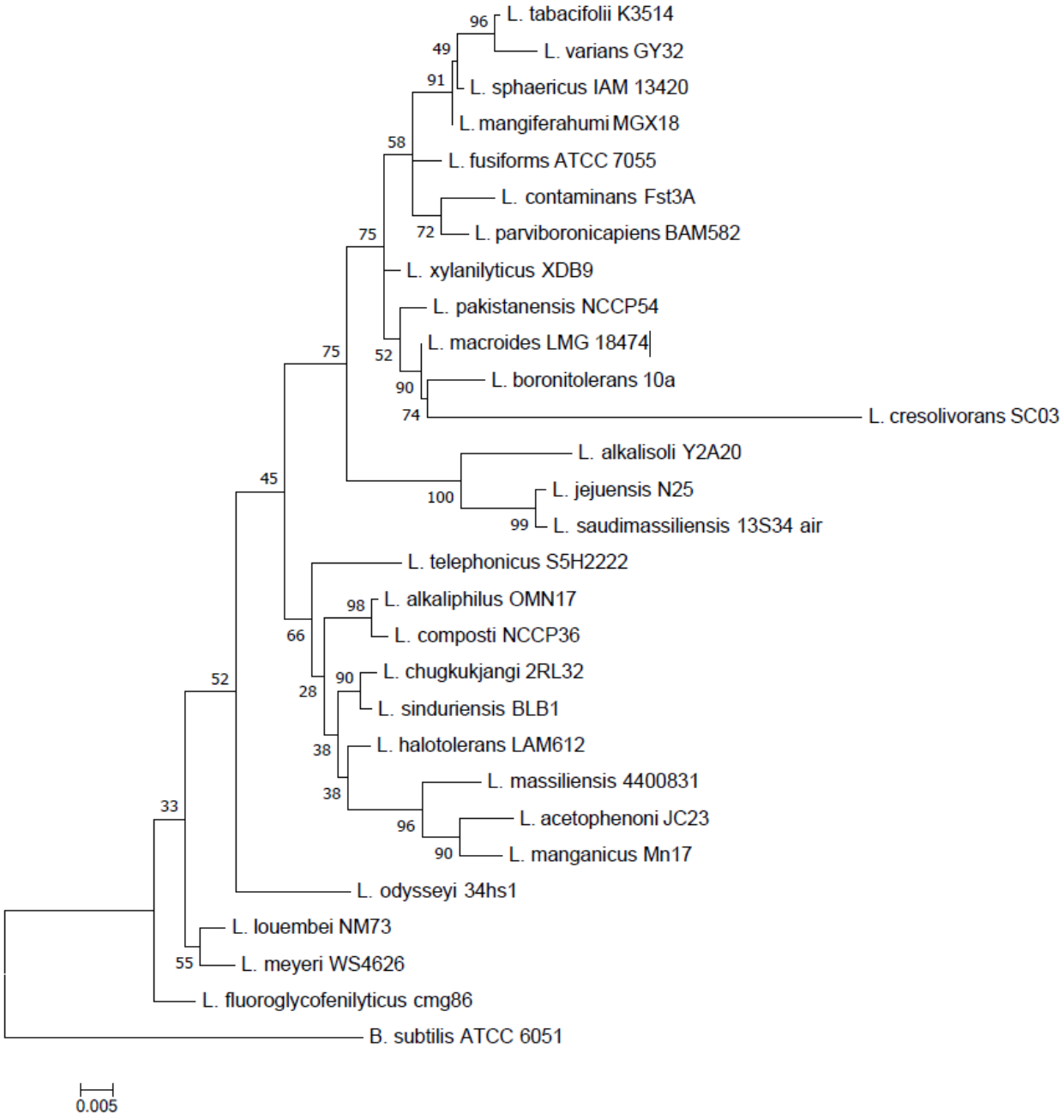

L. tabacifolii K3514
L. varians GY32
L. sphaericus IAM 13420
L. mangiferahumi MGX18
L. fusiforms ATCC 7055
L. contaminans Fst3A
L. parviboronicapiens BAM582
L. xylanilyticus XDB9
L. pakistanensis NCCP54
L. macroides LMG 18474
L. boronitolerans 10a
L. cresolivorans SC03
L. alkalisoli Y2A20
L. jejuensis N25
L. saudimassiliensis 13S34 air
L. telephonicus S5H2222
L. alkaliphilus OMN17
L. composti NCCP36
L. chugkukjangi 2RL32
L. sinduriensis BLB1
L. halotolerans LAM612
L. massiliensis 4400831
L. acetophenoni JC23
L. manganicus Mn17
L. odysseyi 34hs1
L. louembei NM73
L. meyeri WS4626
L. fluoroglycofenilyticus cmg86
B. subtilis ATCC 6051
96
49
91
58
72
75
75
52
90
74
45
100
99
66
98
28
90
52
38
38
33
96
90
55
0.005

Figure 2. Evolutionary relationships between Lysinibacillus *strains based on the Average Nucleotide Identity of available genome sequences*. 52 *Lysinibacillus* strains rooted with *Bacillus subtilis subsp. subtilis* strain 168 was generated based on the alignment of 72 core genes (33,047 polymorphic sites). The tree is drawn to scale and branch length represents the number of base substitutions per site. Nodes annotated with a circle are supported by bootstrap values above 70%.

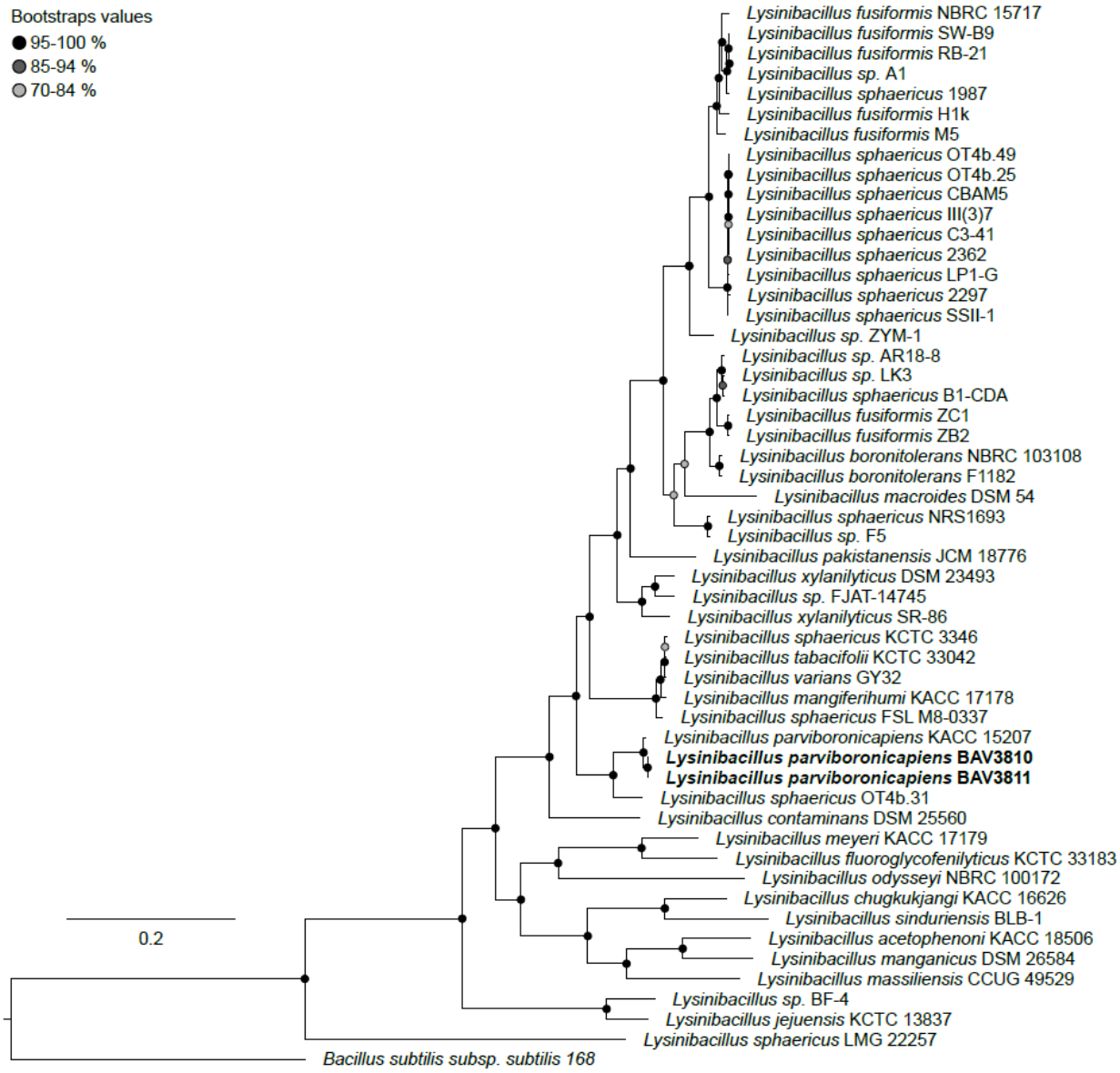
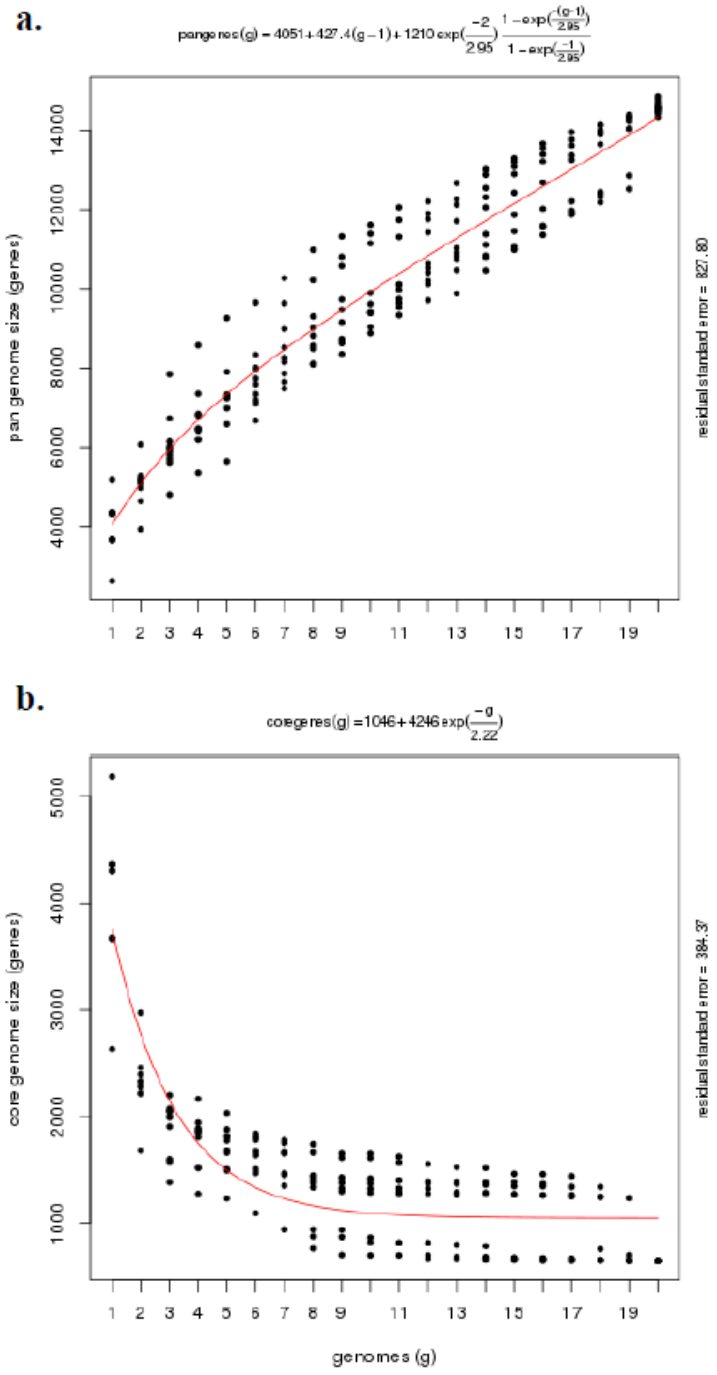


Figure 3. Rarefaction curves of the pan- and core-genomes of 20 Lysinibacillus *type strains.* (a.) Rarefaction curve of the *Lysinibacillus* pangenome showing an open pangenome, and (b.) a rarefaction curve of the core genome. Core genome begins leveling off at eight genomes, suggesting a core genome size of approximately 644 genes.



Chapter Five: Conclusions and future directions

Through the course of this work, several key findings have been made which have served to improve the understanding of bacterial ice nucleation and the distribution of ice nucleation active (ice+) species within precipitation. Several species within well-known clades of ice+ genera, were identified including: *Pseudomonas trivalis*, *P. costantinii*, *P. orientalis*, *P. frederiksbergensis*, and *P. lini*. For many of these species, INA had not been characterized in detail, and some had not been reported from precipitation samples. Furthermore, this work represents the first report that *P. aeruginosa* has been shown to exhibit ice nucleation activity (INA). Previously, the majority of known INA strains outside of the genus *Pseudomonas*, such as strains in the genera *Pantoea*, *Erwinia*, and *Xanthomonas*, had been isolated from plant surfaces. Thus, our finding of strains of these species from precipitation samples contributes knew knowledge on the abundance of INA bacteria in the environment. Additionally, both *Stenotrophomonas*, a close relative of the known ice+ containing genus *Xanthomonas*, and *Lysinibacillus* were identified for the first time as including at least one species with INA. Importantly, the identification and confirmation of the *Lysinibacillus* INA marks the first confirmed non-gammaproteobacteria to exhibit INA.

Despite finding several new ice+ species during the initial precipitation collection and demonstrating that we sampled deeply enough to isolate the vast majority of ice+ species in the region (Supplemental Figure S2, appendix one), several questions remain unanswered with even more being raised over the course of the project.

While we showed that the sampling campaign undertaken in Blacksburg, VA allowed us to culture the majority of culturable ice+ bacteria for the area, we do not know the extent to which these bacteria travel long distance through the atmosphere and how representative our samples are for a larger region. It is possible that the samples taken as part of this campaign are representative

of a much larger geographic area. For each of the precipitation events captured in this study, air mass trajectories were analyzed and the terrain height of each trajectory was analyzed for any potential impact on the total culturable bacteria and total ice nuclei. Though no correlation was found among our samples, future collections from various locations within and outside of this region may better resolve the relationships among our samples and larger geographic regions outside of our collection area.

Another major concern brought up numerous times throughout this campaign was the uncertainty of the source(s) of the isolated bacteria. Because of the limitations of the experiment, there is some degree of ambiguity as to whether or not the bacteria were responsible for the ice nucleation in the cloud layer, and in turn deposited with the rain, present in the clouds but not responsible for any INA therein, scavenged out of the atmosphere during the precipitation event, and/or splashed into the collection cans during the precipitation event and not associated with the precipitation or the column of air between the clouds and the collection cans. The cans themselves were set up to minimize the potential for any splashing and were set up apart from each other and any other surrounding obstacles and the cans present at Kentland Farm were elevated slightly to place the opening 1m above the ground. Furthermore, the cans at the farm were placed on a gravel patch to minimize nearby vegetation, and those present on the roof of Latham Hall were five stories above ground and centered as far from any electrical or maintenance outlets as possible. By arranging the cans in this manner, any splashing during heavy rain should be minimized, thereby minimizing the likelihood of splashing and very local (<5m) vegetation as a potential source of contamination. However, this does not conclusively demonstrate that bacteria cannot splash into our collection cans, nor does it help to answer if the bacteria were from the clouds or from the air column.

In an attempt to provide additional answers to this question, another set of experiments was run to determine if ice+ bacteria could be scavenged out of the air column by falling rain. This work (Appendix Four) utilized simulated rainfall traveling through an approximate 50m column of air at the Virginia Tech Transportation Institute Smart Road Bridge. 8,331 colonies were screened for INA, and 121 demonstrated initial ice nucleation activity. However, like the initial precipitation study, many of these strains did not show confirmed activity (34/121) (Hanlon *et al.* 2017). While this study helped to show that indeed ice+ bacteria can be isolated out of the air, the numbers isolated are significantly lower than those present in the initial study. Furthermore, due to highly variable wind and weather conditions, successful recapture of the simulated rain may have introduced unintentional variability between collections during the same day as well as collections across the multiple events described in this paper. Additional simulated rain events could provide additional resolution to the extent that ice+ bacteria are present in the air column, as well as providing a comparison between samples taken immediately before and/or after a natural precipitation event since it has been shown that there is an increase in ice nucleating particles present in the atmosphere after precipitation (Huffman *et al.* 2013).

Despite the initial preparations made to the collection apparatus to minimize splashing contamination and the follow up investigation to better understand scavenging from the air column, we still do not have a clear answer as to the initial source of the ice+ and the role, if any, these bacteria play in the formation of precipitation. While we know many of these bacteria can reside, at least for short periods, within the could layer (Amato *et al.* 2005, Amato *et al.* 2007, Morris *et al.* 2014), and that they are capable of ice nucleation, (Amato *et al.* 2015, Joly *et al.* 2013), the question remains if these bacteria are aiding in the formation of precipitation. We can, however, conclude that the number of bacteria cultured from our precipitation samples fall below

the estimated 10 ice nuclei per meter of cloud, as outlined by Crawford et al (Crawford *et al.* 2012), if we make several key assumptions: 1. Ice+ bacteria are neither lost nor scavenged while precipitation falls, 2. expression of genes that determine INA in the clouds is similar to that under laboratory growth conditions, 3. all bacteria in precipitation are culturable, and 4. one Ice+ colony plated from precipitation corresponds to one IN in the clouds.

Through the follow-up simulated rain capture study, we were able to show that some bacteria may be scavenged from the atmosphere during deposition, however, we do not know the rate at which bacteria may be lost during descent (gravitational settling) through the atmosphere. Because of this, we cannot confidently estimate the impact these results have on assumption number one listed above. Furthermore, due to the low rate of culturability of bacterial species, it is possible that there are ice+ bacteria that we were unable to culture due to their inability to grow on the media we selected. This would give strong circumstantial evidence against assumption four, but as of yet, no unculturable ice+ bacteria have been identified. This is, in part, due to the highly repetitive nature of the gene, making for the sequencing and subsequent identification in metagenome data difficult. The two inaZ fragments available in the NCBI database are both very short sequences (accession numbers LC142695 and LC142696); as such, these sequences would not be specific enough to accurately predict the presence of the gene in unculturable bacterial sequences. The *Xanthomonas* ice nucleation gene, inaX (accession number X52970), and *Pseudomonas* genes inaQ and inaW (accession numbers EU360731 and X04501), however, do have a full sequence available, and could be used to screen for the gene. Upon running any of these strains through NCBI's BLAST search, no uncultured bacterial sequences are found, and the only hits are from close relatives of the reference strain.

The identification of INA in *Lysinibacillus* and the identification of the *Lysinibacillus* ice+ (LINA) molecule as a nanometer-sized, heat stable, proteinase and lysozyme resistant, secreted molecule raises the possibility that the LINA molecule may be shed from the cells and may remain active in the cloud layer. If this mechanism occurs, one bacterium could produce many ice nuclei per bacterial cell directly refuting the fourth assumption. As of yet however, we do not know the extent to which this molecule is produced and secreted by the bacteria in the atmosphere, or how abundant it is in the environment.

Assumption two, however, remains the most ambiguous of the four presented assumptions. Not only do we not know the specific environmental conditions required for the production of the protein used by the gammaproteobacteria, we also do not understand the pathway responsible for the LINA molecule and its regulation. We do know that limited nutrient deprivation and cold temperatures can increase the production of the ice nucleating protein in *P. syringae* (Nemecek-Marshall *et al.* 1993) but, for *Lysinibacillus*, growth at relatively warm temperatures increased INA. Therefore, it is very challenging to recreate conditions in the lab that induce ice nucleation in bacteria. In fact, only a fraction of the bacteria isolated from precipitation and initially identified as ice+ were confirmed to be ice+ in repeated tests (52% for the initial collection and 28% of scavenged ice nucleators). While some of the bacteria that tested ice+ initially may have been false positives, others may be *de facto* ice+ bacteria that were simply not ice+ under the conditions we used for testing. Therefore, in order to determine the exact extent that bacteria induce precipitation within the clouds, we must better understand the specific environmental conditions necessary to induce ice nucleation and recreate them in a laboratory setting.

With the identification of the ice+ *Lysinibacillus* we wanted to better understand the diversity of ice nucleation within the genus. We were able to determine that only a single species,

L. parviboronicapiens, exhibits ice nucleation activity. Currently, however, we do not know the primary function of INA for this species. Unlike many of the gammaproteobacteria, which are thought to use the activity as a means to better invade host plants and cause disease, *Lysinibacillus* shows no phytopathogenicity. This species may use INA solely as a bioprecipitation mechanism to return to terrestrial surfaces after becoming suspended in the atmosphere, or it could use it as a secondary method of cold-tolerance in case it cannot form a spore due to other environmental conditions. Of course, it is also possible that the INA of the molecule is a secondary characteristic that is independent of the primary function of the molecule.

L. parviboronicapiens contains approximately 240 genes unique to the species. Among these genes is a type one polykeytide synthase/non-ribosomal peptide synthase (T1PKS/NRPS) gene cluster which may play a role in the species' INA based on the results of our mutagenesis screen. The product of the T1PKS/NRPS may be the causative agent for this activity or may be involved in the anchoring and arrangement of another ice+ molecule in a way that allows for effective ice nucleation around the cell. Preliminary work has been done to identify the LINA molecule and a preliminary structure has been generated based on the protein sequence of the predicted T1PKS/NRPS. Despite this, however, no other chemical analysis has been successful in further resolving the molecule's structure, in part due to a lack of UV reactivity. As such, further work must be undertaken to identify the molecule via means other than high performance liquid chromatography and liquid chromatography–mass spectrometry. This includes further collaboration with Hinrich Grothe (Institute of Materials Chemistry, Vienna University of Technology) who has performed some preliminary infrared spectroscopy on the semi-pure molecule preparation based on his previous experience with pollen INA. One of the main limitations we are currently facing is the inability to produce large quantities of the molecule.

Under our current estimates, we are able to produce mere nanograms of LINA molecule in a semi-pure preparation. In order to run nuclear magnetic resonance spectroscopy to find the specific structure, we would need to be able to produce milligrams of reasonably high purity molecule. Fortunately, we have had some recent success with growing the samples in liquid Nutrient Yeast Dextrose media, which should help in preparing larger quantities. Based on some of the suggestions by Brian Bachman (Vanderbilt University, Nashville, Tennesse), who provided the preliminary structure of the LINA molecule, we may be able to perform an extraction using chloroform or other, non-water soluble solution in order to further purify our molecule based on the long lipid tail.

Despite the slightly lower temperature that *Lysinibacillus* can nucleate ice at, compared to *P. syringae* (-6 versus -1 to -3°C), our hope is to utilize the LINA molecule for artificial snow production, or possibly, cloud seeding at some point in the future. In order to accomplish this however, we must maximize the efficiency of LINA molecule production, as well as verify the safety of the molecule. Since many of the known polyketides are toxic to one or more organisms (Bender *et al.* 1999, Cortes *et al.* 1990, Yu *et al.* 2007), we must verify that the LINA molecule is safe to expose not only to humans, but also to the environment. Since the only pathogenic species of *Lysinibacillus* are insect or nematode pathogens (Kellen *et al.* 1965, Yang *et al.* 2012) and do not have any INA (chapters three and four), it is possible that the LINA molecule has no pathogenic properties and provides some other survival advantage (survival at cold temperatures, perhaps) for the bacteria.

The identification of the *Lysinibacillus* INA presents a unique issue for one of the current methods of identifying the biogenic fraction of ice nuclei within environmental samples. Under the current model, ice nuclei resistant to boiling and lysozyme treatments are considered non-

biogenic (Christner *et al.* 2008). However, since the LINA molecule shows resistance to both boiling and lysozyme, LINA would be excluded from the list of biogenic ice nuclei. This would further apply to the birch pollen which shares may of the characteristics of the LINA molecule (O'Sullivan *et al.* 2015, Pummer *et al.* 2012). To counter this, simple tests would need to be devised that could effectively identify proteinaceous, polysaccharide, and polyketide based ice nuclei among other non-biogenic ice nuclei. Ultimately, identifying more stringent tests to identify biogenic versus non-biogenic ice nuclei will aid in the identification of more potential ice nuclei across a wider variety of species and classes of molecules.

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Appendix One: Supplemental material for Chapter Two: Ice nucleation active bacteria in precipitation are genetically diverse and nucleate ice employing different mechanisms

Supplemental Methods

Sampling of precipitation and sample processing

Field studies were conducted at different sites from February 2013 to April 2014 in Virginia, USA (Table 1). A series of cans, 52 cm wide and 1 m tall, and placed at least 1m from each other, were either installed on a flat roof of the 5-story Latham Hall building on the Virginia Tech campus or on gravel pads surrounded by mowed grassy areas to avoid contamination from natural debris (See Supplementary Figure S1 for precise locations and an image of one of the collection sites). Precipitation was collected in sterile autoclave bags (polypropylene biohazard autoclave bag [01 830 E], Fisherbrand) placed in each container. Lids were cleaned with 70% ethanol and placed on the containers until the start of each precipitation event and after the end of each event. Bags were retrieved from the containers while wearing latex gloves that had been sprayed with 70% ethanol, ensuring that water was only collected from the inside of the bottom of the bags. The corner of the bag was cut with scissors that had been sprayed with 70% ethanol, and the water was then transferred to 4L sterile bottles. Bottles were transported on ice to the lab and stored at 4°C until processing. Aliquots of the unprocessed samples were used to measure the electrical conductivity (EC, ± 0.1 µS.cm), total dissolved solids (TDS, ± 0.1 ppm), salinity (± 0.1 ppm) and pH (± 0.01 pH) with a multi-parameter unit (PCSTestr 35, Eutech Instruments/Oakton). The number (K) of total ice nucleation active particles at temperatures warmer than −8°C was determined immediately after sample processing by a droplet-freezing assay (Vali 1971) as described below. For each

rain event, an unexposed bag inside an unopened can was rinsed with 1L of sterile water, which served as negative control.

Characterization of trajectories and climatic conditions of air masses

The climatic conditions experienced by air masses along their trajectories during the 24h period preceding the precipitation event were determined with the NOAA HYSPLIT model version 4 (Draxler and Hess 1998) and the GDAS meteorological database as described in Monteil *et al.* (2014). Three elevations within the planetary boundary layer were considered as air mass altitude at the endpoint location (50m, 500m and 1000m above ground level). Hourly values for the following variables were obtained; altitude of the air mass (m), pressure (hPa), potential and ambient temperature (°C), mixed layer depth (m), relative humidity (%), terrain height (m), downward solar radiation fluxes ($W.m^{-2}$) and the total distance traveled by air masses (km).

Quantification of culturable bacteria

Precipitation samples were stored on ice after collection and were usually processed within 6 hours as described in Morris *et al.* (2008) unless hazardous weather conditions delayed sample retrieval for up to 24 hours (VTP03, 04, 12, 19, 21, and 28). Each sample was concentrated 100 times with a vacuum filtration system and a nitrocellulose membrane (0.22µm porosity, GWSP, Millipore) before dilution plating. Seven different types of culture media were used to enumerate total culturable bacteria and to maximize genetic diversity: Columbia Agar and Tryptic Soy Agar 100% and 10%, Eosin Methylene Blue Agar, Marine Agar 2216, Reasoner's 2A Agar (see Supplementary Table S1 for details). Plates were

incubated at 20°C for 3 to 5 days, and then individual colonies were counted and analyzed for INA (see below). The method allowed a minimal detection threshold of 10 colony-forming units (CFU) per liter for each medium. Water from negative controls was processed like the samples and plated on Tryptic Soy Agar (TSA) only. On average, 36 ± 2 CFU were cultured from negative control samples (corresponding to 0.05% of CFU cultured on average from rain).

Characterization of bacterial INA

Individual colonies from the plated samples were tested for their ability to freeze sterile water at −8°C with a droplet-freezing assay (Vali 1971). For each medium, a minimal threshold of detection of 0.25% of culturable Ice^+ bacteria was obtained. Well-isolated individual colonies were randomly picked with a sterile tip and deposited in a 96-well plate containing 140µL of sterile water. On average, 200 colonies were tested per medium and sample giving a total of over 33,000 colonies tested in this study. Plates were then incubated at 4°C for at least one hour and three 30µL droplets of each suspension were deposited onto a large parafilm plate floating on a −8°C glycerol bath in a cooling thermostat (Lauda Alpha RA24, LAUDA-Brinkmann, USA). After 10 minutes, the number of frozen drops per suspension was recorded for each colony and for positive and negative controls: *P. syringae* strain CC94 (Morris *et al.* 2000) and sterile distilled water, respectively. Any isolate with minimal activity (*i.e.*, one frozen drop) was considered as a potential ice nucleator, and was purified onto fresh medium. Then, strains were stored at −72°C in 25% glycerol for further characterization.

A second step of semi-quantitative INA characterization was performed with standardized suspensions for all strains that had shown at least minimal activity in the first assay. This test consisted in testing 6 drops of 30µL suspensions made of 48h old pure cultures incubated at 20°C and adjusted to an optical density (OD) at 600nm of 0.2. Calibration curves performed with *Bacillus sp.*, *Pantoea sp.*, *Pseudomonas sp.*, *Sphingomonas sp.*, *Xanthomonas sp.*, *Pedobacter sp.*, *Erwinia sp.* and *Rhodococcus sp.* strains showed that an OD_{600} of 0.2 corresponded to a mean of $1.31 \times 10^8 \pm 2.12 \times 10^7$ CFU mL-1. Four temperatures were tested; −4°C, −8°C, −10°C and −12°C. After 10 minutes of incubation at each temperature, the number of frozen drops was recorded and strains were considered as Ice^+ at a given temperature if at least half of the drops froze at that temperature.

Quantification of total IN in precipitation water

The total number of IN active at −8°C present in precipitation was measured for each sample with the same droplet-freezing assay (Vali 1971) described above. Thirty droplets of 20µL of the concentrated sample and thirty droplets of four series of 10−fold dilutions were tested. The cumulative nucleus concentration K at −8°C was determined using the equation $K(\theta) = [\ln N_0 - \ln N(\theta)]/V$, where N_0 is the number of droplets tested, $N(\theta)$ is the number of unfrozen droplets at temperature θ, and V is the droplet volume.

Cumulative nucleus spectra of Lysinibacillus sp. strains

The cumulative IN concentrations per cell of *Lysinibacillus* sp. were determined at every temperature degree from −2°C to −12°C as for precipitation samples. Cumulative IN concentrations were then compared to *P. syringae* CC94 after both species were grown under the

same conditions. For each strain, 30 droplets of 20µL of initial suspensions of 10^8 cells per mL and six 10−fold serial dilutions were tested for four different glycerol stocks for each strain. The average number of cells per drop (*A*) was determined by dilution-plating. The cumulative IN concentrations per cell was determined using the equation given above replacing the component *V* of the equation with *A*. Several treatments and culture conditions were tested to decipher the nature of the ice nucleation active molecule produced by the *Lysinibacillus* sp. strains (LINA). INA tests were performed using cultures grown for 24h at 28°C for all treatments. Treatments consisted of: (i) boiling suspensions for 1 hour at 99°C, (ii) filtering suspensions through a nitrocellulose membrane (0.22µm porosity, Millipore), (iii) filtering the 0.22µm filtrate through a 100kDa membrane and then testing the washed filter (defined here as the retentate) or the filtrate, and (iv) incubating suspensions for 60 minutes either with 87.4µM of lysozyme (Sigma-Aldrich) at 37°C or with 4.5mM proteinase K enzyme (Roche Diagnostics) at 65°C.

Identification of Ice+ bacteria

All strains for which the initial colony showed at least minimal activity were genotyped to determine their affiliation to a taxonomic group. After heating bacterial suspensions at 99°C for ten minutes, we amplified a fragment of the *cts* gene with primers specific to the *Pseudomonas* genus (Sarkar and Guttman 2004): cts-Fp (AGTTGATCATCGAGGGCGCWGCC) and cts-Rp (TGATCGGTTTGATCTCGCACGG). Strains for which the gene was amplified were considered to belong to the genus *Pseudomonas*. For the 498 strains for which no amplification product was obtained, the 16S rDNA gene was amplified using universal primers 1492R (ATCGGYTACCTTGTTACGACTTC) and the reverse complement of 518R (CCAGCAGCCGCGGTAATACG) as described in Lane (1991) and Muyzer *et al.* (1993). All

amplifications were performed using 2x ImmoMix (Bioline BIO-2520), 58°C annealing temperature, and a primer concentration of 10µM. PCR products were cleaned for sequencing using rShrimp Alkaline Phosphatase (Affymetrix 78390) and Exonuclease I (USB 70073X). Products were sequenced at the Biocomplexity Institute of Virginia Tech using Sanger technology. Sequences were trimmed and blasted against the non-redundant nucleotide database in GenBank. A strain was assigned to a species when sequence similarity with a type strain was above 99% over a minimum sequence alignment of 99%. Below these thresholds, strains were identified at either the genus or family level (Supplementary Tables S2 and S3). Phylogenetic trees were built with sequences of strains and of the most similar sequences based on the BLAST results. Our dataset was finally enriched with 902 sequences of type strain sequences downloaded from the public database of the Ribosomal Database Project (RDP) (Cole *et al.* 2014). Sequences were aligned using MUSCLE (Edgar 2004) and phylogenetic trees were built using MEGA6 software (Tamura *et al.* 2013). Trees were built with the maximum likelihood algorithm using the GTR model with uniform rates as nucleotide substitution model and 100 bootstrap replicates were ran to test robustness of tree topologies. All 16S rDNA sequences were deposited in GenBank: KY073884-KY074547.

Statistical analyses

All statistical analyses were performed with R version 3.1.2 (R Core Team 2014). Bacterial concentrations were log transformed to obtain a normal distribution before statistical analyses. Average concentrations of total culturable bacteria and concentrations and frequencies of Ice^+ bacteria were compared pairwise between media types either by the Welch's t-test or by the non-parametric Mann Whitney U-test, if assumptions for the Welch's

test were not met (Table 2). Averages were considered significantly different if *P* values were under 0.05. Correlations between population sizes of total culturable bacteria, concentrations and frequencies of Ice$^+$ bacteria, concentrations of total IN, and all HYSPLIT data (see above) and water chemistry (Table S4) were tested by building a linear model and analyzed using Pearson's method or Spearman's method depending on population distribution. Correlations were considered significantly different if *P* values were below 0.05. A positive value for coefficients "r" or "rho" indicates that both variables decrease or increase together, while a negative value indicates that both variables tend to decrease when the other increases.

Finally, to determine if data collected 14km away from each other reflect a single precipitation event, we compared averages calculated from four events as described previously.

Supplemental Results

Climatic data and precipitation chemistry associated with the deposition of Ice$^+$ bacteria and IN. For total ice nuclei (IN), the most significant associations were observed for backward trajectories computed at 1000m (**Table 3**) while for biological variables associations were found for backward trajectories computed at 50m. Analyses of backward trajectories identified air mass features associated with higher concentrations of total IN active at warm temperatures in precipitation (**Table 3**): total IN were more likely to be more numerous in precipitation whose air masses experienced lower air pressures over longer distances and at high altitude during the preceding 24h. Surprisingly, air masses with lower total IN concentrations were also significantly associated with air masses that experienced higher relative humidity and had been exposed to higher downward radiative fluxes.

These results contrasted with those obtained for frequencies and concentrations of Ice^+ bacteria for which relationships with other climatic variables were observed considering air masses with an initial altitude of 50m elevation at 24h. Indeed, frequencies of culturable Ice^+ bacteria obtained for most of the media had a positive relationship with air pressure and a negative relationship with terrain height (see examples in **Table 4**). Moreover, for Ice^+ bacteria grown on CA, high frequencies seemed to be also associated to higher depths of the mixed layer of the atmosphere, which corresponds to the zone of turbulences near the ground. And air masses having experienced higher air temperatures, or received more sun fluxes carried more numerous Ice^+ bacteria grown on R2A or TSA (**Table S2**).

Finally, we found also significant positive linear relationships between pH, conductivity, and concentration in total dissolved solids of water and concentrations of total culturable bacteria and total ice nuclei (Pearson's and Spearman's correlation tests, coefficients of 0.62 and 0.79, $P < 0.05$). Therefore, their deposition would rather be associated with more alkaline precipitation carrying minerals and matter. Relationships with water conductivity or concentration in total dissolved solids were converse for several media considering only frequencies and concentrations of culturable Ice^+ bacteria (**Table S5)**. Therefore, data gave us clues about the specific conditions benefiting the deposition of total IN and culturable Ice^+ bacteria selected by each medium.

To determine how similar data collected at two different sites within the same precipitation event are, we sampled four events at two locations 14km away from each other. We did not find any significant differences for any chemical or biological variable determined here: total IN numbers, pH, conductivity or bacterial concentrations remained the same between sites (t-tests and Mann Whitney U tests, $P < 0.05$). Therefore, these data support the conclusion that our observations can be generalized to an entire precipitation event over a range of several kilometres.

Table 3. Correlations between IN concentrations with air mass properties for an elevation of 1000m over the 24h preceding the event and with precipitation water chemistry.

Climatic or chemical variables		Number of total Ice Nuclei (Nb Particles/mL)	
		r	*rho*
Correlations tested with precipitation water chemistry			
pH		0.63 *	0.51 .
Conductivity (µS.cm)		0.58 *	0.79 **
Total Disolved Solids (ppm)		0.53 .	0.79 **
Salinity(ppm)		0.55 .	0.56 .
Correlations tested with backward trajectories computed at 1000 m			
Altitude (m, agl)	Min	0.37	0.22
	Max	0.58 *	0.55 **
	Mean	0.39	0.24
Pressure (hPa)	Min	-0.67 **	-0.73 ***
	Max	-0.44 .	-0.22
	Mean	-0.43 .	-0.31
Potential temperature (°C)	Min	-0.11	-0.05
	Max	-0.29	-0.17
	Mean	-0.15	-0.07
Ambient temperature (°C)	Min	-0.38	-0.26
	Max	-0.34	-0.06
	Mean	-0.33	-0.18
Sum_Rainfall (mm/hour)		-0.19	0.16
Mixed Layer Depth (m)	Min	0.42 .	0.43 .
	Max	-0.37	-0.23
	Mean	0.03	-0.13
Relative humidity (%)	Min	-0.56 *	0.04
	Max	-0.75 ***	-0.10
	Mean	-0.66 **	0.08
Terrain Height (m, agl)	Min	-0.05	0.01
	Max	0.00	0.21
	Mean	-0.04	0.03
Sum of downward radiative flux ($W.m^{-2}$)	Sum	-0.54 *	-0.47 .
	Max	-0.45 .	-0.28
	Mean	-0.54 *	-0.47 .
Distance traveled (km)		0.93 ***	0.50 *

r and *rho* represent the coefficients of the Pearson's and Spearman's correlation tests. The significance of the correlations are given by symbols (.P < 0.10 *P < 0.05, **P < 0.01, ***P < 0.001, n=23).

[a] Ice nucleation activity of bacteria has been confirmed with purified cultures incubated 48h at 20°C and tested at -8°C.

Table 4. Correlations between IN concentrations with air mass properties for an elevation of 50m over the 24h preceding the event, and with precipitation water chemistry. Only results for four media types are displayed.

Climatic or chemical variables		Frequency of culturable Ice⁺ bacteria[a] (%)							
		CA		EMB		R2A		TSA	
		r	*rho*	*r*	*rho*	*r*	*rho*	*r*	*rho*
Correlations tested with precipitation water chemistry									
pH		-0.26	0.01	-0.27	-0.26	0.06	-0.11	0.08	-0.02
Conductivity (µS.cm)		-0.32	-0.55 *	-0.47 .	-0.60 *	-0.35	-0.46 .	-0.41	-0.55 *
Total Disolved Solids (ppm)		-0.39	-0.29	-0.48 .	-0.53 *	-0.33	-0.43	-0.35	-0.41
Salinity (ppm)		-0.31	-0.36	-0.43	-0.33	-0.25	-0.05	-0.35	-0.30
Correlations tested with backward trajectories computed at 50 m									
Altitude (m, agl)	Min	-0.04	0.21	-0.07	0.07	0.20	0.20	0.01	0.08
	Max	-0.01	-0.21	-0.10	-0.40 .	-0.01	-0.18	-0.09	-0.24
	Mean	-0.02	-0.34	-0.10	-0.41 .	0.04	-0.15	-0.08	-0.32
Pressure (hPa)	Min	0.19	0.42 *	0.41 .	0.53 **	0.37 .	0.37 .	0.46 *	0.48 *
	Max	0.52 **	0.64 ***	0.55 **	0.58 **	0.46 *	0.38 .	0.55 **	0.51 **
	Mean	0.52 **	0.62 **	0.56 **	0.60 **	0.46 *	0.38 .	0.59 **	0.55 **
Potential temperature (°C)	Min	0.02	0.13	0.01	0.24	0.07	0.27	-0.05	0.17
	Max	0.01	0.10	0.01	0.24	0.09	0.26	0.02	0.23
	Mean	0.03	0.18	0.02	0.26	0.08	0.27	-0.02	0.24
Ambient temperature (°C)	Min	0.15	0.29	0.14	0.33 .	0.17	0.32	0.08	0.30
	Max	0.10	0.28	0.11	0.35 .	0.18	0.35	0.11	0.38 .
	Mean	0.12	0.23	0.11	0.28	0.16	0.29	0.08	0.29
Sum_Rainfall (mm/hour)		-0.03	-0.10	-0.09	-0.12	-0.18	-0.07	-0.20	-0.24
Mixed Layer Depth (m)	Min	0.55 **	0.20	0.34	-0.02	0.23	-0.03	0.23	0.05
	Max	-0.01	0.02	-0.10	0.06	-0.03	0.18	-0.04	0.13
	Mean	0.35	0.13	0.13	0.12	0.08	0.22	0.08	0.20
Relative humidity (%)	Min	0.16	0.02	0.19	0.08	0.09	0.11	0.01	-0.14
	Max	0.26	0.14	0.29	0.11	0.25	0.19	0.18	0.07
	Mean	0.23	0.07	0.22	0.01	0.13	0.07	0.05	-0.13
Terrain Height (m, agl)	Min	-0.54 **	-0.64 **	-0.51 *	-0.55 **	-0.42 *	-0.40 .	-0.49 *	-0.52 **
	Max	-0.24	-0.57 **	-0.36	-0.57 **	-0.33	-0.39 .	-0.39 .	-0.53 **
	Mean	-0.53 **	-0.63 **	-0.50 *	-0.56 **	-0.41 *	-0.35	-0.51 **	-0.49 *
Sum of radiative sun flux ($W.m^{-2}$)	Sum	0.03	0.09	0.00	0.14	0.11	0.21	0.06	0.23
	Max	0.14	0.12	0.08	0.14	0.16	0.22	0.11	0.25
	Mean	0.03	0.09	0.00	0.14	0.11	0.21	0.06	0.23
Distance traveled (km)		0.20	-0.23	0.06	-0.31	-0.06	-0.17	-0.04	-0.21

CA,EMB, R2A and TSA are the abbrebiations for Columbia Agar, Ethylen-Methyl-Blue Agar, Reasoner's 2A Agar, and Tryptic Soy Agar, respectively. Since no correlation was significantly significant for Columbia Agar diluted 10 times, results have not been displayed for this medium.

r and *rho* represent the coefficients of the Pearson's and Spearman's correlation tests. The significance of the correlations are given by symbols (.P < 0.10 *P < 0.05, **P < 0.01, ***P < 0.001).

[a] Ice nucleation activity of bacteria has been confirmed with purified cultures incubated 48h at 20°C and tested at -8°C.

Supplemental Tables

Supplementary Table S1. Media used in this study to enumerate total cultivable bacteria.

Medium	Whole name	Particularity	General Use	Source
CA	Columbia Agar	Rich in peptones from animal proteins	Medical microbiology	Difco
CA 10%	Columbia Agar 10%	Columbia Broth base diluted 10 folds with 15 g of Agar	/	/
EMB	Ethyl-Methyl-Blue Agar	Rich in Gelatine and lactose with eosin and methylene blue, inhibiting Gram positive bacteria	Enteric bacilli	Sigma
MA	Marine Agar 2216	High Salt and minerals content	Marine microbiology	Difco
R2A	Reasoner's 2A Agar	Oligotrophic medium	Water samples & atmospheric microbiology	Teknova
TSA	Tryptic Soy Agar	Rich in peptones from casein and soybean	General purposes	Difco
TSA 10%	Tryptic Soy Agar 10%	Tryptic Soy Broth diluted 10 folds with 15g of Agar	/	/

Supplementary Table S2. Number and list of strains isolated per precipitation sample in Virginia, USA, that had at least minimal INA during the initial testing.

Sample ID[a]	Media used for isolating strains[b]							
	CA		CA 10%		EMB		MA	
	Number of strains	strains ID	Number of strains	strains ID	Number of strains	strains ID	Number of strains	strains ID
VTP01	1	VT0005	0	/	0	/	3	VT0006 to VT0008
VTP02	11	VTP0021 to VTP0026 and VT0051 to VT0054	0	/	6	VT0034 to VT0039	10	VT0027 to VT0033, VT0049, VT0050, and VT0057
VTP03	2	VT0069 and VT0070	0	/	1	VT0075	2	VT0067 and VT0068
VTP05	31	VT0117 to VT0123, VT0188 to VT0206, and VT0219 to VT0222	10	VT0244 to VT0249 and VT0270 to VT0273	42	VT0083 to VT0086, VT0088 to VT0090, VT0124 to VT0156 and VT0287	34	VT0223 to VT0243 and VT0276 to VT0286
VTP06	44	VT0288 to VT0301 and VT0523 to VT0552	24	VT0341 to VT0349 and VT0451 to VT0465	40	VT0302 to VT0311, VT0323, VT0324, and VT0389 to VT0416	29	VT0325 to VT0340 and VT0417 to VT0429
VTP07	0	/	0	/	2	VT0553 and VT0554	0	/
VTP08	4	VT0575, VT0576, VT0585, and VT0586	4	VT0567, VT0568, VT0583, and VT0584	12	VT0560 to VT0664, VT0572 to VT0574, and VT0587 to VT0590	8	VT0569 to VT0571, and VT0593 to VT0597
VTP09	0	/	2	VT0600 and VT0602	1	VT0601	0	/
VTP10	6	VT0613, VT0614, and VT0628 to VT0631	1	VT0617	11	VT0605 to VT0608 and VT0620 to VT0626	9	VT0632 to VT0640
VTP11	4	VT0651 to VT0653 and VT0662	2	VT0654 and VT0668	7	VT0655 to VT0659, VT0663, and VT0669	1	VT0670
VTP12	12	VT0676 to VT0680, VT0768 to VT0770, and VT0789 to VT0792	23	VT0681 to VT0683, VT704 to VT0719, and VT743 to VT0746	14	VT0720 to VT0733	8	VT0700 to VT0703 and VT0747 to VT0750
VTP13	2	VT0835, VT0836	2	VT0819, VT0839	5	VT0830 to VT0834	0	/
VTP14a	0	/	3	VT0846 to VT0848	11	VT0851 to VT0861	3	VT0844, VT0845, and VT0862
VTP14b	2	VT0841, VT0877	1	VT0840	2	VT0849 and VT0850	0	/
VTP17	8	VT1012 to VT1019	0	/	37	VT0954 to VT0990	33	VT0905 to VT0937
VTP19	2	VT1031 and VT1032	4	VT1020 to VT1022 and VT1033	6	VT1023 to VT1025 and VT1035 to VT1037	2	VT1026 and VT1034
VTP21a	2	VT1040 and VT1041	1	VT1042	0	/	2	VT1043, 1044
VTP21b	0	/	3	VT1052 to VT1054	1	VT1055	0	/
VTP24	0	/	0	/	0	/	1	VT1062
VTP26a	2	VT1067 and VT1068	0	/	1	VT1063	0	/
VTP26b	0	/	3	VT1069 to VT1071	3	VT1072 to VT1074	3	VT1075 to VT1077
VTP28a	5	VT1089 to VT1093	3	VT1104 to VT1106	12	VT1134 to VT1145	10	VT1094 to VT1103
VTP28b	10	VT1163 to VT1172	8	VT1173 to VT1180	17	VT1146 to VT1162	21	VT1208 to VT1228

[a] Samples characteristics are given in Table 1

[b] CA, CA10%, EMB, MA, R2A, TSA and TSA10% are the abbrebiations for Columbia Agar, Columbia Agar diluted 10 times, Eosin-Methyl-Blue Agar, Marine Agar, Reasoner's 2A Agar, Tryptic Soy Agar, and Tryptic Soy Agar diluted 10 times, respectively

R2A		TSA		TSA 10%	
Number of strains	strains ID	Number of strains	strains ID	Number of	strains ID
4	VT0001 to VT0004	0	/	0	/
18	VT0011, VT0040 to VT0048, VT0059 to VT0064, and VT0066	12	VT0012 to VT0016, VT0018 to VT0020, VT0055, VT0056, VT0058, and VT0065	0	/
0	/	1	VT0078	3	VT0079 to VT0081
17	VT0091 to VT0102b	26	VT0103 to VT0116 and VT0207 to VT0218	19	VT0250 to VT0258b, VT0260, VT0261, and VT0266 to VT0269
17	VT0466 to VT0482	32	VT0312 to VT0322,VT0483 to VT0497, VT0517 to VT0522	39	VT0350 to VT0388
2	VT0555 and VT0557	1	VT0556	0	/
4	VT0565, and VT0578 to VT0580	0	/	8	VT0558, VT0559, VT0566, VT0577, VT0581, VT0582, VT0591, and VT0592
1	VT0598	1	VT0599	0	/
4	VT0603, VT0604, VT0615, and VT0619	5	VT0609 to VT0612, and VT0618	3	VT0616, VT0627, and VT0641
4	VT0664, VT0665, VT0671, and VT0672	4	VT0650 and VT0673 to VT0675	4	VT0660, VT0661, VT0666, and VT0667
33	VT0697 to VT0699, VT734 to VT0742, VT765 to VT0767 and VT0771 to VT0788	8	VT0811 to VT0818	45	VT0684 to VT0696, VT751 to VT0764, and VT0793 to VT0810
5	VT0825 to VT0829	5	VT0822 to VT0824, VT0837, and VT0838	2	VT0820 and VT0821
0	/	0	/	1	VT0842
0	/	2	VT0875 and VT0876	13	VT0843 and VT0863 to VT0874
21	VT0991 to VT1011	26	VT0879 to VT0904	16	VT0938 to VT0953
3	VT1027 to VT1029	1	VT1030	4	VT1038 to VT1039c
2	VT1045, 1046	2	VT1047, 1048	3	VT1049 to VT1051
0	/	1	VT1056	1	VT1057
0	/	0	/	2	VT1060 VT1061
1	VT1064	0	/	2	VT1065 and VT1066
3	VT1078 to VT1079b	3	VT1080 to VT1082	0	/
14	VT1107 to VT1120	6	VT1083 to VT1088	13	VT1121 to VT1133
11	VT1181 to VT1191	18	VT1229 to VT1246	16	VT1192 to VT1207

Supplementary Table S3. List of 16S rDNA sequences of VT strains that had at least minimal INA at -8°C during the initial test.

	Strain sequence							Description BLAST Hit #1											Description BLAST Hit #2		Description BLAST Hit #3	
VT name[a]	Sample	BAV #	INA (°C)[b]	Tree[c]	Trimmed 16S sequence	Query length	Class	Family	Genus	Description	Accession number	Total score	Query Coverage %	Identities	Number of gaps	Alignment length	Frequency number of identities (%)	Frequency number of gaps (%)	Description	Accession number	Description	Accession number
VT0001	VTP001	2469	-	Y	GCGCGCGC	926	Bacilli	Bacillaceae	Bacillus	Bacillus pseu	KJ767330.1	1703	100	925	1	926	99.9	0.1	Bacillus mycc	KJ721201.1	Bacillus sp. A	JF825987.1
VT0002a	VTP001	2470	-	-	GGGAACTTC	494	Bacilli	Paenibacillaceae	Paenibacillus	Paenibacillus	JF683659.1	913	100	494	0	494	100.0	0.0	-	-	-	-
VT0003	VTP001	2471	-	Y	GGAATTCCA	814	Bacilli	Bacillaceae	Bacillus	Bacillus sp. A	KC236744.1	1469	100	814	0	814	100.0	0.0	Bacillus mega	KT580611.1	-	-
VT0004	VTP001	2472	-	Y	GCGCGCGC	927	Bacilli	Bacillaceae	Bacillus	Bacillus sp. B	KR998244.1	1712	100	927	0	927	100.0	0.0	Bacillus ocea	KJ575007.1	Bacillus firm	KF228909.1
VT0005	VTP001	2487	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM913961.1	1711	100	926	0	926	100.0	0.0	Pseudomonas	AM933521.1	Pseudomonas	AM421016.2
VT0006	VTP001	2497	-	Y	GGAATTCCA	830	Bacilli	Bacillaceae	Bacillus	Bacillus simp	KJ567108.2	1533	100	830	0	830	100.0	0.0	Brevibacteriu	KT003248.1	-	-
VT0007	VTP001	2498	-	-	GAGCTCGT/	910	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	CP012299.1	1681	100	910	0	910	100.0	0.0	Microbacteri	KR085857.1	-	-
VT0008	VTP001	2499	-	Y	GGAATTCCA	928	Bacilli	Bacillaceae	Bacillus	Bacillus safer	KR085891.1	1517	100	826	2	828	99.8	0.2	Bacillus pumi	KP975931.1	-	-
VT0011	VTP002	2527	-	-	GCGCACGT/	929	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	CP009571.1	5079	100	926	4	931	99.5	0.4	-	-	-	-
VT0012	VTP002	2479	-	Y	GGGCTCGC/	770	Bacilli	Bacillaceae	Bacillus	Bacillus pumi	JX049349.1	1377	100	768	1	770	99.7	0.1	Bacillus safer	KR140378.1	-	-
VT0013	VTP002	2500	-	-	AATGGAATT	632	Actinobacteria	Microbacteriaceae	Leifsonia	Leifsonia soli	KM019849.1	1168	100	632	0	632	100.0	0.0	-	-	-	-
VT0014	VTP002	2501	-	-	TTCCGAGTG	722	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	KM187518.1	1303	100	722	0	722	100.0	0.0	-	-	-	-
VT0015	VTP002	2484	-	Y	GGGCTCGC/	926	Bacilli	Bacillaceae	Bacillus	Bacillus safer	KR085891.1	1705	100	925	1	926	99.9	0.1	Bacillus pumi	KP975931.1	Bacillus sp. E	KP702915.1
VT0016	VTP002	2480	-	Y	GGGCTCGC/	910	Bacilli	Bacillaceae	Bacillus	Bacillus sp. K	KR149606.1	1642	100	910	0	910	100.0	0.0	-	-	-	-
VT0018	VTP002	2481	-	Y	GGGCTCGC/	926	Bacilli	Bacillaceae	Bacillus	Bacillus sp. A	FR821149.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
* VT0019b	VTP002	4579	-	Y	GCGCGCGT/	927	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC901542.1	1660	100	925	1	927	99.8	0.1	-	-	-	-
VT0020	VTP002	2482	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1362	100	925	0	926	99.9	0.0	Pseudomonas	HF558390.1	-	-
VT0021	VTP002	2477	-8	Y	GCGCGCGT/	912	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR476390.1	1645	100	912	0	912	100.0	0.0	Pseudomonas	KR476390.1	-	-
VT0022a	VTP002	2534	-	-	TGGAGGGT/	751	Bacilli	Staphylococcaceae	Staphylococcus	Staphylococc	KR059856.1	1387	100	751	0	751	100.0	0.0	-	-	-	-
VT0023	VTP002	2478	-	Y	GGGCTCGC/	896	Bacilli	Bacillaceae	Bacillus	Bacillus aeri	KR140379.1	1611	100	895	0	896	99.9	0.0	Bacillus safer	KT380675.1	Bacillus aero	KR085933.1
VT0024	VTP002	2503	-	Y	GCGCGCGC	927	Bacilli	Bacillaceae	Lysinibacillus	Lysinibacillus	FN397524.1	1673	100	927	0	927	100.0	0.0	-	-	-	-
VT0025	VTP002	2488	-	-	GAGTTCGT/	882	Actinobacteria	Nocardioidaceae	Rhodococcus	Rhodococcus	KR085921.1	1629	100	882	0	882	100.0	0.0	Rhodococcus	LC020106.1	Rhodococcus	KR085866.1
VT0026	VTP002	2483	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074598.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT0027	VTP002	2504	-	-	GCGCACGC/	878	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteri	KP279978.1	1611	100	876	2	878	99.8	0.2	Pantoea aggl	DQ307454.1	-	-
VT0028	VTP002	2505	-12	Y	GGGAACTG	708	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP418810.1	1308	100	708	0	708	100.0	0.0	Pseudomonas	KP212100.1	Pseudomonas	KP762269.1
VT0029	VTP002	2528	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1665	100	926	0	926	100.0	0.0	-	-	-	-
VT0030	VTP002	2506	-	-	GAGCTCGT/	924	Actinobacteria	Microbacteriaceae	Frigoribacterium	Frigoribacteri	EU584512.1	1701	100	923	0	924	99.9	0.0	-	-	-	-
VT0031	VTP002	2507	-	Y	GGAATTCCA	828	Bacilli	Bacillaceae	Bacillus	Bacillus aeri	KR140379.1	1494	100	828	0	828	100.0	0.0	Bacillus aero	KR085933.1	Bacillus strat	KR085786.1
VT0032	VTP002	2508	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	AY574914.1	-	-
VT0033	VTP002	2509	-	Y	GCGCGCGT/	927	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067667.1	1683	100	922	1	927	99.5	0.1	-	-	-	-
VT0034	VTP002	2515	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074597.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0035	VTP002	2495	-	Y	GCGTGCGT/	859	γ-proteobacteria	Xanthomonadaceae	Pseudoxanthomonas	Pseudoxanth	LN613115.1	1517	100	854	2	860	99.3	0.2	Uncultured Xa	JX093167.1	-	-
VT0036	VTP002	2525	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074597.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0037b	VTP002	4484	-	Y	GCGCGCGT/	676	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	avellanae par	1182	100	669	1	677	98.8	0.1	-	-	-	-
VT0037d	VTP004	4522	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1663	100	557	0	557	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0038a	VTP002	2493	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC901542.1	1705	100	925	0	926	99.9	0.0	-	-	-	-
VT0039b	VTP002	4463	-8	Y	GCGCGCGT/	657	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KM187433.1	1197	100	654	0	657	99.5	0.0	Pseudomonas	KP796138.1	-	-
VT0040	VTP002	2510	-	Y	GGGCTCGC/	926	Bacilli	Bacillaceae	Bacillus	Bacillus sp. K	LN874212.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0041	VTP002	2490	-	-	AGTTAGAGC	909	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	CP009571.1	4914	100	903	3	909	99.3	0.3	-	-	-	-
VT0042	VTP002	2511	-	-	GGGTGCGC	912	Flavobacteria	Cytophagaceae	Spirosoma	Spirosoma ri	NR_113978.1	1633	100	899	0	907	99.1	0.0	-	-	-	-
VT0043	VTP002	2491	-	-	GAGCTCGT/	891	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	CP012299.1	3292	100	891	0	891	100.0	0.0	-	-	-	-
VT0044	VTP002	2512	-	-	GAGCTCGT/	776	Actinobacteria	Microbacteriaceae	Kocuria	Kocuria sedi	KF668243.1	1410	100	773	0	777	99.5	0.0	-	-	-	-
VT0045	VTP002	2538	-	-	GCGTGCGC	924	β-proteobacteria	Comamonadaceae	Acidovorax	Acidovorax sp	Y18617.1	1635	100	923	0	924	99.9	0.0	Uncultured Cc	HQ418441.1	-	-
VT0046	VTP002	2513	-	-	GCGCACGT/	927	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	JQ660208.1	1670	100	921	2	929	99.1	0.2	-	-	-	-
VT0047	VTP002	2514	-	-	GAGCTCGT/	929	Actinobacteria	Sanguibacteraceae	Sanguibacter	Sanguibacter	Y09657.1	1700	100	926	0	929	99.7	0.0	-	-	-	-
VT0048	VTP002	2489	-	-	GCGCACGT/	915	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	CP009571.1	5001	100	912	0	915	99.7	0.0	-	-	-	-
VT0049	VTP002	2529	-	-	GAGCTCGT/	929	Actinobacteria	Nocardioidaceae	Rhodococcus	Rhodococcus	LN832018.1	1667	100	927	0	929	99.8	0.0	-	-	-	-
VT0050	VTP002	2530	-	-	GAGCTCGT/	894	Actinobacteria	Microbacteriaceae	Rathayibacter	Rathayibacter	KF441602.1	1652	100	894	0	894	100.0	0.0	-	-	-	-
VT0051	VTP002	2531	-	-	GAGCTCGT/	897	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	CP012299.1	3315	100	897	0	897	100.0	0.0	-	-	-	-
VT0052	VTP002	2539	-	Y	GGGCTCGC/	926	Bacilli	Bacillaceae	Bacillus	Bacillus safer	KR085891.1	1671	100	926	0	926	100.0	0.0	Bacillus pumi	KP975931.1	Bacillus sp. N	KP307835.1
VT0053	VTP002	2540	-4	Y	GCGTGCGT/	820	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	KP704433.1	1469	100	819	1	821	99.8	0.1	Xanthomonas	AY994101.1	-	-
VT0054	VTP002	2532	-	Y	GGGCTCGC/	926	Bacilli	Bacillaceae	Bacillus	Bacillus safer	KR085891.1	1671	100	926	0	926	100.0	0.0	Bacillus pumi	KP975931.1	Bacillus sp. N	KP307835.1
VT0055	VTP002	2521	-	Y	GGGCTCGC/	926	Bacilli	Bacillaceae	Bacillus	Bacillus aeri	KR140379.1	1665	100	925	0	926	99.9	0.0	Bacillus aero	KR085933.1	Bacillus strat	KR085786.1
VT0056	VTP002	2522	-	Y	GGGCTCGC/	926	Bacilli	Bacillaceae	Bacillus	Bacillus aeri	KR140379.1	1665	100	924	0	926	99.8	0.0	Bacillus aero	KR085933.1	Bacillus strat	KR085786.1
VT0057	VTP002	2536	-	-	GAGCTCGT/	929	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	HF571532.1	1705	100	927	0	929	99.8	0.0	Microbacteri	FJ449650.1	Microbacteri	HF571530.1
VT0058	VTP002	2523	-	Y	GGAATTCCA	701	Bacilli	Bacillaceae	Bacillus	Bacillus aero	KR085933.1	1295	100	701	0	701	100.0	0.0	Bacillus strat	KR085786.1	Bacillus altit	KP790040.1
VT0059a	VTP002	2516	-	Y	GGAATTCCA	786	Bacilli	Bacillaceae	Bacillus	Bacillus aero	KR085933.1	1452	100	786	0	786	100.0	0.0	Bacillus strat	KR085786.1	Bacillus altit	KP790040.1
VT0060a	VTP002	2517	-	Y	GGAATTCCA	828	Bacilli	Bacillaceae	Bacillus	Bacillus aeri	KR140379.1	1530	100	828	0	828	100.0	0.0	Bacillus aero	KR085933.1	Bacillus strat	KR085786.1
* VT0060b	VTP002	4469	-	-	TTCCGAGTG	827	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	CP009571.1	4489	100	822	2	827	99.4	0.2	-	-	-	-
VT0061a	VTP002	2518	-	Y	AATTCCACG	829	Bacilli	Bacillaceae	Bacillus	Bacillus pumi	HQ858057.1	1531	100	829	0	829	100.0	0.0	-	-	-	-
VT0062	VTP002	2537	-	-	GAGCTCGT/	760	Actinobacteria	Microbacteriaceae	Amnibacterium	Amnibacteri	KM507591.1	1371	100	755	2	761	99.2	0.3	-	-	-	-
VT0063a	VTP002	2519	-	Y	GGAATTCCA	832	Bacilli	Bacillaceae	Bacillus	Bacillus sp. B	KF984461.1	1537	100	832	0	832	100.0	0.0	Bacillus altit	HQ843841.1	Bacillus subti	GQ360038.1
VT0064a	VTP002	2520	-	Y	GGAATTCCA	826	Bacilli	Bacillaceae	Bacillus	Bacillus aeri	KR140379.1	1494	100	826	0	826	100.0	0.0	Bacillus aero	KR085933.1	Bacillus strat	KR085786.1
VT0065	VTP002	2526	-8	Y	GCGCGCGT/	813	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC776128.1	1467	100	813	0	813	100.0	0.0	Pseudomonas	KP253043.1	Pseudomonas	KP253040.1
VT0066	VTP002	2533	-12	Y	GCGCGCGT/	859	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ569375.1	1550	100	859	0	859	100.0	0.0	-	-	-	-
VT0067	VTP003	2544	-	Y	AATTCCATG	714	Bacilli	Bacillaceae	Bacillus	Bacillus sp. S	KR088645.1	1319	100	714	0	714	100.0	0.0	Bacillus mycc	KR088567.1	-	-
VT0068	VTP003	2545	-	-	GAGCTCGT/	926	Actinobacteria	Cellulomonadaceae	Oerskovia	Oerskovia eni	AB562466.1	1694	100	924	1	927	99.7	0.1	Oerskovia tur	FM955879.1	Oerskovia pa	NR_025471.1
VT0069	VTP003	2554	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteri	HG799975.1	1709	100	927	0	928	99.9	0.0	Curtobacteri	JX458460.1	-	-
VT0070	VTP003	2546	-	Y	GGAATTCCA	819	Bacilli	Bacillaceae	Bacillus	Bacillus sp. B	KT462724.1	1519	100	819	0	819	100.0	0.0	Bacillus mega	KT580611.1	Bacillus arya	KR085881.1
VT0072	VTP003	2547	-	-	GCGCACGT/	912	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	EF540484.1	1685	100	912	0	912	100.0	0.0	-	-	-	-
VT0073	VTP003	2552	-	-	GAGCTCGT/	835	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteri	KJ184990.1	1543	100	835	0	835	100.0	0.0	-	-	-	-
VT0074	VTP003	2548	-	-	GAGCTCGT/	801	Actinobacteria	Microbacteriaceae	Plantibacter	Plantibacter	HE662660.2	1703	100	926	0	928	99.8	0.0	Plantibacter s	HE662662.2	-	-
VT0075	VTP003	2549	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0078	VTP003	2556	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteri	HG799975.1	1709	100	927	0	928	99.9	0.0	Curtobacteri	JX458460.1	-	-
VT0079	VTP003	2550	-	-	GAATTCCAC	655	Bacilli	Paenibacillaceae	Paenibacillus	Paenibacillus	KF484680.1	1210	100	655	0	655	100.0	0.0	Paenibacillus	AB695341.1	-	-
VT0080	VTP003	2551	-	-	AATTCCACG	826	Bacilli	Paenibacillaceae	Paenibacillus	Paenibacillus	DQ854978.1	1496	100	824	4	830	99.3	0.5	-	-	-	-
VT0081	VTP003	2557	-	-	GCGCACGT/	927	α-proteobacteria	Phyllobacteriaceae	Aquamicrobium	Aquamicrobi	KP170490.1	1649	100	922	0	927	99.5	0.0	Uncultured ba	AB636932.1	-	-
VT0082	VTP004	2553	-	-	GATTGGAAT	448	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	KP980569.1	828	100	448	0	448	100.0	0.0	Microbacteri	KP209429.1	Microbacteri	KP209391.1
VT0086	VTP005	2594	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	DQ402053.1	1705	100	925	0	926	99.9	0.0	-	-	-	-
VT0089	VTP005	2592	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_028987.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0090	VTP005	2591	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM934698.1	1647	100	921	0	926	99.5	0.0	-	-	-	-
VT0091	VTP005	2759	-8	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909655.1	1703	100	925	1	926	99.9	0.1	Pseudomonas	AJ308316.1	-	-
VT0092	VTP005	2760	-8	Y	GTTGAATGT	918	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AY574913.1	1646	100	910	1	919	99.0	0.1	Pseudomonas	CP000075.1	-	-
VT0093	VTP005	2637	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB968092.1	1662	100	924	0	926	99.8	0.0	Uncultured ba	GQ487957.1	-	-
VT0095b	VTP005	2762	-8	Y	AAGCGCAC	917	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	KT075207.1	1693	100	917	0	917	100.0	0.0	-	-	-	-
VT0102b	VTP005	2772	-	-	GGGTGCGT/	911	Sphingobacteria	Sphingobacteriaceae	Pedobacter	Pedobacter di	EF660750.1	1677	100	910	0	911	99.9	0.0	-	-	-	-
VT0103	VTP005	2610	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	KT075174.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0107	VTP005	2607	-	-	GCGTGCGC	919	β-proteobacteria	Oxalobacteraceae	Janthinobacterium	Janthinobacte	AB286844.1	1687	100	918	0	920	99.8	0.0	-	-	-	-
VT0117	VTP005	2657	-	-	GGGTCCGT/	896	Flavobacteria	Flavobacteriaceae	Flavobacterium	Flavobacteri	JN820152.1	1655	100	896	0	896	100.0	0.0	-	-	-	-
VT0118	VTP005	2658	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909655.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	AJ308316.1	-	-
VT0125	VTP005	2559	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909659.1	1711	100	926	0	926	100.0	0.0	Pseudomonas	JN800482.1	-	-

Strain sequence								Description BLAST Hit #1													Description BLAST Hit #2		Description BLAST Hit #3	
VT name[a]	Sample	BAV #	INA (°C)[b]	Tree[c]	Trimmed 16S sequence	Query length	Class	Family	Genus	Description	Accession number	Total score	Query Coverage %	Identities	Number of gaps	Alignment length	Frequency number of identities (%)	Frequency number of gaps (%)	Description	Accession number	Description	Accession number
VT0128	VTP005	2562	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1665	100	925	0	926	99.9	0.0	Pseudomonas	HQ222612.1	Pseudomonas	HQ215545.1
VT0129	VTP005	2563	-	Y	GCGCACGC/	922	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KT580675.1	1683	100	921	3	925	99.6	0.3	Pantoea aggl(	KT075203.1	-	-
VT0131	VTP005	2582	-8	Y	TAAAGCGC(	791	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	1411	100	789	1	792	99.6	0.1	-	-	-	-
VT0132	VTP005	2565	-8	Y	GCGCGCGT/	924	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR233795.1	1692	100	923	0	926	99.7	0.0	Pseudomonas	HF558390.1	-	-
VT0141	VTP005	2584	-	Y	GCGCGCGT/	924	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR233795.1	1649	100	923	0	924	99.9	0.0	Pseudomonas	KP641168.1	-	-
VT0144	VTP005	2589	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Plantibacter	Plantibacter :	HE662660.2	1651	100	923	5	928	99.5	0.5	-	-	-	-
VT0146	VTP005	2586	-	-	GAATTCCAC	623	Bacilli	Paenibacillaceae	Paenibacillus	Paenibacillus	KP852522.1	1119	100	622	1	623	99.8	0.2	-	-	-	-
VT0153	VTP005	2747	-	Y	GCGCACGC/	922	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. V	JX458430.1	1631	100	919	3	925	99.4	0.3	Pantoea aggl(	KF465834.1	-	-
VT0155	VTP005	2573	-	Y	TAAAGCGC(	930	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM913961.1	1678	100	930	0	930	100.0	0.0	Pseudomonas	AM933521.1	Pseudomonas	AM421016.2
VT0158	VTP005	2715	-12	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1662	100	924	0	926	99.8	0.0	Pseudomonas	HQ222612.1	Pseudomonas	HQ215545.1
VT0169	VTP005	2736	-8	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1662	100	925	1	926	99.9	0.1	-	-	-	-
VT0176	VTP005	2716	-8	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074598.1	1658	100	924	1	926	99.8	0.1	-	-	-	-
VT0179	VTP005	2738	-8	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR476390.1	1402	100	759	0	759	100.0	0.0	-	-	-	-
VT0184	VTP005	2722	-4	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909655.1	1703	100	924	1	926	99.8	0.1	Pseudomonas	AJ308316.1	-	-
VT0190	VTP005	2639	-8	Y	GCGCGCGT/	619	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KM187433.1	1108	100	617	0	619	99.7	0.0	Pseudomonas	KP796138.1	-	-
VT0191	VTP005	2640	-8	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909659.1	1703	100	925	1	926	99.9	0.1	Pseudomonas	JN800482.1	-	-
VT0201	VTP005	2645	-	Y	GCGCGCGT/	927	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP211411.1	1663	100	926	1	927	99.9	0.1	-	-	-	-
VT0207	VTP005	2617	-8	Y	GCGCGCGT/	927	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909659.1	1705	100	926	1	927	99.9	0.1	Pseudomonas	JN800482.1	-	-
VT0209	VTP005	2604	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909655.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	AJ308316.1	-	-
VT0213	VTP005	2614	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_028987.1	1705	100	925	0	926	99.9	0.0	Pseudomonas	AJ583090.1	-	-
VT0214	VTP005	2613	-12	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AY131218.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0216	VTP005	2601	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR085862.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KC865280.1	-	-
* VT0217	VTP005	2602	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1665	100	925	0	926	99.9	0.0	Pseudomonas	GU396281.1	-	-
VT0222	VTP005	2599	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR233795.1	1711	100	926	0	926	100.0	0.0	Pseudomonas	KP125318.1	Pseudomonas	LN713316.1
VT0223	VTP005	2685	-10	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0224	VTP005	2686	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JF706525.1	1705	100	925	0	926	99.9	0.0	-	-	-	-
VT0227b	VTP005	2753	-8	Y	GCGCGCGT/	924	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1654	100	924	0	926	99.8	0.0	-	-	-	-
VT0233	VTP005	2692	-	-	GAGCTCGT/	929	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	KT580637.1	1705	100	927	0	929	99.8	0.0	Microbacteri	KR085857.1	Microbacteri	CP012299.1
VT0242	VTP005	2771	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JF313057.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0243	VTP005	2766	-	-	GAGCTCGT/	931	Actinobacteria	Micrococcaceae	Arthrobacter	Arthrobacter	AM237357.1	1709	100	929	0	931	99.8	0.0	-	-	-	-
VT0244	VTP005	2665	-8	-	GGCATTGG1	845	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1517	100	838	1	846	99.1	0.1	Pseudomonas	LN713316.1	-	-
VT0246	VTP005	2667	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	AY574914.1	-	-
VT0249	VTP005	2768	-	Y	GCGTGCGT/	846	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP011946.1	1519	100	846	0	846	100.0	0.0	Xanthomonas	CP012251.1	-	-
VT0251	VTP005	2704	-	Y	GCGCACGC/	922	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia billin	NR_102820.1	1685	100	922	4	926	99.6	0.4	-	-	-	-
VT0252	VTP005	2705	-8	Y	GCGCGCGT/	923	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP005969.1	8454	100	923	3	926	99.7	0.3	-	-	-	-
VT0255	VTP005	2702	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909655.1	1656	100	925	2	927	99.8	0.2	Pseudomonas	AJ308316.1	-	-
VT0256	VTP005	2708	-	Y	GCGCGCGT/	920	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KF147090.1	1624	100	918	5	925	99.2	0.5	-	-	-	-
VT0257b	VTP005	2757	-12	Y	AGCGCGCG	729	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR476391.1	1347	100	729	0	729	100.0	0.0	Pseudomonas	LC010244.1	Pseudomonas	KJ756714.1
VT0258a	VTP005	2754	-4	Y	AGCGCGCG	621	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KF500097.1	1147	100	621	0	621	100.0	0.0	Pseudomonas	LN831999.1	Pseudomonas	KP306738.1
VT0268	VTP005	2706	-8	Y	GCGCGCGT/	908	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1665	100	908	0	912	99.6	0.0	-	-	-	-
VT0269	VTP005	2758	-	-	GAATTCCGA	828	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	GU321356.1	1513	100	825	0	828	99.6	0.0	-	-	-	-
VT0271	VTP005	2765	-	-	GCGCACGT/	927	α-proteobacteria	Rhizobiaceae	Agrobacterium	Agrobacteriu	NR_114990.1	1707	100	926	0	927	99.9	0.0	-	-	-	-
VT0274	VTP005	2773	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	KP208636.1	1698	100	926	1	929	99.7	0.1	-	-	-	-
VT0278	VTP005	2674	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP005970.1	8500	100	924	0	926	99.8	0.0	-	-	-	-
VT0281	VTP005	2677	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT0286	VTP005	2678	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JN800482.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0288	VTP006	2774	-12	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1663	100	925	0	926	99.9	0.0	Pseudomonas	HF558390.1	-	KP253040.1
VT0290	VTP006	2776	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909659.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0301	VTP006	2787	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1663	100	924	0	925	99.9	0.0	Pseudomonas	HF558390.1	-	-
VT0305	VTP006	2791	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JN800482.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0312	VTP006	2798	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia rhap(	NR_118858.1	1705	100	925	0	926	99.9	0.0	Enterobacteri	JX067718.1	Enterobacteri	JX067710.1
VT0325	VTP006	2811	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP005970.1	8328	100	925	0	926	99.9	0.0	-	-	-	-
VT0333	VTP006	2819	-4	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1703	100	925	1	926	99.9	0.1	Pseudomonas	AY574914.1	-	-
VT0336	VTP006	2822	-4	Y	GCGCGCGT/	738	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KF146999.1	1332	100	738	0	738	100.0	0.0	-	-	-	-
VT0338	VTP006	2824	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909659.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT0341	VTP006	2827	-	-	ATTCCACGT	368	Bacilli	Paenibacillaceae	Paenibacillus	Paenibacillus	HG942127.1	680	100	368	0	368	100.0	0.0	-	-	-	-
VT0342	VTP006	2828	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1703	100	926	0	926	100.0	0.0	Pseudomonas	AY574914.1	-	-
VT0346	VTP006	2832	-	Y	AGAATTCCA	828	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KT580675.1	1522	100	827	1	828	99.9	0.1	Pantoea aggl(	KT075203.1	-	-
VT0350	VTP006	2836	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0361	VTP006	2847	-	-	GGGTGCGT/	908	Sphingobacteria	Sphingobacteriaceae	Pedobacter	Pedobacter s	AB682400.1	1650	100	903	0	908	99.4	0.0	Pedobacter te	NR_044005.1	-	-
VT0367	VTP006	2853	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0369	VTP006	2855	-	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JN800482.1	1698	100	924	1	926	99.8	0.1	-	-	-	-
VT0370	VTP006	2856	-10	Y	GCGCGCGT/	894	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1595	100	893	0	896	99.7	0.0	-	-	-	-
VT0371	VTP006	2857	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Plantibacter	Plantibacter :	AM396918.1	1709	100	927	0	928	99.9	0.0	Plantibacter f	HE716918.1	-	-
VT0372	VTP006	2858	-	Y	GCGCACGC/	923	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl(	HE716932.1	1700	100	925	1	926	99.9	0.1	-	-	-	-
VT0374	VTP006	2860	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JN800482.1	1660	100	925	1	927	99.8	0.1	-	-	-	-
VT0376	VTP006	2862	-	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1703	100	925	1	926	99.9	0.1	-	-	-	-
VT0378	VTP006	2864	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP211411.1	1711	100	925	0	926	99.9	0.0	Pseudomonas	GU396281.1	-	-
VT0379	VTP006	2865	-	Y	GCGTGCGT/	921	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP012251.1	1656	100	920	1	921	99.9	0.1	Xanthomonas	CP012146.1	-	-
VT0381	VTP006	2867	-	Y	GCGCGCGT/	927	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1705	100	925	0	926	99.9	0.0	-	-	-	-
VT0382	VTP006	2868	-	Y	GCGTACGC/	927	γ-proteobacteria	Enterobacteriaceae	Sodalis	Sodalis praec	CP006569.1	1588	100	913	10	930	98.2	1.1	-	-	-	-
VT0383	VTP006	2869	-	-	AATTCCACG	604	Bacilli	Paenibacillaceae	Paenibacillus	Paenibacillus	KP852522.1	1077	100	602	2	604	99.7	0.3	-	-	-	-
VT0389	VTP006	2875	-4	Y	GCGCGCGT/	615	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC776128.1	1086	100	610	0	615	99.2	0.0	-	-	-	-
VT0392	VTP006	2878	-	Y	GCGTACGC/	925	γ-proteobacteria	Enterobacteriaceae	Sodalis	Sodalis praec	CP006569.1	#####	100	908	10	929	97.7	1.1	-	-	-	-
VT0395	VTP006	2881	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia aphic	AB907777.1	1711	100	926	0	926	100.0	0.0	Erwinia sp. hc	AB893199.1	-	-
VT0417	VTP006	2903	-10	Y	TAAAGCGC(	930	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1678	100	930	0	930	100.0	0.0	-	-	-	-
VT0426	VTP006	2912	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT0434	VTP006	2920	-4	Y	GCGCGCGT/	912	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	HE716923.1	1685	100	912	0	912	100.0	0.0	-	-	-	-
VT0435	VTP006	2921	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KT580675.1	1692	100	922	0	925	99.7	0.0	Pantoea aggl(	KT075203.1	Pantoea aggl(	KJ395361.1
VT0436	VTP006	2922	-	-	GCGCGCGT/	450	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP005970.1	798	100	447	0	450	99.3	0.0	-	-	-	-
VT0439	VTP006	2925	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Frigoribacterium	Frigoribacteri	EU584512.1	1690	100	924	1	928	99.6	0.1	Frigoribacteri	HE716910.1	-	-
VT0442	VTP006	2928	-10	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909655.1	1703	100	925	1	926	99.9	0.1	Pseudomonas	AJ308316.1	-	-
VT0444	VTP006	2930	-8	Y	GCGCGCGT/	927	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1660	100	925	1	927	99.8	0.1	-	-	-	-
VT0445	VTP006	2931	-8	Y	GCGCGCGT/	922	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ569377.1	1663	100	922	0	922	100.0	0.0	-	-	-	-
VT0448	VTP006	2934	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KT580675.1	1709	100	925	0	925	100.0	0.0	Pantoea aggl(	KT075203.1	Pantoea aggl(	KJ395361.1
VT0452	VTP006	2938	-	Y	GCGTACGC/	926	γ-proteobacteria	Enterobacteriaceae	Sodalis	Sodalis praec	CP006569.1	1618	100	913	9	929	98.3	1.0	-	-	-	-
VT0456	VTP006	2942	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl(	KT075209.1	1709	100	925	0	925	100.0	0.0	-	-	-	-
VT0463	VTP006	2949	-	Y	GCGTGCGT/	921	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP011946.1	3313	100	920	0	921	99.9	0.0	Xanthomonas	CP012251.1	-	CP008989.1
VT0467	VTP006	2953	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	AY574914.1	-	-
VT0468	VTP006	2954	-	-	GCGCACGC/	412	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia sp. C-	GQ183858.1	761	100	412	0	412	100.0	0.0	Erwinia typog	KJ781888.1	Bacterium MF	JN194132.1
VT0469	VTP006	2955	-8	Y	GCGTGCGT/	921	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP011946.1	3313	100	920	0	921	99.9	0.0	Xanthomonas	CP012251.1	Xanthomonas	KP419715.1

Strain sequence								Description BLAST Hit #1						Description BLAST Hit #1							Description BLAST Hit #2		Description BLAST Hit #3	
VT name[a]	Sample	BAV #	INA (°C)[b]	Tree[c]	Trimmed 16S sequence	Query length	Class	Family	Genus	Description	Accession number	Total score	Query Coverage %	Identities	Number of gaps	Alignment length	Frequency number of identities (%)	Frequency number of gaps (%)	Description	Accession number	Description	Accession number
VT0471	VTP006	2957	-	Y	GCGTGCGT/	631	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP011946.1	2263	100	631	0	632	99.8	0.0	Xanthomonas	KR005845.1	Xanthomonas	CP012251.1
VT0473	VTP006	2959	-12	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909659.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0481	VTP006	2967	-	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP211411.1	1692	100	923	1	926	99.7	0.1	Pseudomonas	KP211410.1	-	-
VT0483	VTP006	2969	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	AY574914.1	-	-
VT0486	VTP006	2972	-	-	GAGTTCGT/	929	Actinobacteria	Nocardioidaceae	Rhodococcus	Rhodococcus	KR007616.1	1291	100	929	0	929	100.0	0.0	Rhodococcus	KR085921.1	Rhodococcus	KR085866.1
VT0488	VTP006	2974	-12	Y	GGATACTG(	692	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	JX069941.1	1262	100	690	1	693	99.6	0.1	Xanthomonas	JQ614017.1	Xanthomonas	KP419717.1
VT0492	VTP006	2978	-	-	GAGCTCGT/	927	Actinobacteria	Microbacteriaceae	Frigoribacterium	Frigoribacteri	EU584512.1	1667	100	926	1	927	99.9	0.1	-	-	-	-
VT0496	VTP006	2982	-12	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia persi	AB907778.1	1696	100	924	2	927	99.7	0.2	Erwinia aphic	AB907777.1	-	-
VT0499	VTP006	2985	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	KP279966.1	1698	100	923	0	925	99.8	0.0	-	-	-	-
VT0501	VTP006	2987	-	Y	GCGCACGC/	924	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	KP279966.1	1698	100	923	0	925	99.8	0.0	-	-	-	-
VT0510	VTP006	2996	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia aphic	AB907777.1	1703	100	762	0	762	100.0	0.0	Erwinia sp. hc	AB893199.1	-	-
VT0522	VTP006	3008	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia persi	AB907778.1	1707	100	925	0	926	99.9	0.0	Erwinia aphic	AB907777.1	-	-
VT0523	VTP006	3009	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	AY574914.1	-	-
VT0524	VTP006	3010	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteriu	HG799975.1	1709	100	927	0	928	99.9	0.0	Curtobacteriu	JX458460.1	-	-
VT0553	VTP007	3039	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	KT075209.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0554	VTP007	3040	-	Y	CTGGGCGT/	931	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KF057955.1	1703	100	929	1	932	99.7	0.1	Enterobacteri	LC007859.1	Pantoea anar	KC355302.1
VT0555	VTP007	3041	-12	Y	CTGGGCGT/	933	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	AB911210.1	1718	100	933	1	934	99.9	0.1	Xanthomonas	KF270091.1	Xanthomonas	KF057196.1
VT0556	VTP007	3042	-8	Y	GCGCGCGT/	634	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KT223376.1	1139	100	633	0	634	99.8	0.0	-	-	-	-
VT0557	VTP007	3043	-	-	TGGGCGTA/	542	Actinobacteria	Microbacteriaceae	Schumannella	Schumannell	KF057956.1	933	100	531	3	543	97.8	0.6	Plantibacter f	LN774386.1	Plantibacter s	KM253171.1
VT0558	VTP008	3044	-4	Y	CTGGGCGT/	932	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR476390.1	1722	100	932	0	932	100.0	0.0	Pseudomonas	KJ756715.1	Pseudomonas	FJ755788.1
VT0559	VTP008	3045	-4	Y	CTGGGCGT/	932	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR476390.1	1705	100	930	2	933	99.7	0.2	Pseudomonas	KJ756715.1	Pseudomonas	FJ755788.1
VT0560	VTP008	3046	-	Y	CTGGGCGT/	933	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	AB911210.1	1718	100	933	1	934	99.9	0.1	Xanthomonas	KF270091.1	Xanthomonas	KF057196.1
VT0561	VTP008	3047	-10	Y	CTGGGCGT/	931	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB680543.1	1711	100	930	1	932	99.8	0.1	Pseudomonas	KR476390.1	Pseudomonas	KJ756715.1
VT0562	VTP008	3048	-12	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KM675660.1	1712	100	930	1	931	99.9	0.1	Pantoea sp. L	KJ584027.1	Pantoea anar	DQ133546.1
VT0563	VTP008	3049	-	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. 4	JX566609.1	1668	100	922	1	931	99.0	0.1	Erwinia persi	EU681952.1	Pantoea sp. 1	KC236466.1
VT0564	VTP008	3050	-	-	TGGGCGTA/	941	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteriu	JX458460.1	1635	100	929	12	948	98.0	1.3	Curtobacteriu	KF479687.1	Curtobacteriu	KF040989.1
VT0565	VTP008	3051	-10	Y	CTGGGCGT/	931	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KM675660.1	1709	100	930	2	932	99.8	0.2	Pantoea sp. L	KP162076.1	Pantoea sp. L	KJ584027.1
VT0566	VTP008	3052	-	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. L	KP162076.1	1707	100	929	1	931	99.8	0.1	Pantoea sp. B	JX266366.1	Pantoea anar	GQ383910.1
VT0567	VTP008	3053	-10	Y	CTGGGCGT/	932	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR476390.1	1711	100	931	2	933	99.8	0.2	Pseudomonas	KJ756715.1	Pseudomonas	FJ755788.1
VT0568	VTP008	3054	-8	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	LC015551.1	1712	100	930	1	931	99.9	0.1	Pantoea anan	NR_103927.1	Pantoea anar	NR_074740.1
VT0569	VTP008	3055	-8	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	LC015551.1	1707	100	929	1	931	99.8	0.1	Pantoea anan	NR_103927.1	Pantoea anar	NR_074740.1
VT0570	VTP008	3056	-4	Y	CTGGGCGT/	932	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909655.1	1714	100	931	1	932	99.9	0.1	Pseudomonas	AJ308316.1	P.syringae 16	Z76669.1
VT0571	VTP008	3057	-4	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	LC015551.1	1707	100	929	1	931	99.8	0.1	Pantoea anan	NR_103927.1	Pantoea anar	NR_074740.1
VT0572	VTP008	3058	-	Y	CTGGGCGT/	935	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	AB911210.1	1698	100	932	6	937	99.5	0.6	Xanthomonas	KF270091.1	Xanthomonas	KF057196.1
VT0573	VTP008	3059	-	Y	GTCTGTTGT	724	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP012251.1	1297	100	723	0	724	99.9	0.0	Xanthomonas	KP731996.1	-	-
VT0574	VTP008	3060	-12	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. L	KP162076.1	1712	100	930	1	931	99.9	0.1	Pantoea sp. B	JX266366.1	Pantoea aggl	AY741162.1
VT0575	VTP008	3061	-	-	TTGGGCGT/	927	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	KC900892.1	1609	100	917	12	937	97.9	1.3	Microbacteri	KF010642.1	Microbacteri	KF010638.1
VT0576	VTP008	3062	-	-	TGGGCGTA/	944	Actinobacteria	Micrococcaceae	Micrococcus	Micrococcus	JQ658403.1	1727	100	942	1	945	99.7	0.1	Micrococcus	KF142392.1	Micrococcus	EF114312.3
VT0577	VTP008	3063	-10	Y	CTGGGCGT/	931	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. L	KP162076.1	1720	100	931	0	931	100.0	0.0	Pantoea sp. B	JX266366.1	Pantoea aggl	AY741162.1
VT0578	VTP008	3064	-	Y	GCGTGCGT/	920	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	JN872548.1	1595	100	909	4	920	98.8	0.4	Luteibacter sp	FR714940.1	-	-
VT0579	VTP008	3065	-8	Y	CTGGGCGT/	931	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074598.1	1709	100	930	1	932	99.8	0.1	Pseudomonas	AB680548.1	Pseudomonas	AB682254.1
VT0580	VTP008	3066	-	-	TGGGCGTA/	943	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	EU714367.1	1725	100	941	1	944	99.7	0.1	Microbacteri	AB042072.1	Microbacteri	KM884890.1
VT0581	VTP008	3067	-12	Y	CTGGGCGT/	931	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1714	100	931	1	932	99.9	0.1	Pseudomonas	AY574914.1	Pseudomonas	HE716923.1
VT0582	VTP008	3068	-8	Y	CTGGGCGT/	931	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1714	100	931	1	932	99.9	0.1	Pseudomonas	AY574914.1	Pseudomonas	HE716923.1
VT0583	VTP008	3069	-10	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. L	KP162076.1	1712	100	930	1	931	99.9	0.1	Pantoea sp. B	JX266366.1	Pantoea aggl	AY741162.1
VT0584	VTP008	3070	-10	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. L	KP162076.1	1712	100	930	1	931	99.9	0.1	Pantoea sp. B	JX266366.1	Pantoea aggl	AY741162.1
* VT0585	VTP008	3071	-	-	TGGGCGTA/	942	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteriu	JX458460.1	1729	100	941	1	943	99.8	0.1	Curtobacteriu	KF479687.1	Curtobacteriu	KF040989.1
VT0586	VTP008	3072	-4	Y	CTGGGCGT/	931	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1714	100	931	1	932	99.9	0.1	Pseudomonas	AY574914.1	Pseudomonas	HE716923.1
VT0587	VTP008	3073	-10	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. L	KP162076.1	1712	100	930	1	931	99.9	0.1	Pantoea sp. B	JX266366.1	Pantoea aggl	AY741162.1
VT0588	VTP008	3074	-8	Y	CTGGGCGT/	928	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KM675660.1	1683	100	925	3	931	99.4	0.3	Pantoea sp. L	KJ584027.1	Pantoea anar	DQ133546.1
VT0589	VTP008	3075	-	Y	GGAATTGC/	619	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP012251.1	1117	100	619	0	619	100.0	0.0	Xanthomonas	KP731996.1	-	-
VT0590	VTP008	3076	-8	Y	CTGGGCGT/	931	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	GQ383910.1	1698	100	928	2	932	99.6	0.2	Pantoea anan	DQ517335.1	Pantoea anar	DQ195525.1
VT0591	VTP008	3077	-10	Y	CTGGGCGT/	930	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	GQ383910.1	1701	100	928	1	931	99.7	0.1	Pantoea anan	DQ517335.1	Pantoea anar	DQ195525.1
VT0592	VTP008	3078	-	-	GAGCTCGT/	930	Actinobacteria	Nocardioidaceae	Rhodococcus	Rhodococcus	EF612310.1	1678	100	930	0	930	100.0	0.0	-	-	-	-
VT0593	VTP008	3079	-	Y	GGAATTCCA	619	Bacilli	Bacillaceae	Bacillus	Bacillus safe	KM374729.1	1110	100	619	1	620	99.8	0.2	Bacillus pumi	KM096605.1	-	-
VT0594	VTP008	3080	-10	Y	CTGGGCGT/	931	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1714	100	931	1	932	99.9	0.1	Pseudomonas	AY574914.1	Pseudomonas	HE716923.1
VT0595	VTP008	3081	-10	Y	CTGGGCGT/	930	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR476390.1	1709	100	930	2	932	99.8	0.2	Pseudomonas	KJ756715.1	Pseudomonas	FJ755788.1
VT0596	VTP008	3082	-8	Y	CTGGGCGT/	929	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KM675660.1	1701	100	928	2	931	99.7	0.2	Pantoea sp. L	KJ584027.1	Pantoea anar	DQ133546.1
VT0597	VTP008	3083	-4	Y	CTGGGCGT/	931	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	LC015551.1	1703	100	929	2	932	99.7	0.2	Pantoea anan	NR_103927.1	Pantoea anar	NR_074740.1
VT0598	VTP009	3084	-12	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KR296701.1	1703	100	924	0	925	99.9	0.0	-	-	-	-
VT0599	VTP009	3085	-	Y	AGCGCACG(	829	γ-proteobacteria	Enterobacteriaceae	Serratia	Serratia fonti	KP089980.1	1531	100	829	0	829	100.0	0.0	Serratia sp. Z	KM117223.1	Serratia sp. K	JF327450.1
VT0600	VTP009	3095	-8	Y	TAGAATTCC	828	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KR296701.1	1530	100	828	0	828	100.0	0.0	-	-	-	-
VT0601	VTP009	3096	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KR296701.1	1703	100	924	0	925	99.9	0.0	-	-	-	-
VT0602	VTP009	3097	-12	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1660	100	923	0	925	99.8	0.0	Pantoea aggl	AY741162.1	-	-
VT0603	VTP010	3094	-10	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1705	100	716	0	716	100.0	0.0	Pseudomonas	HF558390.1	Pseudomonas	AM909659.1
VT0604	VTP010	3108	-8	Y	GCGCGCGT/	920	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1700	100	920	0	920	100.0	0.0	Pseudomonas	KR476390.1	-	-
VT0605	VTP010	3109	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0606	VTP010	3110	-8	Y	GCGCGCGT/	924	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1707	100	924	0	924	100.0	0.0	-	-	-	-
VT0607	VTP010	3111	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KF057955.1	1677	100	920	0	926	99.4	0.0	Pantoea vaga	CP002206.1	-	-
VT0608	VTP010	3112	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KR296701.1	1660	100	923	0	925	99.8	0.0	-	-	-	-
VT0609	VTP010	3113	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074598.1	1705	100	925	0	926	99.9	0.0	Pseudomonas	AM909656.1	-	-
VT0610	VTP010	3114	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_028987.1	1711	100	926	0	926	100.0	0.0	-	-	-	KC306426.1
VT0611	VTP010	3115	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_028987.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT0612	VTP010	3116	-12	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1703	100	924	0	925	99.9	0.0	Pantoea anar	LC015551.1	-	-
VT0613	VTP010	3117	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0614	VTP010	3118	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0615	VTP010	3119	-8	Y	GCGTGCGT/	929	γ-proteobacteria	Xanthomonadaceae	Stenotrophomonas	Stenotrophon	NR_121739.1	1714	100	929	0	929	100.0	0.0	Stenotrophon	HF548417.1	-	-
VT0616	VTP010	3120	-4	Y	TAAAGCGC(	930	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1718	100	930	0	930	100.0	0.0	-	-	-	-
VT0618	VTP010	3122	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0619	VTP010	3123	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0621	VTP010	3125	-	Y	GCGCGCGT/	913	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	LN794844.1	1676	100	911	0	913	99.8	0.0	Pseudomonas	KJ452464.1	-	-
VT0622	VTP010	3126	-8	Y	GCGCACGC/	912	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KF057955.1	1674	100	910	0	912	99.8	0.0	-	-	-	-
VT0623	VTP010	3127	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anar	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0624	VTP010	3128	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea eucri	HQ706113.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0625	VTP010	3129	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP986948.1	#####	100	922	0	926	99.6	0.0	Pseudomonas	CP009747.1	Pseudomonas	KP640640.1
VT0626	VTP010	3130	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0627	VTP010	3131	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0628	VTP010	3132	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AJ970167.1	1711	100	926	0	926	100.0	0.0	Pseudomonas	KP796138.1	-	-
VT0629	VTP010	3133	-4	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1709	100	925	0	925	100.0	0.0	Pantoea anar	LC015551.1	-	-
VT0630	VTP010	3134	-	-	GGAATTGC(	825	α-proteobacteria	Rhizobiaceae	Rhizobium	Rhizobium sp	HM196763.1	1524	100	825	0	825	100.0	0.0	-	-	-	-
VT0631	VTP010	3135	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067667.1	1705	100	925	0	926	99.9	0.0	-	-	-	-

	Strain sequence							Description BLAST Hit #1					Description BLAST Hit #1							Description BLAST Hit #2		Description BLAST Hit #3	
VT name[a]	Sample	BAV #	INA (°C)[b]	Tree[c]	Trimmed 16S sequence	Query length	Class	Family	Genus	Description	Accession number	Total score	Query Coverage %	Identities	Number of gaps	Alignment length	Frequency number of identities (%)	Frequency number of gaps (%)	Description	Accession number	Description	Accession number
VT0632	VTP010	3136	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0633	VTP010	3137	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0634	VTP010	3138	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP009533.1	#####	100	926	0	926	100.0	0.0	-	-	-	-
VT0635	VTP010	3139	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KF057955.1	1480	100	924	0	926	99.8	0.0	-	-	-	-
VT0636	VTP010	3140	-10	Y	AACTGCATT	796	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	LC015551.1	1471	100	796	0	796	100.0	0.0	-	-	-	-
VT0637	VTP010	3141	-12	-	GCGCGCGT/	411	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KT693288.1	733	100	409	0	411	99.5	0.0	Pseudomonas	AB968092.1	-	-
VT0638	VTP010	3142	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1709	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0639	VTP010	3143	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0640	VTP010	3144	-8	-	GCGCGCGT/	315	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP794940.1	569	100	315	0	315	100.0	0.0	-	-	-	-
VT0641	VTP010	3145	-	Y	AGAATTCCA	827	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067596.1	1528	100	827	0	827	100.0	0.0	Pantoea anan	KM675660.1	-	-
VT0650	VTP011	3168	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JQ659775.1	1617	100	914	0	926	98.7	0.0	-	-	-	-
VT0652	VTP011	3170	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC901542.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
VT0654	VTP011	3172	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JQ659775.1	1617	100	914	0	926	98.7	0.0	-	-	-	-
VT0656	VTP011	3174	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1698	100	923	0	925	99.8	0.0	-	-	-	-
VT0659	VTP011	3177	-10	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC901542.1	1703	100	925	1	926	99.9	0.1	-	-	-	-
VT0661	VTP011	3179	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JQ659775.1	1617	100	914	0	926	98.7	0.0	-	-	-	-
VT0664	VTP011	3182	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JQ659775.1	1617	100	914	0	926	98.7	0.0	-	-	-	-
VT0666	VTP011	3184	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JQ659775.1	1617	100	914	0	926	98.7	0.0	-	-	-	-
VT0670	VTP011	3188	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1709	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0673	VTP011	3191	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1709	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0676	VTP012	3205	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia aphic	AB907777.1	1709	100	925	0	925	100.0	0.0	Erwinia persi	NR_026049.1	-	-
VT0679	VTP012	3208	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1709	100	925	0	925	100.0	0.0	Pantoea aggl	AY741162.1	-	-
VT0680	VTP012	3209	-12	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0681	VTP012	3210	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0682	VTP012	3211	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	NR_119362.1	1660	100	923	0	925	99.8	0.0	Pantoea sp. X	KR296703.1	-	-
VT0683	VTP012	3212	-12	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0684	VTP012	3213	-4	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067596.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	KM675660.1	-	-
VT0685	VTP012	3214	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067596.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	KM675660.1	-	-
VT0686	VTP012	3215	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1654	100	922	0	925	99.7	0.0	-	-	-	-
VT0687	VTP012	3216	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1703	100	924	0	925	99.9	0.0	-	-	-	-
VT0688	VTP012	3217	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1709	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0689	VTP012	3218	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KT580675.1	1669	100	925	0	925	100.0	0.0	Pantoea aggl	KT075203.1	-	-
VT0690	VTP012	3219	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0691	VTP012	3220	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KT580675.1	1709	100	925	0	925	100.0	0.0	Pantoea aggl	KT075203.1	Curtobacteriu	KP099433.1
VT0694	VTP012	3223	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1709	100	925	0	925	100.0	0.0	-	-	-	-
VT0696	VTP012	3225	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1709	100	925	0	925	100.0	0.0	-	-	-	-
VT0697	VTP012	3226	-	Y	GCGCGCGT/	927	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ831070.1	1663	100	926	1	927	99.9	0.1	-	-	-	-
VT0700	VTP012	3229	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
* VT0701	VTP012	3230	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1703	100	924	0	925	99.9	0.0	-	-	-	-
VT0702	VTP012	3231	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0704	VTP012	3233	-4	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074598.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0706	VTP012	3235	-	Y	GCGCACGC/	912	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1672	100	910	0	912	99.8	0.0	-	-	-	-
VT0708	VTP012	3237	-8	Y	AGCGCACG	716	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	LC015551.1	1323	100	716	0	716	100.0	0.0	Pantoea sp. U	KM253209.1	Pantoea anan	KM091726.1
VT0713	VTP012	3242	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KF147112.1	1707	100	925	0	926	99.9	0.0	-	-	-	-
VT0714	VTP012	3243	-4	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1709	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0715	VTP012	3244	-4	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0717	VTP012	3246	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KF057955.1	1705	100	925	0	926	99.9	0.0	-	-	-	-
VT0720	VTP012	3249	-	-	AGCGCGCG	277	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP453682.1	512	100	277	0	277	100.0	0.0	Pseudomonas	KM019875.1	Pseudomonas	KJ854409.1
VT0721	VTP012	3250	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0722	VTP012	3251	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1709	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	Pantoea anan	KM091726.1
VT0724	VTP012	3253	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1698	100	923	0	925	99.8	0.0	-	-	-	-
VT0726	VTP012	3255	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0728	VTP012	3257	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1709	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	Pantoea anan	KM091726.1
VT0729	VTP012	3258	-	Y	GCGCGCGT/	927	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KF956613.1	1667	100	926	0	927	99.9	0.0	-	-	-	-
VT0731	VTP012	3260	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1709	100	925	0	925	100.0	0.0	-	-	-	-
VT0735	VTP012	3264	-	-	GAATTCCAG	514	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KP325093.1	928	100	514	0	514	100.0	0.0	Pantoea vaga	KF986850.1	-	-
VT0736	VTP012	3265	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	DQ307454.1	1653	100	923	1	926	99.7	0.1	-	-	-	-
VT0737	VTP012	3266	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0738	VTP012	3267	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0739	VTP012	3268	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	KT075209.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0740	VTP012	3269	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1663	100	924	0	925	99.9	0.0	Pantoea anan	LC015551.1	-	-
VT0743	VTP012	3272	-	Y	GCGTGCGT/	859	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP011946.1	3100	100	859	0	859	100.0	0.0	Xanthomonas	CP012251.1	-	-
VT0744	VTP012	3273	-	-	GAGCTCGT/	929	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	KM884890.1	1671	100	928	0	929	99.9	0.0	-	-	-	-
VT0745	VTP012	3274	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC901542.1	1705	100	925	0	926	99.9	0.0	-	-	-	-
VT0746	VTP012	3275	-8	Y	GCGCACGC/	924	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1690	100	922	1	925	99.7	0.1	-	-	-	-
VT0747	VTP012	3276	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0748	VTP012	3277	-10	Y	GCGCACGC/	916	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1653	100	916	0	916	100.0	0.0	-	-	-	-
* VT0749	VTP012	3278	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. L'	DQ869046.1	1660	100	923	0	925	99.8	0.0	Pantoea anan	KM675660.1	-	-
VT0750	VTP012	3279	-10	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP762566.1	1711	100	926	0	926	100.0	0.0	-	-	-	-
* VT0752	VTP012	3281	-	Y	GCGTGCGT/	928	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP012251.1	1674	100	928	0	928	100.0	0.0	Xanthomonas	CP012146.1	-	-
VT0753	VTP012	3282	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP762566.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0755	VTP012	3284	-	Y	GCGCACGC/	921	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	KT075174.1	1647	100	921	4	925	99.6	0.4	Xanthomonas	CP012146.1	-	-
VT0756	VTP012	3344	-	-	AGGGAGCG	750	α-proteobacteria	Caulobacteriaceae	Brevundimonas	Brevundimon	KF975414.1	1343	100	744	2	752	98.9	0.3	Brevundimon	KF006808.1	-	-
VT0757	VTP012	3285	-8	Y	AGGCGGTC	797	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1467	100	796	0	797	99.9	0.0	-	-	-	-
VT0759	VTP012	3287	-	Y	GCGCACGC/	912	γ-proteobacteria	Enterobacteriaceae	Ewingella	Ewingella am	FN811904.1	1672	100	910	1	912	99.8	0.1	Rahnella sp. (	AJ415576.1	-	-
VT0760	VTP012	3288	-8	Y	AGCGCACG	763	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KF971769.1	1410	100	763	0	763	100.0	0.0	-	-	-	-
VT0761	VTP012	3289	-	Y	GGAATTCCA	701	Bacilli	Bacillaceae	Bacillus	Bacillus pumi	KM096605.1	1265	100	701	0	701	100.0	0.0	Bacillus safer	KR140378.1	-	-
VT0765	VTP012	3293	-10	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AJ970167.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KP796138.1	-	-
VT0766	VTP012	3294	-	-	AATTCCGAG	315	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	KP980568.1	577	100	314	0	315	99.7	0.0	Sphingomona	KR080483.1	Sphingomona	KP216807.1
* VT0767	VTP012	3295	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	AY741161.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0768	VTP012	3296	-	Y	GCGCACGC/	924	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	921	1	924	99.7	0.1	-	-	-	-
VT0769	VTP012	3297	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Enterobacter	Enterobacter	KF145192.1	1660	100	923	0	925	99.8	0.0	Salmonella b	KC329819.1	Escherichia h	JX968499.1
VT0770	VTP012	3298	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1703	100	924	0	925	99.9	0.0	Pantoea anan	LC015551.1	-	-
VT0771	VTP012	3299	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC901542.1	1705	100	925	0	926	99.9	0.0	-	-	-	-
VT0773	VTP012	3301	-	-	GAGCTCGT/	929	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	NR_025368.1	1671	100	928	0	929	99.9	0.0	-	-	-	-
VT0774	VTP012	3302	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Enterobacter	Enterobacter	HQ407245.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0775	VTP012	3303	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0776	VTP012	3345	-	-	GAATTCCGA	692	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	GU086453.1	1249	100	692	0	692	100.0	0.0	-	-	-	-
VT0778	VTP012	3305	-	Y	GCGCCGCT	923	γ-proteobacteria	Enterobacteriaceae	Enterobacter	Enterobacter	HQ407245.1	1669	100	919	2	923	99.6	0.2	-	-	-	-
VT0779	VTP012	3306	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	DQ512490.1	1654	100	922	0	925	99.7	0.0	-	-	-	-
VT0780	VTP012	3307	-4	Y	TCCGTTGCG	917	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	KR067599.1	1618	100	910	1	917	99.2	0.1	Xanthomonas	CP010409.1	-	-

	Strain sequence							Description BLAST Hit #1					Description BLAST Hit #1							Description BLAST Hit #2		Description BLAST Hit #3	
	VT name[a]	Sample	BAV #	INA (°C)[b]	Tree[c]	Trimmed 16S sequence	Query length	Class	Family	Genus	Description	Accession number	Total score	Query Coverage %	Identities	Number of gaps	Alignment length	Frequency number of identities (%)	Frequency number of gaps (%)	Description	Accession number	Description	Accession number
	VT0781	VTP012	3308	-	Y	GCGCACGC	911	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KT580675.1	1683	100	911	0	911	100.0	0.0	Pantoea aggl	KT075203.1	Pantoea aggl	KJ395361.1
*	VT0782	VTP012	3309	-10	Y	AGAATTCCA	827	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1703	100	827	0	827	100.0	0.0	Pantoea anan	LC015551.1	-	-
	VT0783	VTP012	3310	-	Y	GCGCACGC	924	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KT580675.1	1660	100	924	1	925	99.9	0.1	Pantoea aggl	KT075203.1	Pantoea aggl	KJ395361.1
*	VT0785	VTP012	3346	-10	Y	GCGCACGC	826	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	LC015551.1	1526	100	826	0	826	100.0	0.0	-	-	-	-
	VT0787	VTP012	3313	-8	Y	GCGCACGC	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	AY741161.1	1669	100	922	1	926	99.6	0.1	Pantoea anan	KJ472830.1	Pantoea anan	KJ655537.1
	VT0788	VTP012	3314	-	-	GAGCTCGT	929	Actinobacteria	Brevibacteriaceae	Brevibacterium	Brevibacteriu	JX393074.1	1705	100	927	0	929	99.8	0.0	Brevibacteriu	LC082101.1	-	-
	VT0789	VTP012	3315	-	Y	GCGCACGC	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1658	100	924	1	926	99.8	0.1	-	-	-	-
	VT0790	VTP012	3316	-	Y	GCGCGCGT	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074659.1	1663	100	924	0	926	99.8	0.0	Pseudomonas	NR_043115.1	Pseudomonas	AB060135.1
	VT0791	VTP012	3317	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	KT075174.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
	VT0792	VTP012	3318	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1654	100	924	2	926	99.8	0.2	-	-	-	-
	VT0793	VTP012	3319	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	AY741161.1	1703	100	924	0	925	99.9	0.0	-	-	-	-
	VT0795	VTP012	3321	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1663	100	923	0	925	99.8	0.0	Pantoea anan	KR296701.1	-	-
*	VT0796	VTP012	3322	-	Y	GCGCACGC	908	γ-proteobacteria	Enterobacteriaceae	Enterobacter	Enterobacter	KC355281.1	1555	100	898	6	914	98.2	0.7	Enterobacter	KR189491.1	Enterobacteri	LC007875.1
	VT0798a	VTP012	3324	-10	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
	VT0798b	VTP012	4461	-8	Y	GCGTGCGT	925	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP011946.1	3338	100	925	0	925	100.0	0.0	Xanthomonas	CP012251.1	-	-
	VT0799	VTP012	3325	-10	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	DQ512490.1	1654	100	922	0	925	99.7	0.0	-	-	-	-
	VT0801	VTP012	3327	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	DQ512490.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
	VT0802	VTP012	3328	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1654	100	922	0	925	99.7	0.0	Pantoea anan	KR296701.1	-	-
	VT0803	VTP012	3329	-	-	GAGCTCGT	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteriu	KM378597.1	1709	100	927	0	928	99.9	0.0	Curtobacteriu	KC178601.1	-	-
	VT0805	VTP012	3331	-	-	GAGCTCGT	915	Actinobacteria	Brevibacteriaceae	Brevibacterium	Brevibacteriu	EU099374.1	1690	100	915	0	915	100.0	0.0	-	-	-	-
	VT0807	VTP012	3333	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1660	100	923	0	925	99.8	0.0	Pantoea anan	KR296701.1	-	-
	VT0808	VTP012	3334	-	-	GAGCTCGT	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteriu	KM378597.1	1669	100	927	0	928	99.9	0.0	Curtobacteriu	KC178601.1	-	-
	VT0809	VTP012	3335	-	-	GCGTGCGC	919	β-proteobacteria	Alcaligenaceae	Achromobacter	Achromobact	HG324053.1	1692	100	919	1	920	99.9	0.1	Achromobact	KF150361.1	-	-
	VT0810	VTP012	3336	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1709	100	923	0	925	99.8	0.0	Pantoea anan	KR296701.1	-	-
	VT0811	VTP012	3337	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	DQ512490.1	1698	100	923	0	925	99.8	0.0	-	-	-	-
	VT0812	VTP012	3338	-8	Y	GCGTGCGT	925	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP011946.1	1660	100	923	0	925	99.8	0.0	Xanthomonas	CP012251.1	-	-
	VT0813	VTP012	3339	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1660	100	923	0	925	99.8	0.0	Pantoea anan	KR296701.1	-	-
	VT0814	VTP012	3347	-	-	GAGCTCGT	929	Actinobacteria	Nocardioidaceae	Rhodococcus	Rhodococcus	EF612310.1	1711	100	929	1	930	99.9	0.1	-	-	-	-
	VT0816	VTP012	3341	-	-	AATTCCGAG	822	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	GU086453.1	1561	100	816	5	827	98.7	0.6	-	-	-	-
	VT0817	VTP012	3342	-12	Y	GCGCACGC	924	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067596.1	1701	100	924	1	925	99.9	0.1	Pantoea anan	KM675660.1	-	-
	VT0818b	VTP012	4478	-	-	GAGCTCGT	930	Actinobacteria	Nocardioidaceae	Rhodococcus	Rhodococcus	EF612310.1	1673	100	929	0	930	99.9	0.0	-	-	-	-
	VT0819	VTP013	3348	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	AY741161.1	1651	100	921	0	925	99.6	0.0	Pantoea sp. X	KR296703.1	-	-
	VT0821	VTP013	3350	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1709	100	925	0	925	100.0	0.0	-	-	-	-
	VT0822	VTP013	3351	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	NR_119362.1	1703	100	924	0	925	99.9	0.0	-	-	-	-
	VT0823	VTP013	3352	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
	VT0824	VTP013	3353	-4	Y	GCGCGCGT	604	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KM187157.1	1077	100	602	1	604	99.7	0.2	-	-	-	-
	VT0825	VTP013	3354	-	-	GCGCGCGT	926	γ-proteobacteria	Moraxellaceae	Acinetobacter	Acinetobacter	EU373343.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
	VT0826	VTP013	3355	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	GU324264.1	1692	100	922	0	925	99.7	0.0	-	-	-	-
	VT0827	VTP013	3356	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	AY741161.1	1651	100	923	0	925	99.8	0.0	Pantoea sp. X	KR296703.1	-	-
	VT0828	VTP013	3357	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. L	KJ584033.1	1690	100	922	1	925	99.7	0.1	-	-	-	-
	VT0829	VTP013	3358	-10	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1709	100	925	0	925	100.0	0.0	-	-	-	-
*	VT0832	VTP013	3361	-4	-	GCGCGCGT	503	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Uncultured Ps	EU665123.1	868	100	494	1	503	98.2	0.2	Pseudomonas	KT350501.1	-	-
	VT0833	VTP013	3362	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. 4	JX566609.1	1627	100	916	0	925	99.0	0.0	Pantoea anan	GU339282.1	-	-
	VT0834	VTP013	3363	-8	Y	AATTCCAGG	827	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. L'	DQ869046.1	1660	100	825	0	827	99.8	0.0	Pantoea sp. C	KR067596.1	-	-
	VT0835	VTP013	3364	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	AY741161.1	1654	100	922	0	925	99.7	0.0	Pantoea sp. X	KR296703.1	-	-
	VT0836	VTP013	3365	-10	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	HE617160.1	1660	100	922	1	925	99.7	0.1	-	-	-	-
	VT0837	VTP013	3366	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	JF756691.1	1685	100	921	1	925	99.6	0.1	-	-	-	-
	VT0838	VTP013	3367	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	JF756691.1	1656	100	923	1	925	99.8	0.1	-	-	-	-
	VT0839	VTP013	3368	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
	VT0840	VTP014b	3369	-4	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
	VT0841	VTP014b	3370	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1703	100	924	0	926	99.8	0.0	Pseudomonas	AY574914.1	-	-
	VT0842	VTP014a	3371	-10	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
	VT0843	VTP014b	3372	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
	VT0844	VTP014a	3373	-8	Y	AGAATTCCA	829	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1513	100	826	2	829	99.6	0.2	-	-	-	-
	VT0845	VTP014a	3374	-	-	GAGCTCGT	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteriu	HG799975.1	1669	100	927	0	928	99.9	0.0	-	-	-	-
	VT0846	VTP014a	3375	-	-	GAGCTCGT	926	Actinobacteria	Corynebacteriaceae	Corynebacterium	Corynebacter	KP114217.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
	VT0847a	VTP014a	3376	-12	Y	GCGTGCGT	928	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP012251.1	1674	100	928	0	928	100.0	0.0	Xanthomonas	CP012146.1	-	-
	VT0848	VTP014a	3377	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
	VT0849	VTP014b	3378	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
	VT0850	VTP014b	3379	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
	VT0851	VTP014a	3380	-	-	GGGTCCGT	906	Flavobacteria	Flavobacteriaceae	Chryseobacterium	Chryseobacte	AB682423.1	1599	100	898	0	906	99.1	0.0	-	-	-	-
	VT0852	VTP014a	3381	-	-	GCGCACGT	929	α-proteobacteria	Rhizobiaceae	Rhizobium	Rhizobium sp	AM083999.1	1595	100	911	0	929	98.1	0.0	-	-	-	-
	VT0853	VTP014a	3382	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC822785.1	1667	100	925	0	926	99.9	0.0	-	-	-	-
	VT0854	VTP014a	3383	-	Y	GCGCACGC	926	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia aphic	AB907777.1	1662	100	924	0	926	99.8	0.0	Erwinia persi	NR_026049.1	-	-
	VT0855	VTP014a	3384	-	Y	GCGCACGC	926	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia aphic	AB907777.1	1665	100	925	0	926	99.9	0.0	Erwinia persi	NR_026049.1	-	-
	VT0856	VTP014a	3385	-	Y	GCGCACGC	926	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia aphic	AB907777.1	1665	100	925	0	926	99.9	0.0	Erwinia persi	NR_026049.1	-	-
	VT0857	VTP014a	3386	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	KT075199.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
	VT0858	VTP014a	3387	-	-	GCGCGCGT	926	γ-proteobacteria	Moraxellaceae	Acinetobacter	Acinetobacter	EU373343.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
	VT0859	VTP014a	3388	-	Y	GCGCACGC	926	γ-proteobacteria	Enterobacteriaceae	Erwinia	Erwinia aphic	AB907777.1	1662	100	924	0	926	99.8	0.0	Erwinia aphic	NR_117000.1	-	-
	VT0860	VTP014a	3389	-	Y	GCGCACGC	917	γ-proteobacteria	Enterobacteriaceae	Buttiauxella	Buttiauxella i	NR_025331.1	1602	100	908	10	926	98.1	1.1	-	-	-	-
	VT0861	VTP014a	3390	-	-	GCGCACGT	931	α-proteobacteria	Rhizobiaceae	Rhizobium	Rhizobium sp	AM083999.1	1577	100	910	2	931	97.7	0.2	-	-	-	-
	VT0862	VTP014a	3391	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067667.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
	VT0863	VTP014b	3392	-	-	GAGCTCGT	941	Actinobacteria	Streptomycetaceae	Streptomyces	Streptomyces	GQ863920.1	1698	100	941	0	941	100.0	0.0	-	-	-	-
	VT0864	VTP014b	3393	-	-	GGGCTCGT	936	Actinobacteria	Nocardioidaceae	-	Nocardioidac	FJ200392.3	1680	100	934	0	936	99.8	0.0	-	-	-	-
	VT0865	VTP014b	3394	-	-	GAGCTCGT	928	Actinobacteria	Microbacteriaceae	Pseudoclavibacter	Pseudoclavib	KM199858.1	1644	100	923	2	928	99.5	0.2	Pseudoclavib	NR_029264.1	-	-
	VT0866	VTP014b	3395	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
	VT0867	VTP014b	3396	-	-	GAGCTCGT	927	Actinobacteria	Microbacteriaceae	Frigoribacterium	Frigoribacteri	EU584512.1	1667	100	926	0	927	99.9	0.0	-	-	-	-
	VT0868	VTP014b	3397	-	-	GAGCTCGT	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteriu	HG799975.1	1665	100	926	0	928	99.8	0.0	-	-	-	-
	VT0869	VTP014b	3398	-	-	GAGCTCGT	929	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	KM884890.1	1671	100	928	0	929	99.9	0.0	-	-	-	-
	VT0870	VTP014b	3399	-	-	GTCAGGGG	891	Sphingobacteria	Sphingobacteriaceae	Pedobacter	Pedobacter sp	HM204921.1	1602	100	890	0	891	99.9	0.0	-	-	-	-
	VT0871	VTP014b	3400	-	-	GAATTCCTG	827	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteriu	HG799975.1	1507	100	825	2	829	99.5	0.2	-	-	-	-
	VT0872	VTP014b	3401	-	-	GAGCTCGT	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteriu	HG799975.1	1669	100	927	2	928	99.9	0.2	-	-	-	-
	VT0873	VTP014b	3402	-	-	AGCTCGTAC	928	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	KM884890.1	1665	100	926	0	928	99.8	0.0	-	-	-	-
	VT0874	VTP014b	3403	-	-	ATTCCGAGT	828	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	NR_029111.1	1463	100	823	2	828	99.4	0.2	-	-	-	-
	VT0875	VTP014b	3404	-	-	GAGCTCGT	928	Actinobacteria	Microbacteriaceae	Plantibacter	Plantibacter s	HE662660.2	1660	100	925	0	928	99.7	0.0	-	-	-	-
	VT0876b	VTP014b	4479	-	-	GAGCTCGT	930	Actinobacteria	Nocardioidaceae	Rhodococcus	Rhodococcus	KF441597.1	1718	100	930	0	930	100.0	0.0	Rhodococcus	KC462543.1	Rhodococcus	EU718336.1
	VT0877	VTP014b	3406	-	-	GAGCTCGT	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteriu	HG799975.1	1669	100	927	2	928	99.9	0.2	-	-	-	-
	VT0878	VTP015	3407	-8	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067596.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	KM675660.1	-	-
	VT0879	VTP017	3427	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067596.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
	VT0880	VTP017	3428	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AY604848.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
	VT0882	VTP017	3430	-	Y	GCGCACGC	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KF057955.1	1644	100	921	1	926	99.5	0.1	-	-	-	-

Strain sequence								Description BLAST Hit #1												Description BLAST Hit #2		Description BLAST Hit #3	
VT name[a]	Sample	BAV #	INA (°C)[b]	Tree[c]	Trimmed 16S sequence	Query length	Class	Family	Genus	Description	Accession number	Total score	Query Coverage %	Identities	Number of gaps	Alignment length	Frequency number of identities (%)	Frequency number of gaps (%)	Description	Accession number	Description	Accession number
VT0883	VTP017	3431	-	-	GCGCACGT/	927	α-proteobacteria	Rhizobiaceae	Agrobacterium	Agrobacteriur	JQ809690.1	1667	100	926	0	927	99.9	0.0	Agrobacteriur	NR_114990.1	-	-
VT0884	VTP017	3432	-8	-	GCGCGCGT/	270	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KT362720.1	479	100	268	0	270	99.3	0.0	Pseudomonas	AB968092.1	-	-
VT0885	VTP017	3433	-	Y	GCGCGCGT/	927	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_040860.1	1645	100	921	0	927	99.4	0.0	-	-	-	-
VT0888	VTP017	3436	-	Y	GCGCACGC/	924	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KT580675.1	1707	100	924	0	924	100.0	0.0	Pantoea aggl	KT075203.1	-	-
VT0894	VTP017	3442	-8	Y	GCGCACGC/	936	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1724	100	935	0	936	99.9	0.0	Pantoea anan	NR_103927.1	-	-
VT0896	VTP017	3444	-4	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0900	VTP017	3448	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP005970.1	8328	100	925	0	926	99.9	0.0	-	-	-	-
VT0903	VTP017	3451	-12	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0905	VTP017	3453	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067596.1	1662	100	925	1	926	99.9	0.1	Pantoea anan	KM675660.1	-	-
VT0909	VTP017	3457	-10	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067596.1	1662	100	925	0	926	99.9	0.0	-	-	-	-
VT0910	VTP017	3458	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	AY741161.1	1651	100	921	0	925	99.6	0.0	Pantoea sp. X	KR296703.1	-	-
VT0916b	VTP017	4520	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP005970.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT0917	VTP017	3465	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	AY741161.1	1660	100	923	0	925	99.8	0.0	-	-	-	-
VT0920	VTP017	3468	-	Y	GCGCACGC/	933	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1718	100	933	1	934	99.9	0.1	-	-	-	-
VT0925b	VTP017	4580	-8	Y	GCGCACGC/	828	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	LC027549.1	1519	100	827	1	829	99.8	0.1	-	-	-	-
VT0926	VTP017	3474	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0928	VTP017	3476	-8	Y	GCGCACGC/	933	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1662	100	929	1	933	99.6	0.1	-	-	-	-
VT0938	VTP017	3486	-10	Y	GCGCACGC/	934	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1667	100	931	1	934	99.7	0.1	-	-	-	-
VT0939	VTP017	3487	-	Y	GCGCGCGT/	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1663	100	924	0	925	99.9	0.0	Pseudomonas	GU396281.1	-	-
VT0940	VTP017	3488	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0941	VTP017	3489	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0942	VTP017	3490	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296702.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0943	VTP017	3491	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296702.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0946	VTP017	3494	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1665	100	925	0	926	99.9	0.0	Pseudomonas	GU396281.1	-	-
VT0950	VTP017	3498	-8	Y	GCGTGCGT/	929	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	KR067599.1	1658	100	926	0	929	99.7	0.0	Xanthomonas	CP010409.1	-	-
VT0952	VTP017	3500	-	-	GAGCTCGT/	929	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	KT580637.1	1671	100	928	0	929	99.9	0.0	Microbacteri	KR085857.1	-	-
VT0953	VTP017	3501	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1654	100	922	0	925	99.7	0.0	-	-	-	-
VT0954	VTP017	3502	-8	Y	GCGCGCGT/	916	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1653	100	916	0	916	100.0	0.0	-	-	-	-
VT0955	VTP017	3503	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0958	VTP017	3506	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0959	VTP017	3507	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1658	100	924	1	926	99.8	0.1	-	-	-	-
VT0960	VTP017	3508	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0961	VTP017	3509	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KF057955.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT0962	VTP017	3510	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067596.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	KM675660.1	-	-
VT0963	VTP017	3511	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067596.1	1662	100	925	1	926	99.9	0.1	Pantoea anan	KM675660.1	-	-
VT0966b	VTP017	4543	-8	Y	GCGCACGC/	753	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. A	KF003417.1	1350	100	751	0	753	99.7	0.0	Pantoea aggl	JQ613283.1	Pantoea euca	JQ446440.1
VT0968	VTP017	3516	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0969	VTP017	3517	-10	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1658	100	924	1	926	99.8	0.1	-	-	-	-
VT0970	VTP017	3518	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0976	VTP017	3524	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KF057955.1	1656	100	923	0	926	99.7	0.0	-	-	-	-
VT0977	VTP017	3525	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1660	100	923	0	925	99.8	0.0	-	-	-	-
VT0978	VTP017	3526	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT0979	VTP017	3527	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP005970.1	8328	100	925	0	926	99.9	0.0	-	-	-	-
VT0981	VTP017	3529	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	DQ512490.1	1660	100	923	0	925	99.8	0.0	-	-	-	-
* VT0982	VTP017	3530	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	JF756691.1	1654	100	922	0	925	99.7	0.0	-	-	-	-
VT0985	VTP017	3533	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP211411.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0986	VTP017	3534	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1663	100	923	0	925	99.8	0.0	Pantoea anan	LC015551.1	-	-
VT0987	VTP017	3535	-8	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1663	100	923	1	926	99.7	0.1	Pantoea anan	KR296701.1	-	-
VT0988	VTP017	3536	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT0989	VTP017	3537	-8	Y	GCGCACGC/	820	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. R	KP708601.1	1474	100	819	0	820	99.9	0.0	-	-	-	-
VT0990	VTP017	3538	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	KR296703.1	1669	100	925	0	925	100.0	0.0	Pantoea anan	LC015551.1	-	-
VT0995	VTP017	3543	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT0996	VTP017	3544	-10	Y	GCGCACGC/	927	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. G	KF465938.1	1631	100	920	2	928	99.1	0.2	Pantoea aggl	KT075203.1	-	-
VT1003	VTP017	3551	-10	Y	GTCAGATGT	903	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1629	100	903	0	903	100.0	0.0	-	-	-	-
VT1004	VTP017	3552	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP005970.1	8328	100	925	0	926	99.9	0.0	-	-	-	-
VT1005	VTP017	3553	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	DQ512490.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT1006	VTP017	3554	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	DQ512490.1	1654	100	922	0	925	99.7	0.0	-	-	-	-
VT1010	VTP017	3558	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1663	100	924	0	925	99.9	0.0	-	-	-	-
VT1011	VTP017	3559	-10	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	HF558391.1	1665	100	925	0	926	99.9	0.0	Pseudomonas	CP007014.1	-	-
VT1012	VTP017	3560	-10	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KR067595.1	1669	100	925	0	925	100.0	0.0	-	-	-	-
VT1013	VTP017	3561	-10	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074597.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT1014	VTP017	3562	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1651	100	921	0	925	99.6	0.0	-	-	-	-
VT1015b	VTP017	4464	-	-	GGGTCCGT/	911	Flavobacteria	Flavobacteriaceae	Chryseobacterium	Chryseobacte	KF441578.1	1672	100	909	0	911	99.8	0.0	-	-	-	-
VT1017	VTP017	3565	-8	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. X	LC015551.1	1703	100	924	0	925	99.9	0.0	Pantoea anan	LC015551.1	-	-
* VT1018	VTP017	3566	-8	-	GGCGGTCT(	796	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KR296701.1	1173	100	745	4	799	93.2	0.5	Pantoea stew	AB907781.1	-	-
VT1019	VTP017	3567	-4	Y	GCGCGCGT/	655	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KM187433.1	1157	100	651	1	656	99.2	0.2	Pseudomonas	KP796138.1	-	-
VT1020	VTP019	3620	-	Y	GCGTGCGT/	918	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	DQ213024.1	1561	100	897	0	918	97.7	0.0	-	-	-	-
VT1021	VTP019	3621	-	Y	GCGCACGC/	926	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea anan	KF057955.1	1647	100	921	0	926	99.5	0.0	-	-	-	-
VT1022	VTP019	3622	-	Y	GCGTGCGT/	928	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	DQ213024.1	1638	100	920	0	928	99.1	0.0	Luteimonas al	JX501253.1	-	-
VT1023	VTP019	3623	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC822785.1	1649	100	921	0	926	99.5	0.0	-	-	-	-
VT1024	VTP019	3624	-8	-	GCGCGCGT/	556	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB968092.1	976	100	550	0	556	98.9	0.0	-	-	-	-
VT1025	VTP019	3625	-	Y	GCGCACGC/	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea sp. C	KT580675.1	1660	100	923	0	925	99.8	0.0	Pantoea aggl	KT075203.1	-	-
VT1026	VTP019	3626	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Kocuria	Kocuria rose	KR085904.1	1624	100	921	3	931	98.9	0.3	-	-	-	-
VT1027	VTP019	3627	-	Y	TGGAATTCC	834	Bacilli	Bacillaceae	Bacillus	Bacillus sp. S	KF956669.1	1505	100	834	0	834	100.0	0.0	Bacillus cere	KR185833.1	-	-
VT1028	VTP019	3628	-	-	GCGTGCGC/	924	β-proteobacteria	Oxalobacteraceae	Massilia	Massilia sp. !	FR865961.1	1667	100	924	0	924	100.0	0.0	-	-	-	-
VT1029	VTP019	3629	-	Y	GTTGTGAAA	899	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	HQ891021.1	1617	100	898	0	899	99.9	0.0	Xanthomonas	CP012251.1	Xanthomonas	CP012146.1
VT1031	VTP019	3631	-	Y	GCGCGCGC(	928	Bacilli	Bacillaceae	Bacillus	Bacillus sp. R	KF030227.1	1665	100	926	0	928	99.8	0.0	Bacillus simp	KM888120.1	-	-
VT1033	VTP019	3633	-	Y	TACTGGGC(	938	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	DQ133572.1	1649	100	930	0	938	99.1	0.0	-	-	-	-
VT1034	VTP019	3634	-	-	GAGCTCGT/	929	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	KT580637.1	1667	100	927	0	929	99.8	0.0	Microbacteri	KR085857.1	-	-
VT1035	VTP019	3635	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteri	HG799975.1	1665	100	926	0	928	99.8	0.0	-	-	-	-
VT1036	VTP019	3636	-	-	GCGTGCGC/	924	β-proteobacteria	Oxalobacteraceae	Massilia	Uncultured M	HQ264085.1	1653	100	921	0	924	99.7	0.0	-	-	-	-
VT1037	VTP019	3637	-8	-	GCGCGCGT/	535	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB968092.1	977	100	532	0	535	99.4	0.0	-	-	-	-
VT1038	VTP019	3638	-	-	GGGGTGAA	875	α-proteobacteria	Caulobacteraceae	Brevundimonas	Brevundimon	KR085853.1	1573	100	874	0	875	99.9	0.0	-	-	-	-
VT1039b	VTP019	3640	-12	Y	GCGTGCGT/	928	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP012251.1	1674	100	928	0	928	100.0	0.0	Xanthomonas	CP012146.1	-	-
VT1040	VTP021a	3776	-	Y	GCGGTTTCT	913	Bacilli	Bacillaceae	Bacillus	Bacillus pum	KT624198.1	1615	100	906	7	913	99.2	0.8	-	-	-	-
VT1041	VTP021a	3777	-	Y	GGGCTCGC/	925	Bacilli	Bacillaceae	Bacillus	Bacillus sp. S	KF956695.1	1658	100	924	1	926	99.8	0.1	-	-	-	-
VT1042	VTP021a	3778	-	Y	GTCTGATGT	902	Bacilli	Bacillaceae	Bacillus	Bacillus myc	KR233766.1	1609	100	901	2	904	99.7	0.2	Bacillus weih	KP790050.1	-	-
VT1043	VTP021a	3779	-	Y	GTCTGATGT	906	Bacilli	Bacillaceae	Bacillus	Bacillus simp	KJ567108.2	1626	100	904	0	906	99.8	0.0	-	-	-	-
VT1044	VTP021a	3780	-	Y	GGGCTCGC/	926	Bacilli	Bacillaceae	Bacillus	Bacillus sp. K	LN874212.1	1665	100	925	0	926	99.9	0.0	Bacillus subti	KT580625.1	-	-
VT1045	VTP021a	3781	-	Y	GTCTGATGC	905	Bacilli	Bacillaceae	Bacillus	Bacillus megz	KT580611.1	1615	100	901	0	905	99.6	0.0	Bacillus aryal	KR085881.1	-	-
VT1046a	VTP021a	3782	-	-	GTCAGTGG1	892	Sphingobacteria	Sphingomonadaceae	Olivibacter	Olivibacter s	KT328577.1	1501	100	869	0	892	97.4	0.0	-	-	-	-

Strain sequence							Description BLAST Hit #1					Description BLAST Hit #1							Description BLAST Hit #2		Description BLAST Hit #3	
VT name[a]	Sample	BAV #	INA (°C)[b]	Tree[c]	Trimmed 16S sequence	Query length	Class	Family	Genus	Description	Accession number	Total score	Query Coverage %	Identities	Number of gaps	Alignment length	Frequency number of identities (%)	Frequency number of gaps (%)	Description	Accession number	Description	Accession number
VT1047	VTP021a3783	-	-	GAGCTCGTA	929	Actinobacteria	Micrococcaceae	Arthrobacter	Arthrobacter	KR233753.1	1654	100	927	2	931	99.6	0.2	Arthrobacter	NR_025362.1	-	-	
VT1048	VTP021a3784	-	Y	GCGCGCGC	924	Bacilli	Bacillaceae	Bacillus	Bacillus cere	KC434970.1	1660	100	924	1	925	99.9	0.1	-	-	-	-	
VT1049	VTP021a3785	-	-	GAGCTCGTA	928	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	JN644517.1	1665	100	926	0	928	99.8	0.0	Microbacteri	CP012299.1	Microbacteri	KR085857.1	
* VT1050	VTP021a3786	-	-	GAGTTCGTA	888	Flavobacteria	Flavobacteriaceae	Epilithonimonas	Epilithonimor	NR_115989.1	1418	100	850	2	890	95.5	0.2	Epilithonimor	JX293123.1	-	-	
VT1051	VTP021a3787	-	-	GAGCTCGTA	928	Actinobacteria	Microbacteriaceae	Curtobacterium	Curtobacteri	HG799975.1	1662	100	926	0	928	99.8	0.0	-	-	-	-	
VT1052	VTP021t3788	-4	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	AY574914.1	-	-	
VT1054	VTP021t3790	-	Y	GCGTGCGT	928	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP012251.1	1669	100	927	0	928	99.9	0.0	Xanthomonas	CP012146.1	-	-	
VT1055	VTP021t3791	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1627	100	918	0	926	99.1	0.0	Pseudomonas	AY574914.1	-	-	
VT1056	VTP021t3792	-	-	GAGCTCGTA	929	Actinobacteria	Microbacteriaceae	Microbacterium	Microbacteri	KT580637.1	1653	100	924	0	929	99.5	0.0	Microbacteri	KR085857.1	-	-	
VT1057	VTP021t3793	-	Y	GCGTGCGT	893	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP011827.1	3154	100	887	1	894	99.2	0.1	-	-	-	-	
VT1058	VTP022 3794	-	-	GAGCTCGTA	929	Actinobacteria	Micrococcaceae	Arthrobacter	Arthrobacter	FM213390.2	1671	100	928	0	929	99.9	0.0	Arthrobacter	EU710551.1	-	-	
VT1059	VTP022 3795	-	-	GAGCTCGTA	928	Actinobacteria	Microbacteriaceae	Salinibacterium	Salinibacteri	HQ327119.1	1660	100	925	0	928	99.7	0.0	Microbacteri	FJ907242.1	-	-	
VT1060	VTP024 3798	-4	Y	GCGCGCGT	774	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KT223376.1	1397	100	774	0	774	100.0	0.0	-	-	-	-	
VT1061	VTP024 3799	-	Y	GCGCGGGC	927	Bacilli	Bacillaceae	Bacillus	Bacillus sp. B	KR998244.1	1608	100	915	1	927	98.7	0.1	-	-	-	-	
VT1062	VTP024 3800	-	Y	GGAATTCCA	830	Bacilli	Bacillaceae	Bacillus	Bacillus sp. R	KP267844.1	1498	100	830	0	830	100.0	0.0	-	-	-	-	
VT1063	VTP026a3808	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1665	100	925	0	926	99.9	0.0	-	-	-	-	
VT1064	VTP026a3809	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1665	100	925	0	926	99.9	0.0	-	-	-	-	
VT1065	VTP026a3810	-10	Y	GCGCGCGC	927	Bacilli	Bacillaceae	Lysinibacillus	Lysinibacillus	LN870303.1	1685	100	922	0	927	99.5	0.0	Lysinibacillus	JQ744623.1	Acetobacter s	KF956626.1	
VT1066	VTP026a3811	-10	Y	GCGCGCGC	927	Bacilli	Bacillaceae	Lysinibacillus	Lysinibacillus	LN870303.1	1649	100	922	0	927	99.5	0.0	Lysinibacillus	KJ767321.1	-	-	
VT1068	VTP026a3813	-	-	GAGCTCGTA	927	Actinobacteria	Microbacteriaceae	Frigoribacterium	Frigoribacteri	EU584512.1	1663	100	925	0	927	99.8	0.0	-	-	-	-	
VT1069	VTP026t3814	-	-	TGTAGCGGT	169	Bacilli	Bacillaceae	Bacillus	Bacillus megz	JQ833618.1	291	100	165	0	169	97.6	0.0	Bacillus subti	JQ830729.1	Bacillus pumi	JQ830389.1	
VT1072	VTP026t3817	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1640	100	920	1	926	99.4	0.1	Pseudomonas	NR_074597.1	-	-	
VT1076	VTP026t3821	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1665	100	925	0	926	99.9	0.0	Pseudomonas	NR_074597.1	-	-	
VT1078	VTP026t3823	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1665	100	925	0	926	99.9	0.0	Pseudomonas	NR_074597.1	-	-	
* VT1079a	VTP026t3824	-	Y	GGGCTCGC	917	Bacilli	Bacillaceae	Bacillus	Bacillus aero	KC519433.1	1652	100	911	2	919	99.1	0.2	Bacillus pumi	KF956587.1	Bacillus altit	FN668692.1	
VT1081	VTP026t3826	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1653	100	922	0	926	99.6	0.0	Pseudomonas	NR_074597.1	-	-	
VT1083	VTP028a3960	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JF327447.1	1656	100	923	0	926	99.7	0.0	-	-	-	-	
VT1084	VTP028a3961	-8	Y	GCGCGCGT	784	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KT029454.1	1407	100	784	1	785	99.9	0.1	-	-	-	-	
VT1085a	VTP028a3962	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1086	VTP028a3963	-12	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
* VT1087	VTP028a3964	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1089	VTP028a3966	-4	Y	GCGCGCGT	721	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074598.1	1296	100	720	0	721	99.9	0.0	-	-	-	-	
VT1091	VTP028a3968	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1092	VTP028a3969	-	Y	GCGCACGC	925	γ-proteobacteria	Enterobacteriaceae	Pantoea	Pantoea aggl	KT075174.1	1669	100	925	0	925	100.0	0.0	-	-	-	-	
VT1093	VTP028a3970	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1094	VTP028a3971	-4	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AY574913.1	1662	100	924	0	926	99.8	0.0	Pseudomonas	CP000075.1	-	-	
VT1096	VTP028a3973	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1098	VTP028a3975	-4	-	GCGCGCGT	556	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP005970.1	4992	100	555	0	556	99.8	0.0	-	-	-	-	
VT1099	VTP028a3976	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1103	VTP028a3980	-4	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP211411.1	1665	100	925	0	926	99.9	0.0	-	-	-	-	
* VT1104	VTP028a3981	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AY574913.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	CP000075.1	-	-	
VT1105	VTP028a3982	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP796138.1	1671	100	926	0	926	100.0	0.0	-	-	-	-	
VT1107	VTP028a3984	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1108	VTP028a3985	-	-	AAAGCCCAT	213	Flavobacteria	Flavobacteriaceae	Flavobacterium	Flavobacteri	KC912601.1	355	100	206	0	213	96.7	0.0	-	-	-	-	
VT1109	VTP028a3986	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1110	VTP028a3987	-	-	GTAGCGGT	532	Sphingobacteria	Sphingobacteriaceae	Pedobacter	Pedobacter sp	KM035962.1	850	100	526	0	532	98.9	0.0	-	-	-	-	
VT1111	VTP028a3988	-	-	GTCAGTGG	883	Flavobacteria	Flavobacteriaceae	Flavobacterium	Flavobacteri	AM934652.1	1505	100	869	4	887	98.0	0.5	-	-	-	-	
VT1112	VTP028a3989	-	Y	GTTGGATG	904	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	904	0	904	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1113	VTP028a3990	-	-	GTCAGTGG	887	Flavobacteria	Flavobacteriaceae	Flavobacterium	Flavobacteri	AM934652.1	1591	100	885	0	887	99.8	0.0	-	-	-	-	
VT1114	VTP028a3991	-	-	GTCAGAGG	822	Sphingobacteria	Sphingobacteriaceae	Pedobacter	Pedobacter gl	NR_109023.1	1427	100	812	2	823	98.7	0.2	Pedobacter d	EF660750.1	-	-	
VT1115	VTP028a3992	-	-	GAATTCCGA	831	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	KF805085.1	1492	100	831	1	832	99.9	0.1	-	-	-	-	
VT1116	VTP028a3993	-	-	TGGCGAAG	825	α-proteobacteria	Sphingomonadaceae	Sphingomonas	Sphingomona	KM891564.1	839	100	454	0	454	100.0	0.0	-	-	-	-	
VT1117	VTP028a3994	-	-	GCGCGCGT	892	Bacilli	Staphylococcaceae	Staphylococcus	Staphylococc	JX866757.1	1591	100	888	0	892	99.6	0.0	Staphylococc	CP002110.1	-	-	
VT1118	VTP028a3995	-	-	TCGTAGGC	478	Actinobacteria	Micrococcaceae	Kocuria	Kocuria sp. S	KT025921.1	850	100	473	1	479	98.7	0.2	Kocuria palus	KP345961.1	-	-	
VT1119	VTP028a3996	-	-	GTCAGAGG	889	Sphingobacteria	Sphingobacteriaceae	Pedobacter	Pedobacter d	EF660750.1	1599	100	888	0	889	99.9	0.0	-	-	-	-	
VT1120	VTP028a3997	-	-	AATGAGAC	812	Sphingobacteria	Sphingobacteriaceae	Pedobacter	Pedobacter yr	KC119214.1	1436	100	807	1	813	99.3	0.1	-	-	-	-	
VT1121	VTP028a3998	-	-	GTCAGGGG	891	Sphingobacteria	Sphingobacteriaceae	Pedobacter	Pedobacter yr	KC119214.1	1584	100	886	0	891	99.4	0.0	-	-	-	-	
VT1122	VTP028a3999	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909655.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	AJ308316.1	-	-	
VT1123	VTP028a4000	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1124	VTP028a4001	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1125	VTP028a4002	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1126	VTP028a4003	-	-	GGGTCCGT	909	Flavobacteria	Flavobacteriaceae	Flavobacterium	Flavobacteri	AM934652.1	1631	100	907	0	909	99.8	0.0	-	-	-	-	
VT1127	VTP028a4004	-	-	GGGTGCGT	911	Sphingobacteria	Sphingobacteriaceae	Pedobacter	Pedobacter d	EF660750.1	1638	100	910	0	911	99.9	0.0	-	-	-	-	
VT1128	VTP028a4005	-	-	GTCAGAGG	872	Sphingobacteria	Sphingobacteriaceae	Pedobacter	Pedobacter d	EF660750.1	1555	100	871	2	874	99.7	0.2	-	-	-	-	
* VT1129	VTP028a4006	-	-	GGGTCCGT	908	Flavobacteria	Flavobacteriaceae	Flavobacterium	Flavobacteri	AM934652.1	1631	100	906	1	909	99.7	0.1	-	-	-	-	
VT1130	VTP028a4007	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
* VT1131	VTP028a4008	-	-	GGCGGTTT	900	Flavobacteria	Flavobacteriaceae	Flavobacterium	Flavobacteri	AM934652.1	1652	100	898	0	900	99.8	0.0	-	-	-	-	
* VT1132	VTP028a4009	-	Y	GCGCGCGT	933	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KF054779.1	1676	100	933	1	934	99.9	0.1	-	-	-	-	
VT1133	VTP028a4010	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AY574913.1	1671	100	924	0	926	99.8	0.0	Pseudomonas	CP000075.1	-	-	
VT1134	VTP028a4011	-8	Y	GCGCGCGT	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AY574913.1	1649	100	922	1	926	99.6	0.1	-	-	-	-	
* VT1135	VTP028a4012	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1137	VTP028a4014	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1138	VTP028a4015	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1139	VTP028a4016	-4	-	AACTGCATC	549	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP745131.1	985	100	548	0	549	99.8	0.0	Pseudomonas	KM187433.1	-	-	
* VT1141	VTP028a4018	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ843152.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF054779.1	-	-	
VT1143	VTP028a4020	-	Y	GCGCGCGT	827	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KM187378.1	1478	100	826	2	828	99.8	0.2	-	-	-	-	
* VT1146	VTP028t4023	-8	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP000075.1	8330	100	926	0	926	100.0	0.0	-	-	-	-	
VT1147	VTP028t4024	-4	Y	GCGCGCGT	657	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	1180	100	656	0	657	99.8	0.0	-	-	-	-	
VT1149	VTP028t4026	-	Y	GCGCGCGT	927	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AM909656.1	1663	100	926	1	927	99.9	0.1	Pseudomonas	JN800482.1	-	-	
VT1154	VTP028t4031	-	Y	GCGTGCGT	928	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	CP008714.1	3349	100	928	0	928	100.0	0.0	-	-	-	-	
VT1155	VTP028t4032	-	Y	GCGCGCGT	925	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_024912.1	1649	100	922	1	926	99.6	0.1	Pseudomonas	AY689076.1	-	-	
VT1157	VTP028t4034	-8	Y	GCGCGCGT	919	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1606	100	912	3	921	99.0	0.3	-	-	-	-	
VT1161	VTP028t4038	-	Y	GCGCGCGT	826	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	1485	100	825	0	826	99.9	0.0	-	-	-	-	
VT1162	VTP028t4039	-8	Y	TAAAGCGC	723	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	EF540504.1	1290	100	720	0	723	99.6	0.0	Pseudomonas	NR_114482.1	-	-	
VT1164	VTP028t4041	-8	-	GCGTAGGT	302	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	558	100	302	0	302	100.0	0.0	Pseudomonas	KJ720652.1	Pseudomonas	KC786974.1	
VT1167	VTP028t4044	-	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR085910.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KP208622.1	Pseudomonas	CP005975.1	
VT1169	VTP028t4046	-8	-	AACTGCATC	218	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP192860.1	398	100	217	0	218	99.5	0.0	Pseudomonas	KJ830937.1	-	-	
VT1170	VTP028t4047	-	Y	GGGCTCGC	926	Bacilli	Bacillaceae	Bacillus	Bacillus aeri	KR140379.1	1658	100	924	1	926	99.8	0.1	Bacillus aero	KR085933.1	Bacillus strat	KR085786.1	
VT1171	VTP028t4048	-8	Y	GCGCGCGT	922	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	CP007014.1	8132	100	920	3	925	99.5	0.3	-	-	-	-	
VT1172	VTP028t4049	-4	Y	GCGCGCGT	702	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	1258	100	700	0	702	99.7	0.0	Pseudomonas	NR_074597.1	-	-	
VT1173	VTP028t4050	-12	Y	GCGCGCGT	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1665	100	925	0	926	99.9	0.0	Pseudomonas	NR_074597.1	-	-	

Strain sequence							Description BLAST Hit #1											Description BLAST Hit #2		Description BLAST Hit #3	
VT name[a]	Sample	BAV #	INA (°C)[b]	Tree[c]	Trimmed 16S sequence	Query length	Class	Family	Genus	Description	Accession number	Total score	Query Coverage %	Identities	Number of gaps	Alignment length	Frequency number of identities (%)	Frequency number of gaps (%)	Description	Accession number	Description	Accession number
VT1177	VTP028t	4054	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KR080566.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	KF003398.1	-	-
VT1181	VTP028t	4058	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1662	100	924	0	926	99.8	0.0	Pseudomonas	NR_074597.1	-	-
VT1182	VTP028t	4059	-8	Y	GCGCGCGT/	670	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	1189	100	667	1	671	99.4	0.1	Pseudomonas	KC311259.1	-	-
VT1184	VTP028t	4061	-4	-	CAGTGAAAT	204	γ-proteobacteria	Xanthomonadaceae	Xanthomonas	Xanthomonas	KR067599.1	315	99	193	2	203	95.1	1.0	-	-	-	-
VT1187	VTP028t	4064	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074597.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT1191	VTP028t	4068	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	NR_074597.1	1665	1000	925	0	926	99.9	0.0	-	-	-	-
VT1192	VTP028t	4069	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1665	100	925	0	926	99.9	0.0	Pseudomonas	GU396281.1	-	-
VT1193	VTP028t	4070	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1665	100	925	0	926	99.9	0.0	Pseudomonas	GU396281.1	-	-
VT1194	VTP028t	4071	-8	Y	GCGCGCGT/	859	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KC311259.1	1541	100	857	0	859	99.8	0.0	-	-	-	-
VT1196b	VTP028t	4544	-8	Y	GCGTAGGTC	798	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	1463	100	796	0	798	99.7	0.0	Pseudomonas	KC311259.1	-	-
VT1199	VTP028t	4076	-12	-	GGTGGTTTC	448	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	811	100	445	0	448	99.3	0.0	Pseudomonas	KJ720652.1	Pseudomonas	KC311259.1
VT1200	VTP028t	4077	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT1201	VTP028t	4078	-8	Y	GCGCGCGT/	719	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	1288	100	717	0	719	99.7	0.0	Pseudomonas	KC311259.1	-	-
VT1202	VTP028t	4079	-8	Y	GCGCGCGT/	912	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1679	100	911	1	912	99.9	0.1	-	-	-	-
VT1203	VTP028t	4080	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP211411.1	1671	100	926	0	926	100.0	0.0	-	-	-	-
VT1206b	VTP028t	4524	-	-	GCGCGCGT/	441	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB968092.1	791	100	440	0	441	99.8	0.0	-	-	-	-
* VT1208	VTP028t	4085	-10	-	GCGCGCGT/	193	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KT362720.1	349	100	193	0	193	100.0	0.0	-	-	-	-
VT1214	VTP028t	4091	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Plantibacter	Plantibacter :	HE662660.2	1669	100	927	0	928	99.9	0.0	-	-	-	-
* VT1215	VTP028t	4092	-	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	JX067671.1	1705	100	925	0	926	99.9	0.0	Pseudomonas	GU396281.1	-	-
VT1216	VTP028t	4093	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1662	100	924	0	926	99.8	0.0	-	-	-	-
VT1217	VTP028t	4094	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AY604848.1	1671	100	926	0	926	100.0	0.0	Pseudomonas	HE603504.1	-	-
VT1218	VTP028t	4095	-8	Y	GCGCGCGT/	926	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	AB001440.1	1665	100	925	0	926	99.9	0.0	-	-	-	-
VT1219	VTP028t	4096	-8	Y	GCGCGCGT/	722	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	1288	100	719	0	722	99.6	0.0	Pseudomonas	KC311259.1	-	-
VT1223	VTP028t	4100	-8	-	AACTGCATC	304	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	545	100	301	0	304	99.0	0.0	Pseudomonas	KJ720652.1	-	-
VT1226	VTP028t	4103	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Plantibacter	Plantibacter :	HE662660.2	1662	100	926	1	928	99.8	0.1	-	-	-	-
VT1228	VTP028t	4105	-	-	GAGCTCGT/	928	Actinobacteria	Microbacteriaceae	Plantibacter	Plantibacter :	HE662660.2	1660	100	925	1	928	99.7	0.1	-	-	-	-
* VT1242	VTP028t	4119	-4	-	AACTGCATC	218	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KP192860.1	403	100	218	0	218	100.0	0.0	Pseudomonas	KJ830937.1	Pseudomonas	KJ720652.1
VT1243	VTP028t	4120	-12	-	GCGCGCGT/	782	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	1202	100	743	7	785	94.6	0.9	-	-	-	-
VT1244	VTP028t	4121	-8	-	TTTGTTAAG	230	γ-proteobacteria	Pseudomonadaceae	Pseudomonas	Pseudomonas	KJ830937.1	420	100	230	1	231	99.6	0.4	Pseudomonas	KJ720652.1	Pseudomonas	KC311259.1
VT1246	VTP028t	4123	-	Y	GGAATTCCA	829	Bacilli	Bacillaceae	Bacillus	Bacillus mega	KT580611.1	1496	100	829	0	829	100.0	0.0	-	-	-	-

[a] Strains for which sequencing showed a possible culture contamination are labeled with an *

[b] INA was measured with 6 drops of 20µL for a bacterial concentration standardized at an OF of 0.2 and from -4°C to -12°C. When no value is indicated,

[c] Sequences from Enterobacteriaceae, Xanthomonadaceae, Pseudomonadaceae and Bacillaceae with a length up to 600pb were used to build phylogenetic trees.

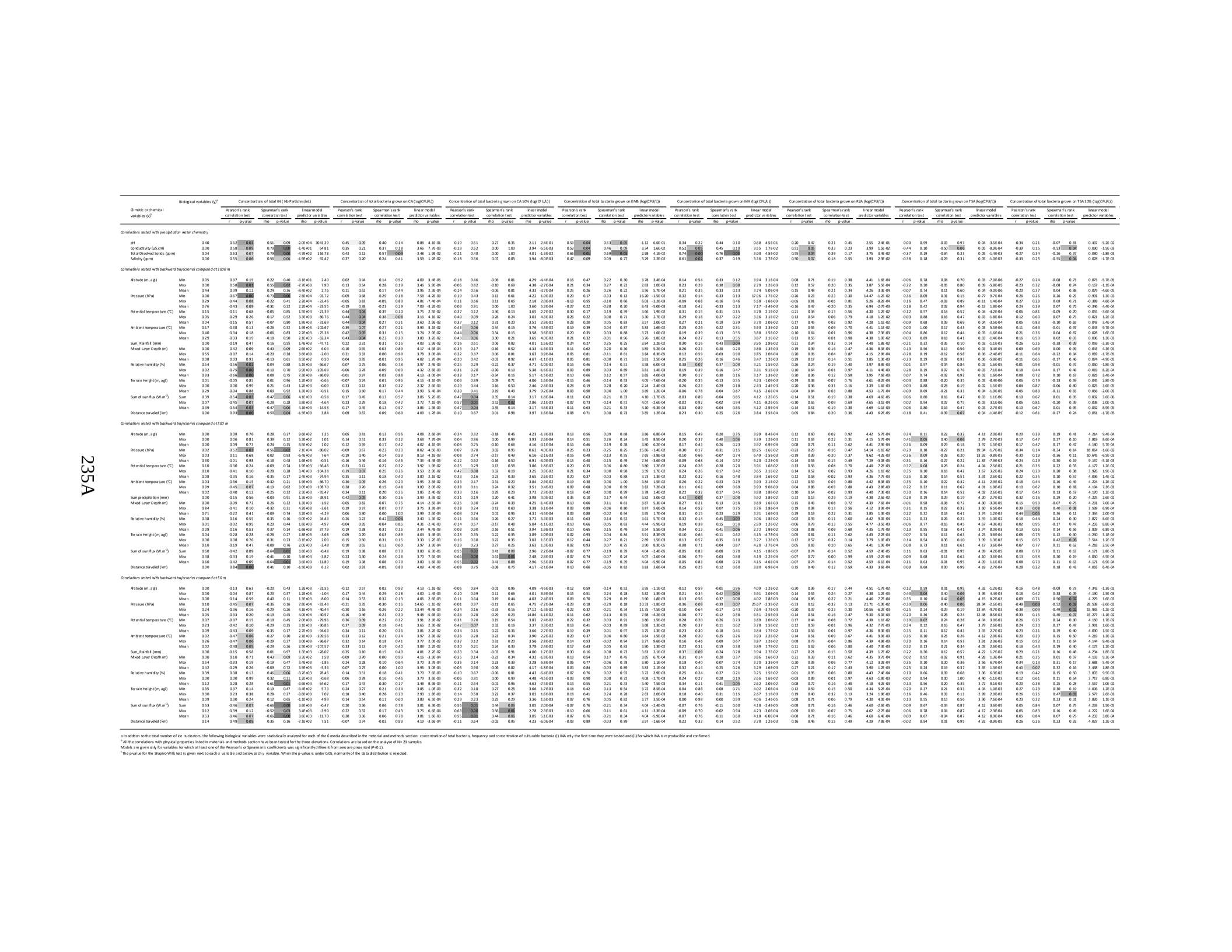
Supplementary Table S4. Estimation of the number of Ice+ bacteria and bacterial IN active at −8°C in clouds based on concentrations of Ice+ bacteria in precipitation.

Variables used	**Min value observed**	**Average**	**Max value observed**
Concentration of Ice^+ bacteria in rain water all samples and media combined (CFU/L)			
Based on initial INA test	40	901 ± 240	21,840
Based on standardized re-testing	0	384 ± 147	13,219
Concentration of IN in rain water (number of IN/L)	11	1117 ± 528	7,440
Number of IN per CFU at −8°C			
Based on *Pseudomonas syringae* CC94	1×10^{-5}		
Based on *Lysinibacillus sp.* strains BAV 3810/3811	4×10^{-8}		

Estimations[a]	**Low estimate**	**Average estimate**	**High estimate**
Number of Ice^+ bacteria active at −8°C in a cloud (CFU/m^3 of cloud)			
(1) Using concentations of Ice+ bacteria from standardized re-resting and assuming that all bacteria are cultivable	0.00	0.37	13.22
(2) Assuming (1) but that cultured Ice^+ bacteria represented only 0.0228% of total bacteria in clouds (according to Tong & Lighthart, 1997)	0.00	16.84	580.73
(3) Assuming (2) but using concentrations of Ice^+ bacteria based on initial INA test	1.75	39.52	1092.00
Number of IN produced by Ice^+ bacteria active at −8°C in a cloud (number of IN/m^3 of cloud)			
Assuming that each Ice^+ CFU corresponds to one active IN in clouds		same values as in (1)	
Assuming the number of bacterial IN produced in clouds by Ice^+ bacteria correspond to the fraction of IN per cell obtained in the lab with pure cultures.	0.00	1.9×10^{-6}	1.3×10^{-4}

[a] Estimations were done using the maximum liquid water content observed in clouds (1 g of water /m3 of cloud) by Crawford *et al.* (2012). For low and high estimates, the lowest and highest concentrations of Ice^+ bacteria observed in precipitation samples were used, while average concentrations of Ice^+bacteria were used for average estimates.

Supplementary Table S5. Correlations between total IN, concentrations and frequencies of culturable Ice+ bacteria, and concentration of total culturable bacteria with air mass properties over the 24h preceding the event and with precipitation water chemistry. Isolates that had a minimal activity during the original INA characterization were considered as potentially ice nucleation active when at least one drop out of three froze after 10 min of incubation at -8°C. Purified isolated were considered fully ice nucleation active when at least 2 drops out of three froze using bacterial suspensions of 0.2 OD600nm.



Climatic or chemical variables (x)[b]		Biological variables (y)[a]	Frequency of Ice+ bacteria grown on CA that had a minimal activity during the original INA characterization (%)						Frequency of of Ice+ bacteria grown on CA 10% that had a minimal activity during the original INA characterization (%)						Frequency of of Ice+ bacteria grown on EMB that had a minimal activity during the original INA characterization (%)					
			Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
			r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry																				
pH		0.40	-0.26	0.35	-0.01	0.97	0.17	-1.9E-02	-0.42	0.14	-0.24	0.41	0.22	-2.7E-02	-0.17	0.56	-0.27	0.33	0.22	-2.1E-02
Conductivity (µS.cm)		0.00	-0.37	0.17	-0.50	0.06	0.04	-6.8E-04	-0.27	0.35	-0.22	0.44	0.03	-4.1E-04	-0.35	0.20	-0.51	0.05	0.09	-1.1E-03
Total Disolved Solids (ppm)		0.04	-0.40	0.16	-0.29	0.31	0.03	-6.7E-04	-0.15	0.64	-0.10	0.74	0.02	-2.6E-04	-0.25	0.40	-0.34	0.24	0.07	-1.3E-03
Salinity (ppm)		0.00	-0.40	0.16	-0.37	0.19	0.03	-5.0E-04	-0.11	0.72	0.01	0.99	0.02	-1.5E-04	-0.26	0.36	-0.26	0.37	0.07	-1.0E-03
Correlations tested with backward trajectories computed at 1000 m																				
Altitude (m, agl)	Min	0.05	0.03	0.88	0.19	0.38	0.02	3.5E-06	-0.20	0.42	0.04	0.86	0.03	-1.8E-05	-0.18	0.43	-0.03	0.90	0.07	-3.2E-05
	Max	0.00	-0.15	0.51	0.02	0.92	0.06	-3.4E-05	-0.25	0.29	-0.15	0.54	0.08	-4.8E-05	-0.23	0.30	-0.11	0.63	0.16	-9.3E-05
	Mean	0.44	-0.01	0.95	0.26	0.24	0.03	-1.7E-06	-0.21	0.39	0.09	0.71	0.04	-2.1E-05	-0.13	0.55	0.09	0.71	0.07	-2.6E-05
Pressure (hPa)	Min	0.00	0.25	0.26	0.25	0.25	-0.47	6.0E-04	0.22	0.36	0.19	0.44	-0.34	4.4E-04	0.30	0.18	0.34	0.13	-0.97	1.2E-03
	Max	0.29	0.10	0.64	-0.08	0.71	-0.06	8.5E-05	0.25	0.30	-0.03	0.91	-0.15	1.8E-04	0.23	0.31	0.03	0.90	-0.26	3.3E-04
	Mean	0.76	0.21	0.33	-0.13	0.55	-0.15	1.9E-04	0.30	0.21	0.00	0.99	-0.18	2.2E-04	0.17	0.45	0.00	0.99	-0.18	2.6E-04
Potential temperature (°C)	Min	0.15	-0.01	0.97	0.15	0.49	0.03	-2.7E-05	-0.09	0.71	-0.09	0.70	0.03	-2.5E-04	-0.14	0.55	-0.05	0.82	0.07	-7.2E-04
	Max	0.05	0.06	0.80	0.24	0.28	0.02	2.7E-04	0.02	0.94	0.01	0.98	0.02	7.4E-05	-0.01	0.97	0.15	0.51	0.06	-6.7E-05
	Mean	0.04	-0.02	0.94	0.08	0.71	0.03	-6.1E-05	-0.03	0.89	-0.11	0.66	0.02	-1.0E-04	-0.07	0.75	-0.01	0.96	0.06	-4.3E-04
Ambient temperature (°C)	Min	0.17	0.11	0.63	0.18	0.40	0.02	4.5E-04	0.07	0.77	-0.07	0.77	0.02	2.8E-04	0.02	0.92	0.15	0.50	0.05	1.7E-04
	Max	0.40	0.04	0.86	0.11	0.62	0.02	1.5E-04	0.11	0.64	0.01	0.97	0.02	3.5E-04	-0.01	0.97	0.13	0.58	0.06	-5.4E-05
	Mean	0.29	0.07	0.77	0.13	0.54	0.02	2.7E-04	0.09	0.71	-0.01	0.97	0.02	3.2E-04	-0.01	0.96	0.13	0.55	0.06	-7.7E-05
Sum_Rainfall (mm)		0.00	-0.17	0.45	-0.23	0.29	0.03	-7.4E-04	-0.24	0.33	-0.44	0.06	0.03	-8.5E-04	-0.21	0.36	-0.13	0.56	0.06	-1.6E-03
Mixed Layer Depth (m)	Min	0.00	0.28	0.19	0.05	0.84	0.02	6.6E-05	0.33	0.17	0.21	0.38	0.01	6.2E-05	0.21	0.35	0.04	0.86	0.04	8.2E-05
	Max	0.55	-0.24	0.26	-0.22	0.31	0.05	-1.9E-05	-0.19	0.44	-0.21	0.39	0.04	-1.3E-05	-0.26	0.25	0.00	0.99	0.09	-3.5E-05
	Mean	0.08	-0.06	0.77	-0.13	0.55	0.03	-1.4E-05	-0.01	0.98	-0.06	0.82	0.02	-1.6E-06	-0.15	0.50	0.15	0.50	0.08	-6.0E-05
Relative humidity (%)	Min	0.54	0.23	0.28	0.05	0.81	0.00	4.2E-04	0.18	0.47	0.01	0.98	0.01	2.7E-04	0.15	0.50	0.24	0.28	0.03	4.6E-04
	Max	0.02	0.29	0.18	0.12	0.59	-0.03	6.5E-04	0.19	0.43	0.11	0.67	-0.01	3.7E-04	0.40	0.07	0.35	0.11	-0.07	1.6E-03
	Mean	0.33	0.24	0.27	0.09	0.68	-0.01	4.8E-04	0.19	0.44	0.06	0.81	0.00	3.3E-04	0.24	0.28	0.28	0.20	0.00	8.2E-04
Terrain Height (m, agl)	Min	0.01	-0.18	0.42	-0.12	0.60	0.03	-4.0E-05	-0.12	0.63	-0.24	0.32	0.03	-2.2E-05	0.09	0.70	-0.01	0.96	0.05	3.4E-05
	Max	0.00	-0.10	0.64	-0.05	0.83	0.06	-4.4E-05	-0.12	0.63	-0.17	0.49	0.06	-5.6E-05	0.26	0.24	0.02	0.93	-0.09	2.1E-04
	Mean	0.29	-0.30	0.17	-0.11	0.60	0.05	-5.8E-05	-0.26	0.28	-0.28	0.24	0.04	-4.6E-05	0.04	0.87	-0.04	0.86	0.05	1.3E-05
Sum of sun flux ($W.m^{-2}$)	Sum	0.39	0.14	0.51	0.08	0.71	0.01	2.4E-06	0.13	0.60	-0.13	0.61	0.01	1.9E-06	0.08	0.72	0.13	0.56	0.04	2.5E-06
	Max	0.07	0.11	0.61	-0.03	0.90	0.02	1.9E-05	0.13	0.61	-0.19	0.43	0.01	1.8E-05	0.04	0.85	0.01	0.95	0.05	1.2E-05
	Mean	0.39	0.14	0.51	0.08	0.71	0.01	6.0E-05	0.13	0.60	-0.13	0.61	0.01	4.7E-05	0.08	0.72	0.13	0.56	0.04	6.2E-05
Distance traveled (km)		0.00	-0.04	0.84	-0.34	0.12	0.03	-3.2E-06	0.04	0.86	-0.11	0.67	0.02	2.5E-06	-0.16	0.49	-0.25	0.26	0.07	-1.9E-05
Correlations tested with backward trajectories computed at 500 m																				
Altitude (m, agl)	Min	0.00	-0.27	0.24	-0.08	0.73	0.07	-5.7E-05	-0.02	0.94	0.06	0.82	0.02	-3.6E-06	0.02	0.91	0.04	0.85	0.05	1.1E-05
	Max	0.00	-0.23	0.32	-0.08	0.74	0.17	-1.1E-04	-0.23	0.33	-0.22	0.36	0.05	-5.4E-05	-0.09	0.68	-0.08	0.72	0.08	-4.5E-05
	Mean	0.00	-0.20	0.37	0.04	0.88	0.08	-4.6E-05	-0.16	0.52	-0.03	0.89	0.03	-2.5E-05	-0.07	0.77	0.02	0.95	0.06	-2.1E-05
Pressure (hPa)	Min	0.02	0.26	0.26	0.26	0.25	-0.99	1.3E-03	0.26	0.29	0.20	0.42	-0.47	5.6E-04	0.35	0.11	0.33	0.14	-1.28	1.5E-03
	Max	0.03	0.27	0.23	0.09	0.71	-0.39	4.6E-04	0.24	0.33	0.13	0.60	-0.21	2.3E-04	0.22	0.32	0.12	0.58	-0.38	4.5E-04
	Mean	0.30	0.24	0.29	0.07	0.75	-0.35	4.4E-04	0.34	0.15	0.23	0.35	-0.29	3.2E-04	0.29	0.19	0.23	0.30	-0.48	5.6E-04
Potential temperature (°C)	Min	0.10	-0.06	0.81	-0.09	0.70	0.05	-3.6E-04	0.05	0.84	-0.04	0.89	0.02	1.3E-04	0.01	0.97	0.17	0.44	0.05	4.1E-05
	Max	0.10	0.12	0.60	0.07	0.75	0.02	1.2E-03	-0.02	0.92	-0.09	0.70	0.02	-8.6E-05	-0.06	0.78	0.12	0.60	0.06	-4.5E-04
	Mean	0.08	0.05	0.83	-0.10	0.65	0.04	3.4E-04	0.01	0.96	0.02	0.95	0.02	3.5E-05	-0.04	0.87	0.21	0.35	0.06	-2.1E-04
Ambient temperature (°C)	Min	0.03	0.11	0.63	-0.01	0.97	0.04	9.7E-04	0.09	0.70	-0.05	0.85	0.02	3.1E-04	0.05	0.83	0.17	0.46	0.05	3.1E-04
	Max	0.39	0.21	0.36	0.04	0.87	0.03	1.6E-03	0.11	0.64	0.01	0.98	0.02	3.9E-04	0.01	0.95	0.15	0.51	0.05	9.0E-05
	Mean	0.02	0.16	0.50	0.02	0.93	0.04	1.3E-03	0.12	0.63	0.02	0.94	0.02	4.0E-04	0.04	0.85	0.12	0.59	0.05	2.8E-04
Sum precipitation (mm)		0.00	-0.26	0.25	-0.38	0.09	0.06	-2.3E-03	-0.16	0.52	-0.45	0.05	0.02	-5.7E-04	-0.23	0.31	-0.17	0.44	0.06	-1.8E-03
Mixed Layer Depth (m)	Min	0.00	0.09	0.69	0.00	0.99	0.04	4.3E-05	0.62	0.00	0.36	0.13	0.01	1.3E-04	0.53	0.01	0.33	0.13	0.03	2.4E-04
	Max	0.84	-0.11	0.64	-0.22	0.34	0.07	-1.7E-05	-0.21	0.40	-0.23	0.34	0.04	-1.7E-05	-0.25	0.26	0.00	1.00	0.10	-4.2E-05
	Mean	0.71	-0.11	0.65	-0.17	0.46	0.07	-4.9E-05	0.14	0.58	0.03	0.89	0.01	3.0E-05	0.01	0.95	0.14	0.52	0.05	6.3E-06
Relative humidity (%)	Min	0.38	-0.01	0.98	-0.01	0.97	0.05	-1.8E-05	0.15	0.53	0.02	0.92	0.00	3.4E-04	0.22	0.32	0.13	0.56	0.00	9.9E-04
	Max	0.01	0.18	0.44	0.17	0.46	-0.02	8.2E-04	0.20	0.41	0.14	0.57	-0.03	6.1E-04	0.30	0.17	0.21	0.35	-0.08	1.6E-03
	Mean	0.29	0.08	0.72	0.10	0.67	0.03	3.4E-04	0.14	0.57	0.02	0.93	0.00	3.4E-04	0.19	0.39	0.08	0.73	-0.01	9.0E-04
Terrain Height (m, agl)	Min	0.04	0.06	0.79	-0.13	0.59	0.04	2.8E-05	-0.29	0.22	-0.34	0.16	0.03	-5.2E-05	-0.22	0.32	-0.17	0.44	0.07	-8.0E-05
	Max	0.00	0.04	0.87	-0.06	0.80	0.02	3.6E-05	-0.21	0.38	-0.23	0.34	0.07	-7.6E-05	-0.24	0.28	-0.27	0.22	0.18	-1.8E-04
	Mean	0.10	-0.05	0.83	-0.11	0.65	0.06	-2.0E-05	-0.37	0.12	-0.43	0.07	0.04	-6.2E-05	-0.26	0.24	-0.32	0.15	0.09	-9.1E-05
Sum of sun flux ($W.m^{-2}$)	Sum	0.60	0.10	0.67	0.01	0.95	0.03	3.6E-06	-0.17	0.48	-0.20	0.42	0.04	-2.7E-06	-0.13	0.57	0.01	0.95	0.08	-4.1E-06
	Max	0.38	0.06	0.81	-0.20	0.39	0.04	2.0E-05	-0.05	0.83	-0.15	0.54	0.03	-8.3E-06	-0.10	0.67	-0.02	0.92	0.07	-3.2E-05
	Mean	0.60	0.10	0.67	0.01	0.95	0.03	8.9E-05	-0.17	0.48	-0.20	0.42	0.04	-6.7E-05	-0.13	0.57	0.01	0.95	0.08	-1.0E-04
Distance traveled (km)		0.00	-0.12	0.61	-0.27	0.24	0.06	-1.7E-05	-0.03	0.92	-0.15	0.55	0.02	-2.7E-06	-0.18	0.42	-0.28	0.21	0.07	-4.0E-05
Correlations tested with backward trajectories computed at 50 m																				
Altitude (m, agl)	Min	0.00	-0.01	0.96	0.17	0.43	0.03	-4.5E-05	-0.10	0.67	-0.19	0.43	0.02	-3.7E-04	-0.04	0.87	0.08	0.73	0.06	-2.9E-04
	Max	0.00	-0.07	0.75	-0.27	0.21	0.03	-3.2E-05	-0.21	0.38	-0.34	0.15	0.03	-7.5E-05	-0.07	0.77	-0.21	0.34	0.06	-5.1E-05
	Mean	0.00	-0.06	0.78	-0.35	0.10	0.03	-6.1E-05	-0.23	0.34	-0.49	0.03	0.03	-1.9E-04	-0.07	0.74	-0.39	0.07	0.06	-1.3E-04
Pressure (hPa)	Min	0.10	0.29	0.18	0.44	0.04	-0.77	8.6E-04	0.27	0.26	0.33	0.17	-0.64	7.1E-04	0.39	0.07	0.40	0.06	-1.82	2.0E-03
	Max	0.24	0.53	0.01	0.54	0.01	-0.85	8.8E-04	0.41	0.08	0.35	0.14	-0.58	6.1E-04	0.51	0.02	0.49	0.02	-1.40	1.5E-03
	Mean	0.05	0.57	0.00	0.57	0.00	-0.99	1.1E-03	0.47	0.04	0.48	0.04	-0.72	7.7E-04	0.54	0.01	0.52	0.01	-1.62	1.7E-03
Potential temperature (°C)	Min	0.07	0.01	0.95	0.08	0.72	0.03	4.5E-05	-0.01	0.97	-0.06	0.80	0.02	-2.4E-05	-0.03	0.89	0.18	0.42	0.06	-1.8E-04
	Max	0.23	0.00	1.00	0.06	0.78	0.03	-2.4E-06	-0.05	0.85	-0.11	0.65	0.03	-1.4E-04	-0.07	0.77	0.07	0.75	0.06	-3.9E-04
	Mean	0.09	0.02	0.92	0.13	0.55	0.02	8.0E-05	-0.01	0.98	-0.03	0.89	0.02	-2.3E-05	-0.04	0.86	0.19	0.40	0.06	-2.4E-04
Ambient temperature (°C)	Min	0.02	0.15	0.49	0.22	0.31	0.02	5.4E-04	0.10	0.67	-0.01	0.98	0.02	3.4E-04	0.08	0.72	0.24	0.27	0.05	5.1E-04
	Max	0.26	0.11	0.63	0.24	0.27	0.02	3.6E-04	0.04	0.87	-0.01	0.97	0.02	1.2E-04	0.04	0.87	0.20	0.37	0.05	2.1E-04
	Mean	0.02	0.12	0.60	0.17	0.43	0.02	4.1E-04	0.07	0.78	-0.02	0.92	0.02	2.1E-04	0.05	0.84	0.22	0.33	0.05	2.8E-04
Sum_Rainfall (mm)		0.00	-0.10	0.64	-0.22	0.32	0.03	-3.5E-04	-0.04	0.86	-0.23	0.35	0.02	-1.2E-04	-0.15	0.51	-0.06	0.79	0.06	-8.7E-04
Mixed Layer Depth (m)	Min	0.00	0.47	0.02	0.13	0.55	0.01	1.1E-04	0.50	0.03	0.28	0.24	0.01	9.6E-05	0.39	0.07	0.13	0.57	0.04	1.6E-04
	Max	0.54	-0.10	0.65	-0.08	0.71	0.03	-8.2E-06	-0.30	0.22	-0.32	0.17	0.05	-2.2E-05	-0.27	0.22	-0.09	0.68	0.10	-4.1E-05
	Mean	0.42	0.21	0.35	-0.05	0.83	0.00	4.8E-05	0.08	0.76	0.01	0.96	0.01	1.6E-05	0.00	1.00	0.21	0.36	0.05	3.0E-07
Relative humidity (%)	Min	0.39	0.16	0.45	0.03	0.89	0.00	4.6E-04	0.29	0.23	0.14	0.55	-0.01	6.8E-04	0.23	0.30	0.13	0.55	0.00	1.1E-03
	Max	0.00	0.20	0.35	0.07	0.77	-0.03	6.2E-04	0.26	0.27	0.24	0.32	-0.05	7.8E-04	0.29	0.20	0.19	0.39	-0.08	1.5E-03
	Mean	0.12	0.19	0.38	-0.01	0.97	-0.01	5.3E-04	0.30	0.21	0.10	0.67	-0.03	7.6E-04	0.24	0.27	0.09	0.68	-0.03	1.2E-03
Terrain Height (m, agl)	Min	0.35	-0.54	0.01	-0.54	0.01	0.06	-1.3E-04	-0.43	0.07	-0.45	0.05	0.04	-8.7E-05	-0.45	0.04	-0.51	0.02	0.10	-1.8E-04
	Max	0.00	-0.31	0.16	-0.56	0.01	0.13	-1.6E-04	-0.25	0.31	-0.35	0.14	0.10	-1.1E-04	-0.32	0.14	-0.49	0.02	0.25	-3.0E-04
	Mean	0.09	-0.58	0.00	-0.56	0.01	0.09	-1.5E-04	-0.47	0.04	-0.45	0.05	0.07	-1.0E-04	-0.47	0.03	-0.52	0.01	0.15	-2.1E-04
Sum of sun flux ($W.m^{-2}$)	Sum	0.53	-0.01	0.98	0.06	0.78	0.03	-8.6E-08	-0.13	0.60	-0.16	0.51	0.03	-1.8E-06	-0.09	0.70	0.08	0.72	0.07	-2.6E-06
	Max	0.12	0.09	0.68	0.08	0.71	0.02	1.5E-05	-0.03	0.89	-0.09	0.72	0.02	-4.7E-06	-0.05	0.83	0.07	0.75	0.06	-1.4E-05
	Mean	0.53	-0.01	0.98	0.06	0.78	0.03	-2.2E-06	-0.13	0.60	-0.16	0.51	0.03	-4.5E-05	-0.09	0.70	0.08	0.72	0.07	-6.4E-05
Distance traveled (km)		0.14	0.10	0.63	-0.28	0.19	0.02	2.7E-05	0.14	0.58	-0.10	0.68	0.02	2.7E-05	0.04	0.87	-0.15	0.50	0.05	1.6E-05

Climatic or chemical variables (x)[b]		Frequency of of Ice+ bacteria grown on MA that had a minimal activity during the original INA characterization (%)						Frequency of of Ice+ bacteria grown on R2A that had a minimal activity during the original INA characterization (%)						Frequency of of Ice+ bacteria grown on TSA that had a minimal activity during the original INA characterization (%)						Frequency of of Ice+ bacteria grown on TSA 10% that had a minimal activity during the original INA characterization (%)					
		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry																									
pH		-0.22	0.43	-0.26	0.34	0.25	-2.7E-02	0.20	0.47	0.21	0.45	2.55	2.4E-01	0.00	0.99	-0.03	0.93	0.04	-3.5E-04	-0.34	0.21	-0.07	0.81	0.437	-5.2E-02
Conductivity (µS.cm)		-0.40	0.14	-0.48	0.07	0.07	-1.2E-03	0.51	0.05	0.33	0.23	3.99	1.5E-02	-0.44	0.10	-0.50	0.06	0.05	-8.9E-04	-0.39	0.15	-0.53	0.04	0.090	-1.5E-03
Total Disolved Solids (ppm)		-0.32	0.27	-0.38	0.18	0.07	-1.8E-03	0.55	0.04	0.39	0.17	3.75	3.4E-02	-0.37	0.19	-0.34	0.23	0.05	-1.4E-03	-0.27	0.34	-0.26	0.37	0.080	-1.8E-03
Salinity (ppm)		-0.32	0.26	-0.27	0.35	0.06	-1.4E-03	0.50	0.07	0.18	0.55	3.93	2.3E-02	-0.38	0.18	-0.29	0.31	0.05	-1.0E-03	-0.33	0.25	-0.55	0.04	0.078	-1.7E-03
Correlations tested with backward trajectories computed at 1000 m																									
Altitude (m, agl)	Min	-0.19	0.39	-0.04	0.86	0.06	-3.2E-05	0.08	0.71	0.19	0.38	4.41	1.6E-04	-0.06	0.78	0.08	0.70	0.03	-7.0E-06	-0.27	0.24	-0.08	0.73	0.073	-5.7E-05
	Max	-0.18	0.41	-0.10	0.63	0.12	-7.0E-05	0.12	0.57	0.20	0.35	3.87	5.5E-04	-0.22	0.30	-0.05	0.80	0.09	-5.8E-05	-0.23	0.32	-0.08	0.74	0.167	-1.1E-04
	Mean	-0.13	0.55	0.05	0.82	0.06	-2.4E-05	0.15	0.48	0.21	0.34	4.26	3.3E-04	-0.07	0.74	0.11	0.60	0.04	-9.0E-06	-0.20	0.37	0.04	0.88	0.079	-4.6E-05
Pressure (hPa)	Min	0.27	0.21	0.16	0.47	-0.86	1.1E-03	-0.26	0.23	-0.23	0.30	14.47	-1.2E-02	0.36	0.09	0.31	0.15	-0.77	9.7E-04	0.26	0.26	0.26	0.25	-0.991	1.3E-03
	Max	0.18	0.41	0.00	1.00	-0.19	2.4E-04	-0.05	0.81	-0.05	0.81	5.26	-8.2E-04	0.16	0.47	-0.03	0.89	-0.11	1.4E-04	0.27	0.23	0.09	0.71	-0.389	4.6E-04
	Mean	0.14	0.52	-0.04	0.86	-0.15	2.1E-04	-0.16	0.47	-0.20	0.35	6.94	-2.7E-03	0.18	0.42	0.02	0.94	-0.13	1.8E-04	0.24	0.29	0.07	0.75	-0.346	4.4E-04
Potential temperature (°C)	Min	-0.13	0.57	-0.17	0.44	0.05	-6.3E-04	0.21	0.34	0.13	0.56	4.30	1.2E-02	-0.12	0.57	0.14	0.52	0.04	-4.2E-04	-0.06	0.81	-0.09	0.70	0.055	-3.6E-04
	Max	0.03	0.90	-0.06	0.79	0.04	2.2E-04	0.13	0.54	0.06	0.79	4.18	1.2E-02	-0.03	0.88	0.16	0.47	0.03	-1.8E-04	0.12	0.60	0.07	0.75	0.021	1.2E-03
	Mean	-0.04	0.86	-0.15	0.48	0.05	-2.2E-04	0.17	0.45	0.02	0.92	4.28	1.1E-02	-0.09	0.68	0.09	0.69	0.04	-3.5E-04	0.05	0.83	-0.10	0.65	0.043	3.4E-04
Ambient temperature (°C)	Min	0.03	0.91	-0.08	0.72	0.04	1.8E-04	0.13	0.55	0.09	0.70	4.41	1.1E-02	0.00	1.00	0.17	0.43	0.03	5.5E-06	0.11	0.63	-0.01	0.97	0.043	9.7E-04
	Max	0.05	0.82	-0.09	0.67	0.04	3.0E-04	0.10	0.64	0.01	0.96	4.38	7.3E-03	-0.04	0.86	0.17	0.44	0.03	-1.6E-04	0.21	0.36	0.04	0.87	0.028	1.6E-03
	Mean	0.01	0.95	-0.12	0.58	0.04	8.9E-05	0.13	0.55	0.01	0.98	4.38	1.0E-02	-0.03	0.89	0.18	0.41	0.03	-1.4E-04	0.16	0.50	0.02	0.93	0.036	1.3E-03
Sum_Rainfall (mm)		-0.26	0.24	-0.21	0.33	0.05	-1.9E-03	0.21	0.34	0.32	0.14	4.40	1.8E-02	-0.21	0.32	-0.35	0.10	0.03	-1.1E-03	-0.26	0.25	-0.38	0.09	0.059	-2.3E-03
Mixed Layer Depth (m)	Min	0.12	0.58	0.04	0.84	0.03	4.6E-05	0.19	0.39	0.16	0.47	4.36	8.3E-04	0.13	0.56	0.13	0.56	0.03	3.4E-05	0.09	0.69	0.00	0.99	0.043	4.3E-05
	Max	-0.17	0.43	-0.19	0.39	0.06	-2.1E-05	0.20	0.35	0.04	0.87	4.15	2.9E-04	-0.28	0.19	-0.12	0.58	0.06	-2.4E-05	-0.11	0.64	-0.22	0.34	0.069	-1.7E-05
	Mean	-0.14	0.53	0.01	0.97	0.07	-5.1E-05	0.29	0.17	0.14	0.51	3.85	1.3E-03	-0.23	0.29	-0.02	0.93	0.06	-5.8E-05	-0.11	0.65	-0.17	0.46	0.074	-4.9E-05
Relative humidity (%)	Min	-0.07	0.75	-0.12	0.60	0.05	-2.1E-04	0.26	0.24	0.19	0.40	3.97	8.8E-03	0.01	0.97	-0.04	0.84	0.03	1.6E-05	-0.01	0.98	-0.01	0.97	0.050	-1.8E-05
	Max	0.21	0.33	0.09	0.68	-0.02	7.9E-04	0.10	0.64	-0.01	0.97	4.11	4.4E-03	0.28	0.19	0.07	0.76	-0.03	7.1E-04	0.18	0.44	0.17	0.46	-0.019	8.2E-04
	Mean	0.04	0.86	-0.03	0.90	0.03	1.3E-04	0.20	0.36	0.12	0.58	3.95	7.6E-03	0.07	0.74	-0.02	0.92	0.02	1.6E-04	0.08	0.72	0.10	0.67	0.025	3.4E-04
Terrain Height (m, agl)	Min	0.18	0.42	0.17	0.44	0.03	6.5E-05	-0.19	0.38	-0.07	0.76	4.61	-8.2E-04	-0.03	0.88	-0.20	0.35	0.03	-8.4E-06	0.06	0.79	-0.13	0.59	0.045	2.8E-05
	Max	0.14	0.53	0.10	0.66	-0.02	9.6E-05	0.20	0.36	0.31	0.16	3.39	1.6E-03	0.03	0.88	-0.28	0.19	0.02	1.5E-05	0.04	0.87	-0.06	0.80	0.025	3.6E-05
	Mean	0.08	0.70	0.08	0.71	0.03	2.7E-05	-0.04	0.84	0.03	0.88	4.53	-1.7E-04	-0.09	0.69	-0.21	0.33	0.04	-1.9E-05	-0.05	0.83	-0.11	0.65	0.056	-2.0E-05
Sum of sun flux ($W.m^{-2}$)	Sum	0.13	0.56	0.07	0.75	0.02	3.5E-06	-0.14	0.51	-0.19	0.38	4.69	-4.6E-05	0.06	0.80	0.16	0.47	0.03	1.1E-06	0.10	0.67	0.01	0.95	0.032	3.6E-06
	Max	0.06	0.77	-0.08	0.71	0.03	1.7E-05	-0.10	0.65	-0.09	0.69	4.65	-3.1E-04	0.02	0.94	0.07	0.75	0.03	3.1E-06	0.06	0.81	-0.20	0.39	0.038	2.0E-05
	Mean	0.13	0.56	0.07	0.75	0.02	8.7E-05	-0.14	0.51	-0.19	0.38	4.69	-1.1E-03	0.06	0.80	0.16	0.47	0.03	2.7E-05	0.10	0.67	0.01	0.95	0.032	8.9E-05
Distance traveled (km)		-0.18	0.42	-0.18	0.40	0.05	-2.1E-05	0.05	0.84	0.20	0.36	4.43	6.2E-05	-0.18	0.41	-0.39	0.07	0.04	-1.4E-05	-0.12	0.61	-0.27	0.24	0.061	-1.7E-05
Correlations tested with backward trajectories computed at 500 m																									
Altitude (m, agl)	Min	0.08	0.72	-0.03	0.88	0.04	3.3E-05	-0.14	0.52	-0.25	0.24	0.04	-3.6E-05	-0.11	0.61	-0.10	0.66	0.03	-3.2E-05	0.04	0.87	-0.05	0.83	0.047	2.0E-05
	Max	-0.07	0.77	-0.03	0.88	0.06	-3.0E-05	-0.24	0.27	-0.27	0.21	0.07	-6.6E-05	-0.18	0.41	-0.22	0.31	0.06	-5.6E-05	-0.23	0.32	-0.16	0.49	0.123	-1.3E-04
	Mean	-0.02	0.95	-0.05	0.83	0.04	-4.6E-06	-0.21	0.33	-0.18	0.40	0.04	-3.8E-05	-0.19	0.38	-0.22	0.32	0.04	-4.0E-05	-0.08	0.75	-0.09	0.70	0.056	-2.8E-05
Pressure (hPa)	Min	0.35	0.10	0.18	0.41	-1.23	1.5E-03	0.53	0.01	0.51	0.01	-1.12	1.3E-03	0.45	0.03	0.37	0.08	-1.07	1.3E-03	0.37	0.10	0.38	0.09	-1.627	1.9E-03
	Max	0.16	0.48	0.09	0.69	-0.25	3.0E-04	0.38	0.07	0.37	0.08	-0.40	4.4E-04	0.40	0.06	0.41	0.05	-0.48	5.2E-04	0.23	0.31	0.35	0.12	-0.497	5.6E-04
	Mean	0.25	0.25	0.18	0.42	-0.40	4.6E-04	0.46	0.03	0.40	0.06	-0.46	5.1E-04	0.46	0.03	0.47	0.02	-0.52	5.8E-04	0.32	0.15	0.40	0.08	-0.670	7.6E-04
Potential temperature (°C)	Min	0.00	0.98	-0.05	0.83	0.04	2.3E-05	-0.06	0.79	0.05	0.84	0.03	-1.7E-04	-0.07	0.75	0.07	0.75	0.03	-2.3E-04	0.10	0.67	0.01	0.96	0.043	6.0E-04
	Max	-0.05	0.81	-0.12	0.58	0.05	-3.5E-04	-0.10	0.66	0.06	0.79	0.04	-3.8E-04	-0.09	0.67	0.10	0.66	0.04	-4.2E-04	0.03	0.90	-0.02	0.94	0.044	2.4E-04
	Mean	-0.02	0.91	-0.02	0.92	0.04	-1.3E-04	-0.08	0.73	0.13	0.55	0.04	-2.5E-04	-0.09	0.69	0.15	0.50	0.03	-3.2E-04	0.06	0.79	0.08	0.72	0.043	4.4E-04
Ambient temperature (°C)	Min	0.04	0.85	-0.08	0.72	0.04	2.5E-04	0.04	0.87	0.17	0.45	0.03	1.3E-04	0.02	0.92	0.18	0.40	0.03	9.3E-05	0.13	0.57	0.03	0.90	0.043	9.8E-04
	Max	0.03	0.90	-0.14	0.53	0.04	1.7E-04	0.07	0.75	0.18	0.41	0.03	2.7E-04	0.05	0.81	0.28	0.20	0.03	2.2E-04	0.15	0.51	0.10	0.68	0.030	1.2E-03
	Mean	0.04	0.85	-0.17	0.45	0.04	2.6E-04	0.04	0.84	0.15	0.50	0.03	1.6E-04	0.03	0.88	0.22	0.31	0.03	1.4E-04	0.16	0.48	0.01	0.96	0.035	1.3E-03
Sum precipitation (mm)		-0.23	0.28	-0.19	0.39	0.05	-1.6E-03	-0.24	0.26	-0.21	0.34	0.04	-1.0E-03	-0.23	0.28	-0.41	0.05	0.03	-1.1E-03	-0.19	0.40	-0.45	0.04	0.055	-1.7E-03
Mixed Layer Depth (m)	Min	0.38	0.08	0.31	0.14	0.02	1.6E-04	0.31	0.16	0.24	0.27	0.02	8.0E-05	0.38	0.07	0.25	0.26	0.02	1.1E-04	0.34	0.13	0.31	0.17	0.029	1.8E-04
	Max	-0.20	0.37	-0.22	0.32	0.07	-2.9E-05	-0.25	0.25	-0.25	0.26	0.06	-2.2E-05	-0.17	0.43	-0.06	0.77	0.05	-1.7E-05	-0.21	0.35	-0.25	0.27	0.096	-4.2E-05
	Mean	-0.10	0.66	-0.09	0.69	0.06	-3.9E-05	-0.17	0.45	-0.15	0.51	0.05	-4.0E-05	-0.02	0.92	0.03	0.89	0.03	-6.0E-06	-0.14	0.54	-0.22	0.34	0.083	-7.1E-05
Relative humidity (%)	Min	0.14	0.53	0.05	0.83	0.01	5.9E-04	0.04	0.87	0.07	0.76	0.03	9.3E-05	0.12	0.58	0.05	0.82	0.01	3.6E-04	0.10	0.68	-0.04	0.85	0.021	5.1E-04
	Max	0.18	0.40	0.14	0.53	-0.04	9.0E-04	0.13	0.56	0.15	0.49	0.00	3.8E-04	0.19	0.37	0.11	0.63	-0.03	6.5E-04	0.15	0.50	0.13	0.58	-0.034	9.7E-04
	Mean	0.06	0.80	-0.02	0.92	0.02	2.4E-04	0.02	0.91	0.05	0.81	0.03	6.2E-05	0.08	0.70	-0.03	0.89	0.01	2.4E-04	0.06	0.81	-0.05	0.82	0.028	3.1E-04
Terrain Height (m, agl)	Min	-0.19	0.38	0.04	0.85	0.05	-6.7E-05	-0.29	0.17	-0.16	0.46	0.04	-6.2E-05	-0.36	0.09	-0.38	0.07	0.05	-8.5E-05	-0.29	0.20	-0.30	0.19	0.073	-1.2E-04
	Max	-0.31	0.14	-0.21	0.34	0.19	-2.2E-04	-0.36	0.09	-0.40	0.06	0.14	-1.6E-04	-0.37	0.08	-0.42	0.04	0.15	-1.8E-04	-0.33	0.14	-0.41	0.07	0.249	-2.9E-04
	Mean	-0.26	0.23	-0.09	0.68	0.07	-8.7E-05	-0.36	0.09	-0.26	0.24	0.06	-7.3E-05	-0.39	0.06	-0.47	0.03	0.06	-8.9E-05	-0.36	0.11	-0.34	0.13	0.104	-1.5E-04
Sum of sun flux ($W.m^{-2}$)	Sum	-0.06	0.78	-0.03	0.89	0.05	-1.8E-06	-0.08	0.72	0.02	0.93	0.04	-1.3E-06	-0.03	0.88	0.12	0.57	0.03	-6.3E-07	-0.22	0.34	-0.11	0.63	0.091	-8.3E-06
	Max	-0.01	0.96	-0.04	0.87	0.04	-2.8E-06	-0.06	0.78	-0.02	0.91	0.04	-1.1E-05	0.00	0.99	0.12	0.58	0.03	3.3E-07	-0.14	0.55	-0.19	0.40	0.080	-5.3E-05
	Mean	-0.06	0.78	-0.03	0.89	0.05	-4.5E-05	-0.08	0.72	0.02	0.93	0.04	-3.4E-05	-0.03	0.88	0.12	0.57	0.03	-1.6E-05	-0.22	0.34	-0.11	0.63	0.091	-2.1E-04
Distance traveled (km)		-0.20	0.37	-0.14	0.54	0.06	-4.1E-05	-0.33	0.13	-0.36	0.09	0.05	-4.1E-05	-0.22	0.31	-0.31	0.16	0.04	-3.1E-05	-0.19	0.41	-0.29	0.21	0.069	-4.8E-05
Correlations tested with backward trajectories computed at 50 m																									
Altitude (m, agl)	Min	-0.14	0.53	0.08	0.71	0.04	-1.0E-03	-0.06	0.77	0.05	0.81	0.03	-2.8E-04	-0.08	0.72	-0.08	0.73	0.03	-4.0E-04	-0.11	0.63	0.03	0.90	0.052	-1.0E-03
	Max	-0.02	0.92	-0.13	0.57	0.04	-1.7E-05	-0.20	0.37	-0.32	0.14	0.04	-8.5E-05	-0.15	0.51	-0.37	0.08	0.04	-7.2E-05	-0.21	0.36	-0.40	0.07	0.065	-1.9E-04
	Mean	-0.04	0.85	-0.29	0.18	0.04	-7.1E-05	-0.19	0.40	-0.52	0.01	0.04	-1.9E-04	-0.16	0.47	-0.41	0.05	0.03	-1.8E-04	-0.21	0.35	-0.43	0.05	0.058	-4.3E-04
Pressure (hPa)	Min	0.34	0.11	0.21	0.34	-1.48	1.6E-03	0.59	0.00	0.64	0.00	-1.55	1.7E-03	0.51	0.01	0.50	0.02	-1.50	1.6E-03	0.40	0.07	0.47	0.03	-2.192	2.4E-03
	Max	0.40	0.06	0.31	0.15	-1.04	1.1E-03	0.57	0.00	0.53	0.01	-0.89	9.3E-04	0.56	0.01	0.46	0.03	-0.99	1.0E-03	0.41	0.07	0.57	0.01	-1.322	1.4E-03
	Mean	0.46	0.03	0.33	0.13	-1.30	1.4E-03	0.68	0.00	0.61	0.00	-1.16	1.2E-03	0.64	0.00	0.63	0.00	-1.24	1.3E-03	0.52	0.02	0.60	0.00	-1.845	2.0E-03
Potential temperature (°C)	Min	-0.04	0.87	-0.08	0.72	0.04	-1.9E-04	-0.10	0.67	0.02	0.91	0.04	-3.2E-04	-0.10	0.65	0.07	0.76	0.03	-3.7E-04	0.04	0.88	-0.05	0.82	0.047	2.5E-04
	Max	-0.05	0.84	-0.16	0.46	0.05	-2.5E-04	-0.07	0.76	0.05	0.84	0.04	-2.3E-04	-0.05	0.81	0.16	0.47	0.03	-2.0E-04	0.00	1.00	-0.08	0.73	0.049	5.9E-06
	Mean	-0.03	0.88	-0.06	0.79	0.04	-1.8E-04	-0.08	0.72	0.07	0.74	0.04	-2.7E-04	-0.08	0.72	0.14	0.52	0.03	-3.1E-04	0.03	0.89	0.04	0.85	0.046	2.3E-04
Ambient temperature (°C)	Min	0.06	0.78	-0.03	0.90	0.04	3.6E-04	0.04	0.84	0.21	0.35	0.03	1.5E-04	0.03	0.88	0.21	0.34	0.03	1.4E-04	0.14	0.53	0.02	0.92	0.042	1.1E-03
	Max	0.04	0.84	-0.05	0.81	0.04	2.4E-04	0.06	0.79	0.21	0.34	0.03	2.0E-04	0.06	0.79	0.30	0.16	0.03	2.2E-04	0.10	0.68	0.08	0.73	0.038	6.7E-04
	Mean	0.04	0.86	-0.05	0.82	0.04	2.3E-04	0.03	0.90	0.13	0.55	0.03	9.3E-05	0.02	0.93	0.18	0.41	0.03	8.1E-05	0.12	0.62	0.03	0.91	0.040	8.4E-04
Sum_Rainfall (mm)		-0.14	0.51	-0.08	0.71	0.05	-7.9E-04	-0.19	0.38	-0.12	0.59	0.04	-6.3E-04	-0.17	0.43	-0.37	0.08	0.03	-6.5E-04	-0.07	0.77	-0.26	0.25	0.051	-4.7E-04
Mixed Layer Depth (m)	Min	0.25	0.25	0.16	0.47	0.03	9.9E-05	0.15	0.49	0.05	0.82	0.03	3.6E-05	0.23	0.29	0.12	0.57	0.02	6.2E-05	0.24	0.30	0.13	0.58	0.035	1.1E-04
	Max	-0.29	0.19	-0.34	0.11	0.08	-3.9E-05	-0.26	0.23	-0.20	0.37	0.06	-2.1E-05	-0.15	0.49	0.08	0.73	0.05	-1.4E-05	-0.35	0.12	-0.21	0.36	0.121	-6.2E-05
	Mean	-0.13	0.55	-0.08	0.70	0.07	-5.0E-05	-0.18	0.42	-0.13	0.57	0.05	-4.0E-05	-0.02	0.92	0.06	0.77	0.03	-5.6E-06	-0.19	0.40	-0.19	0.41	0.095	-9.3E-05
Relative humidity (%)	Min	0.21	0.33	0.13	0.55	-0.01	9.7E-04	0.05	0.81	0.04	0.84	0.02	1.5E-04	0.08	0.73	-0.05	0.83	0.02	2.4E-04	0.30	0.19	0.02	0.94	-0.041	1.7E-03
	Max	0.22	0.32	0.12	0.58	-0.05	1.1E-03	0.12	0.57	0.12	0.58	0.00	3.8E-04	0.16	0.47	0.02	0.91	-0.02	5.4E-04	0.26	0.26	0.06	0.80	-0.094	1.6E-03
	Mean	0.18	0.41	0.03	0.91	-0.02	8.1E-04	0.06	0.79	-0.03	0.90	0.02	1.6E-04	0.07	0.74	-0.11	0.62	0.01	2.2E-04	0.28	0.21	-0.02	0.93	-0.066	1.6E-03
Terrain Height (m, agl)	Min	-0.33	0.12	-0.24	0.26	0.07	-1.3E-04	-0.51	0.01	-0.49	0.02	0.06	-1.2E-04	-0.50	0.02	-0.54	0.01	0.06	-1.3E-04	-0.39	0.08	-0.50	0.02	0.098	-1.9E-04
	Max	-0.32	0.14	-0.28	0.20	0.22	-2.8E-04	-0.47	0.02	-0.64	0.00	0.20	-2.5E-04	-0.46	0.03	-0.63	0.00	0.21	-2.7E-04	-0.40	0.07	-0.60	0.00	0.338	-4.3E-04
	Mean	-0.39	0.06	-0.27	0.21	0.11	-1.6E-04	-0.59	0.00	-0.53	0.01	0.10	-1.5E-04	-0.56	0.01	-0.60	0.00	0.10	-1.6E-04	-0.48	0.03	-0.54	0.01	0.163	-2.5E-04
Sum of sun flux ($W.m^{-2}$)	Sum	-0.02	0.94	0.04	0.85	0.04	-4.8E-07	-0.02	0.93	0.11	0.60	0.03	-3.3E-07	-0.01	0.97	0.17	0.43	0.03	-1.3E-07	-0.16	0.49	-0.09	0.69	0.077	-5.5E-06
	Max	0.00	0.99	0.05	0.81	0.04	4.9E-07	0.01	0.96	0.11	0.61	0.03	1.6E-06	0.05	0.83	0.20	0.36	0.03	8.5E-06	-0.13	0.56	-0.10	0.65	0.075	-4.5E-05
	Mean	-0.02	0.94	0.04	0.85	0.04	-1.2E-05	-0.02	0.93	0.11	0.60	0.03	-8.2E-06	-0.01	0.97	0.17	0.43	0.03	-3.3E-06	-0.16	0.49	-0.09	0.69	0.077	-1.4E-04
Distance traveled (km)		-0.03	0.90	0.00	0.99	0.04	-1.2E-05	-0.14	0.53	-0.23	0.29	0.04	-3.5E-05	-0.04	0.85	-0.23	0.28	0.03	-1.1E-05	-0.08	0.73	-0.25	0.28	0.059	-4.0E-05

a In addtion to the total number of ice nucleators, the following biological variables were statistically analyzed for each of the 6 media described in the material and methods section: concentration of total bacteria, frequency and concentration of culturable bacteria (i) INA only the first time they were tested and (ii) for which INA is reproducible and confirmed.

[b] All the correlations with physical properties listed in materials and methods section have been tested for the three elevations. Correlations are based on the analyse of N= 23 samples

Models are given only for variables for which at least one of the Pearson's or Spearman's coefficients was significantly different from zero are presented (P<0.1).

[c] The p-value for the Shapiro-Wilk test is given next to each *x* variable and below each *y* variable. When the p-value is under 0.05, normality of the data distribution is rejected.

Climatic or chemical variables (x)[b]		Biological variables (y)[a]	Frequency of culturable bacteria grown on CA nucleating ice at > -12°C (%)						Frequency of culturable bacteria grown on CA 10% nucleating ice at > -12°C (%)						Frequency of culturable bacteria grown on EMB nucleating ice at > -12°C (%)						Frequency of culturable bacteria grown on MA nucleating ice at > -12°C (%)						Frequency of culturable bacteria grown on R2A nucleating ice at > -12°C (%)						Frequency of culturable bacteria grown on TSA nucleating ice at > -12°C (%)						Frequency of culturable bacteria grown on TSA 10% nucleating ice at > -12°C (%)					
			Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
			r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry																																												
pH		0.40	-0.24	0.38	0.03	0.92	0.15	-1.7E-02	-0.41	0.14	-0.26	0.38	0.13	-1.6E-02	-0.27	0.34	-0.27	0.34	0.25	-2.9E-02	-0.21	0.46	-0.28	0.31	0.21	-2.3E-02	0.15	0.60	-0.06	0.84	-0.04	7.8E-03	-0.02	0.95	-0.02	0.95	0.04	-1.2E-03	-0.29	0.29	-0.16	0.56	0.220	-2.6E-02
Conductivity (µS.cm)		0.00	-0.34	0.21	-0.50	0.06	0.03	-6.0E-04	-0.29	0.32	-0.31	0.28	0.02	-2.7E-04	-0.46	0.08	-0.58	0.02	0.07	-1.2E-03	-0.41	0.13	-0.55	0.03	0.06	-1.1E-03	-0.36	0.19	-0.44	0.10	0.03	-4.7E-04	-0.44	0.10	-0.55	0.04	0.04	-7.6E-04	-0.40	0.14	-0.54	0.04	0.051	-8.8E-04
Total Disolved Solids (ppm)		0.04	-0.39	0.17	-0.27	0.35	0.02	-5.2E-04	-0.21	0.49	-0.14	0.65	0.01	-2.3E-04	-0.44	0.11	-0.50	0.07	0.06	-1.7E-03	-0.31	0.28	-0.45	0.11	0.06	-1.6E-03	-0.29	0.31	-0.40	0.15	0.03	-7.5E-04	-0.39	0.17	-0.41	0.15	0.04	-1.2E-03	-0.33	0.25	-0.31	0.28	0.045	-1.2E-03
Salinity (ppm)		0.00	-0.39	0.17	-0.38	0.18	0.02	-3.8E-04	-0.11	0.71	0.10	0.74	0.01	-9.2E-05	-0.42	0.14	-0.34	0.24	0.06	-1.2E-03	-0.35	0.23	-0.26	0.38	0.05	-1.3E-03	-0.30	0.30	-0.06	0.84	0.03	-5.6E-04	-0.37	0.20	-0.30	0.29	0.04	-8.4E-04	-0.32	0.26	-0.43	0.13	0.040	-8.5E-04
Correlations tested with backward trajectories computed at 1000 m																																												
Altitude (m, agl)	Min	0.05	-0.01	0.95	0.17	0.45	0.02	-1.2E-06	-0.18	0.46	-0.05	0.83	0.02	-1.0E-05	-0.22	0.33	-0.11	0.62	0.05	-3.3E-05	-0.24	0.28	-0.04	0.84	0.05	-3.7E-05	-0.15	0.49	-0.07	0.75	0.02	-1.1E-05	-0.04	0.85	0.02	0.93	0.02	-4.2E-06	-0.27	0.24	-0.14	0.56	0.042	-3.3E-05
	Max	0.00	-0.15	0.49	-0.03	0.91	0.06	-3.3E-05	-0.26	0.29	-0.04	0.86	0.05	-2.9E-05	-0.25	0.27	-0.17	0.44	0.13	-8.3E-05	-0.19	0.39	-0.04	0.86	0.10	-6.6E-05	-0.18	0.42	-0.04	0.85	0.05	-2.8E-05	-0.22	0.31	-0.14	0.51	0.08	-4.9E-05	-0.28	0.21	-0.13	0.58	0.111	-7.6E-05
	Mean	0.44	-0.07	0.75	0.22	0.32	0.02	-7.4E-06	-0.20	0.40	0.06	0.82	0.02	-1.2E-05	-0.21	0.35	-0.03	0.90	0.06	-3.3E-05	-0.17	0.43	0.06	0.79	0.05	-2.9E-05	-0.11	0.62	0.03	0.90	0.02	-8.3E-06	-0.08	0.71	0.05	0.83	0.03	-8.5E-06	-0.22	0.33	0.00	0.99	0.046	-2.9E-05
Pressure (hPa)	Min	0.00	0.22	0.32	0.24	0.27	-0.39	4.9E-04	0.26	0.28	0.25	0.29	-0.24	3.1E-04	0.34	0.12	0.43	0.05	-0.94	1.2E-03	0.28	0.20	0.24	0.27	-0.80	1.0E-03	0.30	0.17	0.38	0.07	-0.40	5.0E-04	0.34	0.11	0.34	0.12	-0.63	7.8E-04	0.32	0.16	0.40	0.07	-0.719	9.1E-04
	Max	0.29	0.17	0.43	0.01	0.97	-0.11	1.3E-04	0.23	0.34	-0.04	0.86	-0.08	1.0E-04	0.27	0.22	0.15	0.52	-0.28	3.3E-04	0.21	0.33	-0.04	0.85	-0.21	2.6E-04	0.17	0.44	0.03	0.87	-0.08	9.6E-05	0.17	0.44	0.04	0.87	-0.10	1.3E-04	0.31	0.18	0.13	0.58	-0.257	3.0E-04
	Mean	0.76	0.27	0.20	-0.09	0.69	-0.19	2.3E-04	0.30	0.21	0.01	0.98	-0.11	1.3E-04	0.27	0.22	0.09	0.69	-0.28	3.5E-04	0.16	0.46	-0.05	0.83	-0.17	2.2E-04	0.09	0.67	-0.01	0.95	-0.04	5.8E-05	0.21	0.34	0.05	0.82	-0.14	1.8E-04	0.27	0.23	0.08	0.74	-0.228	2.8E-04
Potential temperature (°C)	Min	0.15	-0.01	0.95	0.17	0.43	0.02	-3.7E-05	-0.04	0.88	0.13	0.59	0.01	-6.2E-05	-0.06	0.80	0.14	0.54	0.04	-2.6E-04	-0.08	0.70	0.04	0.86	0.04	-3.9E-04	-0.06	0.77	0.36	0.10	0.02	-1.4E-04	-0.09	0.67	0.16	0.47	0.03	-2.7E-04	-0.11	0.64	0.02	0.94	0.033	-3.8E-04
	Max	0.05	0.06	0.78	0.31	0.15	0.01	2.8E-04	0.08	0.75	0.34	0.15	0.01	1.9E-04	0.08	0.71	0.36	0.10	0.02	5.9E-04	0.08	0.71	0.17	0.44	0.02	6.0E-04	0.05	0.82	0.45	0.03	0.01	1.7E-04	-0.01	0.97	0.23	0.29	0.02	-4.2E-05	0.07	0.78	0.20	0.40	0.019	3.7E-04
	Mean	0.04	-0.03	0.91	0.12	0.58	0.02	-8.4E-05	0.01	0.98	0.15	0.54	0.01	1.2E-05	0.00	1.00	0.20	0.37	0.04	2.3E-06	0.01	0.96	0.06	0.78	0.03	5.2E-05	-0.01	0.96	0.33	0.12	0.02	-2.7E-05	-0.07	0.74	0.15	0.49	0.03	-2.4E-04	-0.02	0.93	-0.01	0.98	0.029	-8.8E-05
Ambient temperature (°C)	Min	0.17	0.12	0.60	0.26	0.22	0.02	4.6E-04	0.13	0.60	0.24	0.33	0.01	3.0E-04	0.13	0.57	0.36	0.10	0.03	8.0E-04	0.08	0.71	0.18	0.42	0.03	5.2E-04	0.06	0.78	0.51	0.01	0.01	1.8E-04	0.05	0.83	0.27	0.21	0.02	1.9E-04	0.08	0.73	0.17	0.47	0.025	4.0E-04
	Max	0.40	0.05	0.84	0.16	0.46	0.02	1.6E-04	0.15	0.55	0.31	0.19	0.01	2.8E-04	0.11	0.63	0.33	0.13	0.03	5.8E-04	0.11	0.61	0.14	0.52	0.02	6.3E-04	0.02	0.93	0.43	0.04	0.01	4.8E-05	-0.03	0.89	0.20	0.36	0.02	-1.1E-04	0.12	0.59	0.18	0.44	0.020	5.5E-04
	Mean	0.29	0.08	0.72	0.19	0.38	0.02	3.0E-04	0.14	0.57	0.29	0.24	0.01	2.9E-04	0.11	0.63	0.35	0.11	0.03	6.4E-04	0.08	0.73	0.13	0.54	0.03	4.6E-04	0.02	0.92	0.46	0.03	0.01	6.5E-05	0.00	1.00	0.25	0.25	0.02	8.5E-07	0.09	0.70	0.18	0.43	0.023	4.2E-04
Sum_Rainfall (mm)		0.00	-0.10	0.65	-0.06	0.79	0.02	-4.2E-04	-0.16	0.50	-0.12	0.64	0.02	-3.6E-04	-0.17	0.44	-0.13	0.55	0.04	-1.1E-03	-0.22	0.31	-0.10	0.64	0.04	-1.5E-03	-0.07	0.74	0.07	0.76	0.02	-2.3E-04	-0.17	0.44	-0.20	0.36	0.02	-7.2E-04	-0.22	0.33	-0.17	0.46	0.032	-1.2E-03
Mixed Layer Depth (m)	Min	0.00	0.31	0.15	0.04	0.85	0.01	6.8E-05	0.34	0.15	0.10	0.68	0.01	3.9E-05	0.11	0.62	-0.16	0.49	0.03	3.8E-05	0.09	0.67	0.04	0.87	0.03	3.2E-05	0.01	0.96	-0.15	0.49	0.02	1.7E-06	0.15	0.49	0.04	0.86	0.02	3.3E-05	0.16	0.50	-0.12	0.60	0.022	4.2E-05
	Max	0.55	-0.22	0.31	-0.23	0.29	0.04	-1.6E-05	-0.17	0.49	-0.02	0.94	0.02	-7.0E-06	-0.18	0.43	-0.01	0.98	0.06	-2.0E-05	-0.14	0.52	-0.03	0.88	0.05	-1.6E-05	-0.24	0.27	0.11	0.63	0.03	-1.3E-05	-0.24	0.27	-0.06	0.80	0.04	-1.7E-05	-0.20	0.38	-0.08	0.72	0.049	-1.9E-05
	Mean	0.08	-0.02	0.92	-0.11	0.62	0.02	-4.5E-06	0.06	0.82	-0.02	0.92	0.01	7.5E-06	-0.16	0.49	-0.01	0.96	0.06	-5.2E-05	-0.14	0.52	0.12	0.60	0.06	-4.8E-05	-0.29	0.19	0.03	0.90	0.04	-4.4E-05	-0.16	0.47	0.05	0.83	0.04	-3.4E-05	-0.15	0.52	-0.16	0.49	0.048	-4.0E-05
Relative humidity (%)	Min	0.54	0.33	0.12	0.24	0.27	-0.01	5.6E-04	0.26	0.29	0.23	0.35	0.00	2.4E-04	0.17	0.44	0.17	0.45	0.01	4.4E-04	-0.02	0.93	0.05	0.81	0.03	-4.8E-05	0.07	0.76	0.25	0.24	0.01	8.5E-05	0.13	0.54	0.12	0.59	0.01	2.3E-04	0.07	0.76	0.21	0.35	0.019	1.5E-04
	Max	0.02	0.38	0.07	0.33	0.12	-0.05	8.0E-04	0.29	0.22	0.28	0.24	-0.01	3.4E-04	0.35	0.11	0.23	0.29	-0.06	1.1E-03	0.24	0.27	0.19	0.39	-0.03	8.0E-04	0.34	0.12	0.23	0.30	-0.03	5.2E-04	0.39	0.07	0.21	0.34	-0.05	8.2E-04	0.28	0.22	0.31	0.18	-0.033	7.4E-04
	Mean	0.33	0.35	0.10	0.29	0.18	-0.03	6.6E-04	0.28	0.25	0.29	0.23	-0.01	3.0E-04	0.23	0.30	0.20	0.36	-0.01	6.6E-04	0.08	0.71	0.11	0.61	0.01	2.5E-04	0.15	0.50	0.24	0.28	0.00	2.0E-04	0.21	0.34	0.10	0.65	-0.01	3.9E-04	0.16	0.49	0.29	0.20	0.001	3.8E-04
Terrain Height (m, agl)	Min	0.01	-0.24	0.28	-0.21	0.34	0.03	-5.0E-05	-0.12	0.64	-0.09	0.72	0.02	-1.3E-05	0.05	0.83	-0.01	0.98	0.04	1.6E-05	0.20	0.36	0.17	0.44	0.02	6.8E-05	0.17	0.45	0.09	0.67	0.01	2.6E-05	-0.08	0.70	-0.13	0.54	0.02	-1.8E-05	0.04	0.86	-0.02	0.92	0.026	1.0E-05
	Max	0.00	-0.08	0.73	-0.01	0.98	0.04	-3.0E-05	-0.13	0.60	-0.13	0.60	0.04	-3.7E-05	0.15	0.50	0.01	0.97	-0.03	1.0E-04	0.17	0.43	0.04	0.86	-0.04	1.1E-04	0.29	0.19	0.05	0.81	-0.04	8.2E-05	0.04	0.87	-0.11	0.61	0.01	1.5E-05	0.11	0.63	0.00	0.99	-0.015	6.2E-05
	Mean	0.29	-0.32	0.14	-0.13	0.54	0.04	-5.8E-05	-0.26	0.29	-0.11	0.64	0.02	-2.7E-05	-0.02	0.93	0.02	0.94	0.04	-5.2E-06	0.12	0.58	0.09	0.67	0.02	3.5E-05	0.18	0.42	0.14	0.54	0.01	2.4E-05	-0.12	0.59	-0.11	0.62	0.03	-2.2E-05	-0.05	0.83	0.00	0.99	0.032	-1.1E-05
Sum of sun flux (W.m^{-2})	Sum	0.39	0.17	0.45	0.08	0.70	0.01	2.6E-06	0.22	0.36	0.25	0.30	0.00	2.0E-06	0.22	0.33	0.29	0.20	0.01	5.5E-06	0.18	0.40	0.27	0.22	0.01	4.6E-06	0.14	0.53	0.51	0.01	0.01	1.6E-06	0.13	0.57	0.24	0.27	0.01	2.0E-06	0.13	0.58	0.19	0.40	0.014	2.6E-06
	Max	0.07	0.15	0.48	0.02	0.93	0.01	2.4E-05	0.20	0.41	0.16	0.51	0.00	1.7E-05	0.19	0.41	0.20	0.36	0.01	4.5E-05	0.13	0.55	0.15	0.50	0.01	3.2E-05	0.11	0.62	0.46	0.03	0.01	1.3E-05	0.08	0.71	0.20	0.37	0.01	1.3E-05	0.09	0.70	0.03	0.91	0.017	1.8E-05
	Mean	0.39	0.17	0.45	0.08	0.70	0.01	6.5E-05	0.22	0.36	0.25	0.30	0.00	4.9E-05	0.22	0.33	0.29	0.20	0.01	1.4E-04	0.18	0.40	0.27	0.22	0.01	1.1E-04	0.14	0.53	0.51	0.01	0.01	4.0E-05	0.13	0.57	0.24	0.27	0.01	5.0E-05	0.13	0.58	0.19	0.40	0.014	6.6E-05
Distance traveled (km)		0.00	0.03	0.91	-0.28	0.19	0.02	1.7E-06	0.01	0.96	-0.30	0.21	0.01	4.2E-07	-0.09	0.68	-0.28	0.21	0.04	-9.6E-06	-0.15	0.49	-0.27	0.22	0.04	-1.6E-05	-0.19	0.39	-0.41	0.05	0.02	-9.3E-06	-0.14	0.53	-0.28	0.20	0.03	-9.2E-06	-0.12	0.60	-0.38	0.09	0.034	-1.0E-05
Correlations tested with backward trajectories computed at 500 m																																												
Altitude (m, agl)	Min	0.00	-0.03	0.88	0.00	0.99	0.02	-8.1E-06	-0.04	0.88	0.14	0.58	0.01	-4.6E-06	-0.02	0.92	0.00	0.98	0.04	-8.5E-06	0.09	0.69	0.05	0.83	0.03	3.4E-05	-0.07	0.76	0.02	0.91	0.02	-1.2E-05	-0.11	0.63	-0.14	0.53	0.02	-2.6E-05	-0.03	0.88	0.01	0.97	0.029	-1.0E-05
	Max	0.00	-0.06	0.80	-0.08	0.71	0.03	-1.5E-05	-0.19	0.45	0.00	1.00	0.03	-2.6E-05	-0.11	0.64	-0.18	0.43	0.06	-4.3E-05	-0.06	0.79	0.06	0.80	0.05	-2.5E-05	-0.03	0.89	0.01	0.97	0.02	-5.8E-06	-0.11	0.61	-0.17	0.43	0.04	-3.0E-05	-0.22	0.33	-0.14	0.54	0.069	-7.2E-05
	Mean	0.00	-0.11	0.62	0.02	0.93	0.02	-1.9E-05	-0.15	0.55	0.09	0.71	0.02	-1.4E-05	-0.10	0.67	-0.04	0.87	0.04	-2.6E-05	0.01	0.97	0.03	0.89	0.03	2.0E-06	-0.06	0.80	0.04	0.84	0.02	-7.0E-06	-0.16	0.46	-0.13	0.57	0.03	-2.8E-05	-0.13	0.58	-0.01	0.98	0.034	-2.8E-05
Pressure (hPa)	Min	0.02	0.23	0.30	0.30	0.17	-0.45	5.3E-04	0.26	0.28	0.17	0.48	-0.29	3.4E-04	0.38	0.08	0.42	0.05	-1.19	1.4E-03	0.34	0.11	0.18	0.41	-1.10	1.3E-03	0.32	0.13	0.21	0.34	-0.48	5.6E-04	0.38	0.08	0.30	0.16	-0.76	8.9E-04	0.39	0.08	0.38	0.09	-0.978	1.1E-03
	Max	0.03	0.31	0.15	0.35	0.10	-0.31	3.4E-04	0.24	0.32	0.12	0.64	-0.13	1.4E-04	0.26	0.24	0.23	0.30	-0.40	4.4E-04	0.15	0.51	0.10	0.66	-0.22	2.5E-04	0.24	0.27	0.10	0.64	-0.17	1.9E-04	0.36	0.09	0.30	0.16	-0.37	4.0E-04	0.28	0.22	0.22	0.34	-0.351	3.9E-04
	Mean	0.30	0.37	0.08	0.33	0.13	-0.36	4.0E-04	0.35	0.14	0.19	0.45	-0.18	2.0E-04	0.32	0.15	0.27	0.22	-0.46	5.2E-04	0.22	0.31	0.18	0.42	-0.32	3.7E-04	0.23	0.29	0.11	0.63	-0.15	1.8E-04	0.41	0.05	0.30	0.16	-0.39	4.3E-04	0.36	0.11	0.28	0.22	-0.436	4.9E-04
Potential temperature (°C)	Min	0.10	0.06	0.78	0.17	0.43	0.02	1.7E-04	0.10	0.69	0.27	0.26	0.01	1.6E-04	0.08	0.72	0.27	0.23	0.03	3.4E-04	0.06	0.78	0.16	0.46	0.03	2.7E-04	0.02	0.92	0.40	0.06	0.01	4.7E-05	-0.02	0.91	0.15	0.50	0.02	-6.6E-05	0.04	0.86	0.19	0.41	0.026	1.5E-04
	Max	0.10	0.03	0.89	0.17	0.45	0.02	1.1E-04	0.04	0.87	0.23	0.35	0.01	8.4E-05	0.04	0.86	0.31	0.16	0.03	2.3E-04	0.01	0.97	0.11	0.60	0.03	5.6E-05	0.01	0.98	0.44	0.03	0.01	1.5E-05	-0.03	0.91	0.21	0.33	0.02	-9.4E-05	-0.02	0.94	0.16	0.48	0.029	-8.0E-05
	Mean	0.08	0.03	0.89	0.24	0.26	0.02	9.6E-05	0.07	0.78	0.36	0.14	0.01	1.2E-04	0.05	0.84	0.37	0.09	0.03	2.3E-04	0.03	0.88	0.22	0.32	0.03	1.6E-04	0.01	0.98	0.46	0.03	0.02	1.4E-05	-0.03	0.90	0.26	0.24	0.02	-9.0E-05	0.01	0.98	0.24	0.29	0.027	2.6E-05
Ambient temperature (°C)	Min	0.03	0.15	0.49	0.28	0.19	0.02	5.2E-04	0.17	0.49	0.31	0.20	0.01	3.4E-04	0.15	0.52	0.36	0.10	0.03	7.7E-04	0.10	0.66	0.18	0.41	0.03	5.4E-04	0.08	0.73	0.49	0.02	0.01	1.9E-04	0.07	0.75	0.25	0.26	0.02	2.5E-04	0.09	0.69	0.20	0.39	0.025	4.0E-04
	Max	0.39	0.13	0.57	0.25	0.25	0.01	4.6E-04	0.18	0.45	0.28	0.24	0.01	3.8E-04	0.13	0.57	0.35	0.12	0.03	7.3E-04	0.07	0.75	0.12	0.59	0.03	4.1E-04	0.06	0.78	0.46	0.03	0.01	1.6E-04	0.09	0.67	0.31	0.14	0.02	3.5E-04	0.10	0.66	0.22	0.34	0.020	4.8E-04
	Mean	0.02	0.14	0.52	0.21	0.33	0.01	5.0E-04	0.18	0.45	0.29	0.23	0.01	3.7E-04	0.14	0.52	0.33	0.14	0.03	7.9E-04	0.10	0.66	0.09	0.68	0.03	5.5E-04	0.07	0.75	0.46	0.03	0.01	1.8E-04	0.08	0.71	0.29	0.18	0.02	2.9E-04	0.11	0.63	0.17	0.46	0.022	5.0E-04
Sum precipitation (mm)		0.00	-0.08	0.72	-0.10	0.64	0.02	-3.1E-04	-0.12	0.62	-0.16	0.51	0.01	-2.7E-04	-0.16	0.49	-0.15	0.51	0.04	-1.0E-03	-0.21	0.34	-0.13	0.56	0.04	-1.3E-03	-0.17	0.43	0.06	0.77	0.02	-5.1E-04	-0.22	0.30	-0.22	0.31	0.03	-8.9E-04	-0.17	0.46	-0.21	0.37	0.030	-8.8E-04
Mixed Layer Depth (m)	Min	0.00	0.70	0.00	0.32	0.13	0.00	1.7E-04	0.64	0.00	0.33	0.17	0.00	8.2E-05	0.47	0.03	0.14	0.53	0.02	1.8E-04	0.37	0.08	0.29	0.18	0.02	1.5E-04	0.27	0.21	0.07	0.77	0.01	5.0E-05	0.43	0.04	0.17	0.43	0.01	1.1E-04	0.45	0.04	0.27	0.24	0.012	1.4E-04
	Max	0.84	-0.10	0.66	-0.16	0.48	0.03	-8.2E-06	-0.15	0.54	-0.05	0.83	0.02	-7.6E-06	-0.14	0.52	0.02	0.94	0.06	-2.0E-05	-0.17	0.44	-0.05	0.82	0.06	-2.3E-05	-0.17	0.44	0.15	0.48	0.03	-1.0E-05	-0.10	0.64	0.00	1.00	0.03	-8.8E-06	-0.24	0.30	-0.09	0.68	0.058	-2.7E-05
	Mean	0.71	0.29	0.18	-0.04	0.86	-0.01	6.6E-05	0.23	0.35	0.08	0.74	0.00	3.0E-05	0.05	0.82	0.03	0.89	0.03	1.9E-05	-0.08	0.70	0.04	0.86	0.05	-3.1E-05	-0.09	0.68	0.18	0.40	0.02	-1.5E-05	0.08	0.71	0.10	0.67	0.01	1.9E-05	-0.06	0.80	-0.04	0.86	0.036	-1.7E-05
Relative humidity (%)	Min	0.38	0.30	0.16	0.25	0.26	-0.02	7.4E-04	0.27	0.26	0.32	0.18	-0.01	3.7E-04	0.27	0.22	0.22	0.32	-0.02	1.0E-03	0.20	0.35	0.17	0.44	-0.01	7.9E-04	0.24	0.28	0.38	0.07	-0.01	4.3E-04	0.16	0.47	0.08	0.73	0.00	3.9E-04	0.17	0.46	0.14	0.56	-0.002	5.2E-04
	Max	0.01	0.32	0.13	0.22	0.32	-0.06	9.1E-04	0.32	0.18	0.30	0.21	-0.04	5.9E-04	0.31	0.15	0.15	0.51	-0.08	1.4E-03	0.23	0.28	0.20	0.37	-0.06	1.0E-03	0.27	0.21	0.24	0.27	-0.03	5.6E-04	0.28	0.20	0.18	0.41	-0.04	7.7E-04	0.25	0.28	0.21	0.37	-0.050	9.0E-04
	Mean	0.29	0.29	0.18	0.21	0.34	-0.03	7.1E-04	0.24	0.32	0.24	0.33	-0.01	3.6E-04	0.24	0.29	0.11	0.63	-0.03	9.2E-04	0.11	0.60	0.03	0.88	0.00	4.5E-04	0.18	0.41	0.25	0.26	-0.01	3.3E-04	0.14	0.51	0.01	0.96	0.00	3.6E-04	0.15	0.53	0.11	0.64	-0.005	4.7E-04
Terrain Height (m, agl)	Min	0.04	-0.37	0.08	-0.31	0.16	0.03	-7.3E-05	-0.30	0.21	-0.32	0.18	0.02	-3.2E-05	-0.26	0.24	-0.23	0.31	0.05	-7.8E-05	-0.19	0.40	-0.03	0.88	0.04	-5.9E-05	-0.15	0.49	-0.07	0.77	0.02	-2.2E-05	-0.32	0.13	-0.22	0.31	0.03	-6.4E-05	-0.28	0.22	-0.22	0.33	0.041	-6.7E-05
	Max	0.00	-0.21	0.33	-0.30	0.16	0.08	-8.6E-05	-0.19	0.44	-0.21	0.40	0.04	-4.0E-05	-0.26	0.25	-0.29	0.19	0.15	-1.6E-04	-0.28	0.19	-0.21	0.35	0.16	-1.8E-04	-0.15	0.51	-0.11	0.61	0.04	-4.3E-05	-0.28	0.20	-0.29	0.17	0.10	-1.1E-04	-0.29	0.20	-0.34	0.13	0.129	-1.5E-04
	Mean	0.10	-0.40	0.06	-0.35	0.10	0.05	-7.6E-05	-0.38	0.11	-0.36	0.13	0.03	-3.9E-05	-0.28	0.21	-0.29	0.19	0.07	-8.1E-05	-0.24	0.27	-0.17	0.45	0.06	-7.2E-05	-0.14	0.51	-0.12	0.58	0.02	-2.0E-05	-0.33	0.12	-0.30	0.16	0.04	-6.4E-05	-0.34	0.13	-0.28	0.22	0.057	-8.0E-05
Sum of sun flux (W.m^{-2})	Sum	0.60	-0.01	0.98	-0.02	0.94	0.02	-8.9E-08	-0.04	0.88	0.12	0.61	0.02	-3.5E-07	-0.03	0.89	0.12	0.60	0.04	-8.6E-07	-0.04	0.87	0.15	0.49	0.04	-9.5E-07	0.05	0.83	0.35	0.10	0.01	5.6E-07	0.07	0.77	0.17	0.43	0.02	1.1E-06	-0.16	0.48	-0.02	0.93	0.045	-3.5E-06
	Max	0.38	0.06	0.77	-0.04	0.84	0.01	1.0E-05	0.05	0.82	0.11	0.65	0.01	5.3E-06	0.03	0.91	0.11	0.64	0.03	7.0E-06	0.02	0.92	0.13	0.56	0.03	5.7E-06	0.04	0.87	0.36	0.10	0.01	4.3E-06	0.08	0.73	0.14	0.53	0.02	1.2E-05	-0.09	0.69	-0.08	0.72	0.039	-2.0E-05
	Mean	0.60	-0.01	0.98	-0.02	0.94	0.02	-2.2E-06	-0.04	0.88	0.12	0.61	0.02	-8.8E-06	-0.03	0.89	0.12	0.60	0.04	-2.1E-05	-0.04	0.87	0.15	0.49	0.04	-2.4E-05	0.05	0.83	0.35	0.10	0.01	1.4E-05	0.07	0.77	0.17	0.43	0.02	2.7E-05	-0.16	0.48	-0.02	0.93	0.045	-8.8E-05
Distance traveled (km)		0.00	-0.07	0.76	-0.38	0.07	0.02	-7.9E-06	-0.05	0.84	-0.20	0.42	0.01	-3.2E-06	-0.19	0.40	-0.38	0.08	0.05	-3.5E-05	-0.19	0.38	-0.17	0.43	0.05	-3.6E-05	-0.24	0.27	-0.38	0.08	0.02	-2.1E-05	-0.19	0.37	-0.29	0.17	0.03	-2.3E-05	-0.19	0.41	-0.40	0.07	0.039	-2.7E-05
Correlations tested with backward trajectories computed at 50 m																																												
Altitude (m, agl)	Min	0.00	-0.02	0.93	0.20	0.37	0.02	-8.7E-05	0.00	0.99	0.18	0.46	0.01	-6.8E-06	-0.04	0.87	0.14	0.52	0.04	-2.3E-04	-0.11	0.63	0.22	0.32	0.03	-7.1E-04	0.10	0.65	0.31	0.15	0.01	3.1E-04	-0.01	0.95	0.08	0.70	0.02	-5.4E-05	-0.07	0.76	0.18	0.43	0.029	-3.7E-04
	Max	0.00	-0.05	0.84	-0.25	0.25	0.02	-1.9E-05	-0.18	0.46	-0.19	0.45	0.02	-3.8E-05	-0.07	0.75	-0.35	0.11	0.04	-4.6E-05	-0.03	0.91	-0.07	0.76	0.03	-1.7E-05	-0.02	0.91	-0.07	0.76	0.02	-7.6E-06	-0.08	0.72	-0.24	0.27	0.02	-3.3E-05	-0.20	0.39	-0.35	0.12	0.036	-9.9E-05
	Mean	0.00	-0.05	0.83	-0.32	0.14	0.02	-4.4E-05	-0.17	0.49	-0.31	0.19	0.02	-8.2E-05	-0.07	0.77	-0.37	0.09	0.04	-9.5E-05	-0.03	0.88	-0.20	0.37	0.03	-4.9E-05	0.01	0.96	-0.06	0.80	0.02	7.8E-06	-0.08	0.71	-0.32	0.13	0.02	-7.7E-05	-0.19	0.41	-0.37	0.10	0.032	-2.2E-04
Pressure (hPa)	Min	0.10	0.24	0.27	0.43	0.04	-0.59	6.6E-04	0.27	0.26	0.36	0.13	-0.39	4.4E-04	0.40	0.06	0.50	0.02	-1.57	1.7E-03	0.33	0.12	0.26	0.23	-1.32	1.5E-03	0.41	0.05	0.32	0.13	-0.76	8.4E-04	0.44	0.04	0.48	0.02	-1.10	1.2E-03	0.42	0.06	0.50	0.02	-1.325	1.5E-03
	Max	0.24	0.54	0.01	0.65	0.00	-0.81	8.4E-04	0.41	0.08	0.29	0.24	-0.35	3.7E-04	0.51	0.02	0.53	0.01	-1.18	1.2E-03	0.38	0.07	0.32	0.14	-0.91	9.5E-04	0.41	0.05	0.24	0.27	-0.45	4.7E-04	0.56	0.01	0.51	0.01	-0.84	8.7E-04	0.47	0.03	0.55	0.01	-0.878	9.2E-04
	Mean	0.05	0.54	0.01	0.62	0.00	-0.88	9.4E-04	0.49	0.03	0.40	0.09	-0.46	4.9E-04	0.53	0.01	0.54	0.01	-1.33	1.4E-03	0.44	0.04	0.38	0.08	-1.12	1.2E-03	0.45	0.03	0.30	0.16	-0.54	5.7E-04	0.59	0.00	0.55	0.01	-0.96	1.0E-03	0.55	0.01	0.55	0.01	-1.140	1.2E-03
Potential temperature (°C)	Min	0.07	0.03	0.88	0.16	0.47	0.02	1.1E-04	0.06	0.81	0.32	0.19	0.01	1.1E-04	0.04	0.84	0.29	0.19	0.04	2.2E-04	0.02	0.91	0.17	0.43	0.03	1.2E-04	0.01	0.98	0.43	0.04	0.02	1.6E-05	-0.05	0.84	0.17	0.44	0.02	-1.5E-04	-0.01	0.98	0.16	0.50	0.028	-2.4E-05
	Max	0.23	0.02	0.91	0.13	0.57	0.02	7.9E-05	0.02	0.93	0.17	0.47	0.01	4.2E-05	0.03	0.88	0.27	0.22	0.03	1.7E-04	0.01	0.96	0.09	0.68	0.03	5.4E-05	0.03	0.89	0.44	0.03	0.01	7.1E-05	0.01	0.95	0.23	0.28	0.02	4.5E-05	-0.03	0.88	0.10	0.68	0.031	-1.4E-04
	Mean	0.09	0.05	0.84	0.20	0.37	0.02	1.5E-04	0.07	0.78	0.32	0.19	0.01	1.3E-04	0.05	0.84	0.30	0.17	0.03	2.3E-04	0.02	0.92	0.19	0.39	0.03	1.2E-04	0.01	0.95	0.46	0.03	0.01	3.4E-05	-0.01	0.95	0.24	0.27	0.02	-4.6E-05	-0.01	0.98	0.22	0.34	0.028	-2.6E-05
Ambient temperature (°C)	Min	0.02	0.17	0.43	0.31	0.15	0.02	5.8E-04	0.18	0.46	0.29	0.23	0.01	3.5E-04	0.16	0.47	0.36	0.10	0.03	8.5E-04	0.12	0.60	0.22	0.32	0.03	6.2E-04	0.10	0.67	0.50	0.02	0.01	2.4E-04	0.09	0.69	0.30	0.16	0.02	3.0E-04	0.11	0.63	0.20	0.38	0.024	4.8E-04
	Max	0.26	0.12	0.59	0.31	0.15	0.01	3.7E-04	0.11	0.64	0.25	0.30	0.01	2.0E-04	0.13	0.56	0.37	0.09	0.03	6.5E-04	0.09	0.68	0.20	0.37	0.02	4.6E-04	0.11	0.63	0.54	0.01	0.01	2.5E-04	0.11	0.60	0.38	0.07	0.02	3.6E-04	0.07	0.78	0.24	0.30	0.023	2.7E-04
	Mean	0.02	0.13	0.54	0.25	0.24	0.01	4.4E-04	0.15	0.55	0.28	0.24	0.01	2.8E-04	0.13	0.56	0.31	0.16	0.03	6.8E-04	0.09	0.67	0.19	0.39	0.03	4.9E-04	0.09	0.70	0.48	0.02	0.01	2.1E-04	0.08	0.72	0.29	0.18	0.02	2.6E-04	0.08	0.73	0.21	0.36	0.024	3.4E-04
Sum_Rainfall (mm)		0.00	-0.04	0.84	-0.13	0.55	0.02	-1.4E-04	-0.02	0.93	0.02	0.95	0.01	-3.4E-05	-0.09	0.70	-0.07	0.74	0.04	-4.3E-04	-0.11	0.60	-0.04	0.87	0.03	-5.7E-04	-0.14	0.52	0.03	0.90	0.02	-3.3E-04	-0.19	0.39	-0.24	0.27	0.02	-6.0E-04	-0.06	0.78	-0.07	0.76	0.029	-2.5E-04
Mixed Layer Depth (m)	Min	0.00	0.53	0.01	0.15	0.49	0.00	1.2E-04	0.50	0.03	0.20	0.41	0.01	5.8E-05	0.31	0.16	-0.07	0.77	0.02	1.1E-04	0.24	0.27	0.11	0.62	0.02	8.7E-05	0.14	0.53	-0.14	0.52	0.01	2.3E-05	0.28	0.20	0.05	0.80	0.01	6.3E-05	0.32	0.15	0.02	0.92	0.017	8.9E-05
	Max	0.54	-0.04	0.86	-0.02	0.92	0.02	-3.1E-06	-0.21	0.38	-0.08	0.75	0.02	-9.7E-06	-0.16	0.49	0.04	0.86	0.06	-2.0E-05	-0.25	0.25	-0.12	0.58	0.07	-3.1E-05	-0.11	0.60	0.25	0.25	0.02	-6.5E-06	-0.04	0.87	0.13	0.56	0.03	-2.9E-06	-0.33	0.14	-0.09	0.71	0.066	-3.4E-05
	Mean	0.42	0.30	0.16	0.07	0.77	-0.01	6.6E-05	0.18	0.47	0.08	0.74	0.00	2.3E-05	0.06	0.80	0.09	0.70	0.03	2.0E-05	-0.11	0.63	0.08	0.71	0.05	-3.7E-05	-0.05	0.81	0.22	0.32	0.02	-8.3E-06	0.12	0.60	0.20	0.37	0.01	2.5E-05	-0.10	0.68	0.00	1.00	0.041	-2.7E-05
Relative humidity (%)	Min	0.39	0.17	0.43	-0.01	0.96	-0.01	4.5E-04	0.34	0.16	0.31	0.19	-0.01	4.8E-04	0.24	0.28	0.14	0.53	-0.01	9.7E-04	0.26	0.24	0.17	0.44	-0.03	1.1E-03	0.11	0.61	0.15	0.50	0.00	2.2E-04	0.02	0.93	-0.14	0.51	0.02	5.3E-05	0.29	0.21	0.09	0.68	-0.022	9.4E-04
	Max	0.00	0.27	0.21	0.13	0.56	-0.05	7.9E-04	0.37	0.12	0.44	0.06	-0.05	6.6E-04	0.31	0.16	0.16	0.48	-0.08	1.4E-03	0.28	0.20	0.22	0.31	-0.08	1.3E-03	0.23	0.29	0.27	0.21	-0.03	4.9E-04	0.19	0.38	0.07	0.76	-0.03	5.6E-04	0.31	0.17	0.19	0.40	-0.072	1.1E-03
	Mean	0.12	0.24	0.27	0.06	0.78	-0.02	6.1E-04	0.35	0.14	0.25	0.30	-0.03	5.4E-04	0.25	0.25	0.06	0.79	-0.03	1.0E-03	0.23	0.29	0.06	0.77	-0.03	9.4E-04	0.12	0.59	0.10	0.65	0.00	2.2E-04	0.06	0.80	-0.13	0.55	0.01	1.4E-04	0.29	0.21	0.08	0.75	-0.040	9.5E-04
Terrain Height (m, agl)	Min	0.35	-0.55	0.01	-0.62	0.00	0.05	-1.2E-04	-0.46	0.05	-0.45	0.05	0.03	-5.6E-05	-0.46	0.03	-0.49	0.02	0.08	-1.6E-04	-0.31	0.14	-0.32	0.14	0.06	-1.1E-04	-0.32	0.13	-0.32	0.14	0.03	-5.2E-05	-0.50	0.01	-0.52	0.01	0.05	-1.1E-04	-0.44	0.05	-0.53	0.01	0.059	-1.2E-04
	Max	0.00	-0.28	0.20	-0.56	0.01	0.11	-1.4E-04	-0.23	0.35	-0.42	0.07	0.05	-6.0E-05	-0.34	0.13	-0.53	0.01	0.21	-2.6E-04	-0.29	0.17	-0.35	0.10	0.19	-2.3E-04	-0.29	0.19	-0.34	0.11	0.08	-1.0E-04	-0.38	0.07	-0.53	0.01	0.15	-1.9E-04	-0.36	0.11	-0.58	0.01	0.179	-2.3E-04
	Mean	0.09	-0.55	0.01	-0.61	0.00	0.08	-1.3E-04	-0.50	0.03	-0.44	0.06	0.04	-6.6E-05	-0.46	0.03	-0.51	0.02	0.11	-1.7E-04	-0.37	0.09	-0.34	0.11	0.09	-1.4E-04	-0.34	0.11	-0.29	0.18	0.04	-6.0E-05	-0.52	0.01	-0.49	0.02	0.08	-1.2E-04	-0.50	0.02	-0.51	0.02	0.095	-1.5E-04
Sum of sun flux (W.m^{-2})	Sum	0.53	0.01	0.97	0.04	0.86	0.02	1.3E-07	0.00	0.99	0.13	0.60	0.01	-1.4E-08	0.00	1.00	0.18	0.44	0.04	-6.6E-11	0.00	1.00	0.21	0.33	0.03	5.1E-09	0.06	0.78	0.41	0.06	0.01	7.0E-07	0.08	0.71	0.23	0.29	0.02	1.3E-06	-0.11	0.65	0.01	0.96	0.038	-2.1E-06
	Max	0.12	0.11	0.60	0.07	0.74	0.01	1.7E-05	0.09	0.73	0.17	0.49	0.01	7.3E-06	0.06	0.78	0.17	0.44	0.03	1.5E-05	0.02	0.92	0.22	0.32	0.03	5.2E-06	0.08	0.72	0.44	0.03	0.01	8.9E-06	0.14	0.54	0.25	0.25	0.01	2.1E-05	-0.06	0.79	0.02	0.95	0.034	-1.2E-05
	Mean	0.53	0.01	0.97	0.04	0.86	0.02	3.1E-06	0.00	0.99	0.13	0.60	0.01	-3.6E-07	0.00	1.00	0.18	0.44	0.04	-1.6E-09	0.00	1.00	0.21	0.33	0.03	1.3E-07	0.06	0.78	0.41	0.06	0.01	1.8E-05	0.08	0.71	0.23	0.29	0.02	3.2E-05	-0.11	0.65	0.01	0.96	0.038	-5.3E-05
Distance traveled (km)		0.14	0.16	0.47	-0.34	0.12	0.01	3.8E-05	0.15	0.53	-0.14	0.56	0.01	1.9E-05	0.02	0.94	-0.33	0.13	0.04	6.0E-06	-0.03	0.87	-0.07	0.75	0.04	-1.3E-05	-0.09	0.67	-0.28	0.19	0.02	-1.6E-05	0.01	0.97	-0.21	0.33	0.02	1.9E-06	-0.01	0.97	-0.32	0.16	0.028	-2.4E-06

a In addtion to the total number of ice nucleators, the following biological variables were statistically analyzed for each of the 6 media described in the material and methods section: concentration of total bacteria, frequency and concentration of culturable bacteria (i) INA only the first time they were tested and (ii) for which INA is reproducible and confirmed.

[b] All the correlations with physical properties listed in materials and methods section have been tested for the three elevations. Correlations are based on the analyse of N= 23 samples

Models are given only for variables for which at least one of the Pearson's or Spearman's coefficients was significantly different from zero are presented (P<0.1).

[c] The p-value for the Shapiro-Wilk test is given next to each *x* variable and below each *y* variable. When the p-value is under 0.05, normality of the data distribution is rejected.

Climatic or chemical variables (x)[b]	Biological variables (y)[a]		Concentration of Ice+ bacteria grown on CA that had a minimal activity during the original INA characterization (%)						Concentration of Ice+ bacteria grown on CA 10% that had a minimal activity during the original INA characterization (%)						Concentration of Ice+ bacteria grown on EMB that had a minimal activity during the original INA characterization (%)						Concentration of Ice+ bacteria grown on MA that had a minimal activity during the original INA characterization (%)					
			Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
			r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry																										
pH		0.40	0.19	0.49	0.19	0.50	-1.46	4.4E-01	0.28	0.33	0.24	0.41	-1.99	5.8E-01	0.09	0.75	0.11	0.69	0.68	1.9E-01	-0.25	0.37	-0.22	0.43	5.51	-5.7E-01
Conductivity (µS.cm)		0.00	-0.40	0.14	-0.22	0.44	2.16	-2.2E-02	0.37	0.19	0.30	0.30	1.81	1.8E-02	0.04	0.89	-0.03	0.91	2.01	2.0E-03	0.26	0.34	0.01	0.96	1.13	1.5E-02
Total Disolved Solids (ppm)		0.04	-0.28	0.33	0.04	0.90	2.10	-3.1E-02	0.51	0.08	0.62	0.02	1.31	4.9E-02	0.19	0.51	0.24	0.41	1.64	2.0E-02	0.35	0.21	0.16	0.60	0.70	3.7E-02
Salinity (ppm)		0.00	-0.41	0.14	-0.37	0.19	2.15	-3.3E-02	0.43	0.14	0.12	0.69	1.57	3.1E-02	0.09	0.77	-0.14	0.63	1.85	6.6E-03	0.41	0.14	0.17	0.57	0.77	3.2E-02
Correlations tested with backward trajectories computed at 1000 m																										
Altitude (m, agl)	Min	0.05	0.26	0.23	0.29	0.18	1.43	9.7E-04	0.45	0.05	0.38	0.10	1.45	1.5E-03	0.09	0.69	0.12	0.59	2.19	2.9E-04	0.30	0.17	0.36	0.09	1.12	1.1E-03
	Max	0.00	0.13	0.57	0.22	0.30	0.68	1.1E-03	0.25	0.30	0.33	0.17	0.22	1.7E-03	-0.18	0.41	0.14	0.52	3.75	-1.3E-03	0.29	0.19	0.24	0.26	-1.00	2.4E-03
	Mean	0.44	0.28	0.20	0.33	0.12	1.11	1.1E-03	0.48	0.04	0.39	0.10	0.96	1.7E-03	0.18	0.42	0.22	0.32	1.91	6.1E-04	0.34	0.12	0.38	0.07	0.72	1.3E-03
Pressure (hPa)	Min	0.00	0.16	0.47	-0.03	0.91	-9.79	1.4E-02	-0.24	0.32	-0.33	0.17	16.76	-1.8E-02	0.17	0.45	0.00	1.00	-8.20	1.3E-02	-0.25	0.26	-0.27	0.22	19.24	-2.1E-02
	Max	0.29	-0.16	0.47	-0.11	0.63	6.27	-4.7E-03	-0.37	0.12	-0.25	0.30	11.23	-9.6E-03	-0.06	0.77	-0.05	0.82	3.89	-1.7E-03	-0.28	0.19	-0.28	0.19	9.46	-8.3E-03
	Mean	0.76	-0.18	0.40	-0.26	0.23	7.30	-6.0E-03	-0.35	0.14	-0.30	0.22	10.63	-9.4E-03	-0.17	0.45	-0.22	0.32	6.51	-4.6E-03	-0.28	0.20	-0.31	0.15	9.62	-8.9E-03
Potential temperature (°C)	Min	0.15	0.16	0.48	0.15	0.51	1.60	1.7E-02	0.21	0.38	-0.04	0.89	1.81	2.1E-02	0.38	0.08	0.22	0.32	1.80	3.6E-02	-0.03	0.89	0.00	1.00	1.64	-3.2E-03
	Max	0.05	0.16	0.46	0.20	0.37	1.17	2.9E-02	0.03	0.90	-0.08	0.74	2.04	4.6E-03	0.37	0.09	0.20	0.38	1.01	5.6E-02	-0.07	0.75	0.01	0.96	1.88	-1.3E-02
	Mean	0.04	0.17	0.45	0.06	0.79	1.46	2.1E-02	0.17	0.48	-0.14	0.58	1.79	1.9E-02	0.39	0.07	0.12	0.61	1.56	4.2E-02	-0.04	0.87	-0.04	0.84	1.67	-4.4E-03
Ambient temperature (°C)	Min	0.17	0.13	0.54	0.09	0.68	1.72	2.1E-02	0.06	0.82	-0.14	0.57	2.10	7.7E-03	0.39	0.07	0.26	0.24	2.00	5.2E-02	-0.08	0.70	-0.06	0.80	1.67	-1.3E-02
	Max	0.40	0.08	0.73	0.05	0.82	1.71	1.0E-02	0.01	0.96	-0.05	0.85	2.14	1.3E-03	0.35	0.11	0.22	0.33	1.80	4.0E-02	-0.17	0.43	-0.13	0.57	1.88	-2.3E-02
	Mean	0.29	0.11	0.61	0.03	0.90	1.68	1.7E-02	0.05	0.83	-0.12	0.61	2.08	6.7E-03	0.38	0.08	0.18	0.42	1.86	4.7E-02	-0.15	0.48	-0.13	0.56	1.80	-2.2E-02
Sum_Rainfall (mm)		0.00	-0.30	0.17	-0.09	0.68	2.04	-4.9E-02	0.00	0.99	0.00	0.99	2.16	-3.0E-04	0.04	0.86	0.24	0.28	2.29	5.5E-03	-0.09	0.67	0.16	0.45	1.65	-1.5E-02
Mixed Layer Depth (m)	Min	0.00	-0.21	0.33	-0.03	0.89	2.09	-1.8E-03	0.37	0.12	0.39	0.10	1.77	2.5E-03	0.12	0.59	0.08	0.71	2.19	8.7E-04	0.33	0.13	0.20	0.36	1.21	2.7E-03
	Max	0.55	-0.25	0.26	-0.27	0.21	2.58	-6.8E-04	-0.29	0.23	-0.27	0.27	3.00	-7.3E-04	0.09	0.68	-0.03	0.88	2.06	2.3E-04	-0.13	0.56	-0.10	0.65	1.98	-3.5E-04
	Mean	0.08	-0.36	0.09	-0.23	0.29	3.29	-2.9E-03	0.08	0.76	-0.05	0.84	1.84	6.0E-04	0.03	0.91	0.01	0.96	2.22	1.9E-04	0.18	0.41	0.13	0.55	0.88	1.5E-03
Relative humidity (%)	Min	0.54	0.01	0.95	0.05	0.84	1.79	9.4E-04	0.27	0.26	0.31	0.19	1.21	1.6E-02	0.37	0.09	0.42	0.05	1.14	2.0E-02	0.16	0.46	0.20	0.35	1.00	1.0E-02
	Max	0.02	0.08	0.73	-0.01	0.95	1.32	6.3E-03	0.11	0.66	0.15	0.53	1.52	7.6E-03	0.40	0.06	0.28	0.21	0.02	2.8E-02	0.11	0.63	0.18	0.42	0.89	8.5E-03
	Mean	0.33	0.04	0.86	0.07	0.74	1.65	2.7E-03	0.22	0.38	0.33	0.17	1.16	1.4E-02	0.38	0.08	0.38	0.08	0.72	2.3E-02	0.18	0.42	0.22	0.31	0.72	1.3E-02
Terrain Height (m, agl)	Min	0.01	0.01	0.97	-0.07	0.74	1.83	7.9E-05	-0.15	0.54	-0.23	0.34	2.31	-1.0E-03	0.00	0.99	-0.04	0.88	2.31	1.4E-05	0.08	0.72	0.08	0.71	1.49	6.4E-04
	Max	0.00	0.22	0.32	0.18	0.41	-0.45	3.3E-03	-0.02	0.93	-0.01	0.95	2.42	-3.9E-04	0.27	0.22	0.33	0.13	-0.36	3.9E-03	0.08	0.73	0.10	0.64	0.80	1.1E-03
	Mean	0.29	0.02	0.93	-0.01	0.96	1.80	1.3E-04	-0.17	0.48	-0.27	0.26	2.50	-1.1E-03	0.08	0.71	0.09	0.70	2.15	5.0E-04	-0.05	0.81	-0.08	0.73	1.71	-3.6E-04
Sum of sun flux ($W.m^{-2}$)	Sum	0.39	-0.06	0.78	-0.11	0.63	2.02	-3.7E-05	-0.30	0.22	-0.38	0.11	2.95	-1.6E-04	0.17	0.46	0.07	0.76	1.87	8.9E-05	-0.09	0.67	-0.12	0.60	1.86	-5.7E-05
	Max	0.07	-0.10	0.66	-0.20	0.35	2.16	-5.8E-04	-0.25	0.29	-0.34	0.15	2.92	-1.3E-03	0.19	0.39	0.07	0.77	1.76	9.9E-04	-0.15	0.49	-0.19	0.37	2.08	-8.9E-04
	Mean	0.39	-0.06	0.78	-0.11	0.63	2.02	-9.3E-04	-0.30	0.22	-0.38	0.11	2.95	-4.0E-03	0.17	0.46	0.07	0.76	1.87	2.2E-03	-0.09	0.67	-0.12	0.60	1.86	-1.4E-03
Distance traveled (km)		0.00	-0.25	0.24	-0.16	0.47	2.26	-6.6E-04	0.02	0.93	0.05	0.83	2.12	4.5E-05	-0.37	0.09	-0.11	0.61	2.82	-8.0E-04	-0.06	0.80	0.05	0.82	1.69	-1.5E-04
Correlations tested with backward trajectories computed at 500 m																										
Altitude (m, agl)	Min	0.00	0.10	0.66	0.05	0.83	1.76	9.0E-04	0.37	0.12	0.36	0.14	1.83	2.9E-03	0.29	0.19	0.15	0.50	2.09	2.3E-03	0.34	0.11	0.24	0.26	1.30	3.1E-03
	Max	0.00	0.09	0.68	0.08	0.70	1.32	9.2E-04	0.09	0.71	0.20	0.41	1.72	7.6E-04	0.13	0.56	0.24	0.28	1.67	1.1E-03	0.33	0.12	0.29	0.18	-0.28	3.3E-03
	Mean	0.00	0.09	0.67	0.05	0.82	1.71	6.3E-04	0.22	0.36	0.23	0.34	1.85	1.3E-03	0.22	0.32	0.25	0.26	2.04	1.3E-03	0.31	0.16	0.23	0.28	1.16	2.0E-03
Pressure (hPa)	Min	0.02	0.32	0.14	0.16	0.46	-23.55	2.9E-02	-0.13	0.60	-0.21	0.39	11.11	-1.0E-02	0.05	0.81	-0.12	0.60	-1.37	4.2E-03	-0.25	0.26	-0.22	0.31	20.99	-2.2E-02
	Max	0.03	0.09	0.69	0.22	0.31	-1.79	3.7E-03	-0.06	0.81	0.07	0.77	4.22	-2.1E-03	-0.22	0.33	-0.15	0.51	10.06	-7.9E-03	-0.28	0.20	-0.17	0.45	13.01	-1.2E-02
	Mean	0.30	0.11	0.60	0.22	0.31	-2.65	4.7E-03	0.02	0.94	0.04	0.86	1.53	6.6E-04	-0.10	0.66	-0.11	0.61	5.61	-3.5E-03	-0.21	0.35	-0.12	0.59	9.48	-8.3E-03
Potential temperature (°C)	Min	0.10	0.16	0.46	0.10	0.64	1.66	1.7E-02	0.23	0.34	0.05	0.84	1.87	2.3E-02	0.40	0.06	0.27	0.22	1.92	3.7E-02	0.05	0.80	0.15	0.49	1.53	5.8E-03
	Max	0.10	0.06	0.77	0.03	0.90	1.66	9.2E-03	-0.01	0.97	-0.18	0.47	2.18	-1.2E-03	0.35	0.11	0.22	0.32	1.44	4.3E-02	-0.11	0.61	-0.09	0.70	1.90	-1.6E-02
	Mean	0.08	0.12	0.60	0.09	0.69	1.64	1.4E-02	0.13	0.59	-0.10	0.68	1.91	1.4E-02	0.39	0.07	0.24	0.28	1.71	4.0E-02	-0.02	0.94	0.04	0.87	1.62	-1.9E-03
Ambient temperature (°C)	Min	0.03	0.18	0.41	0.13	0.57	1.69	2.4E-02	0.18	0.45	-0.07	0.79	1.97	2.2E-02	0.41	0.06	0.22	0.33	2.01	4.6E-02	-0.03	0.88	-0.01	0.98	1.62	-4.2E-03
	Max	0.39	0.14	0.53	0.06	0.79	1.55	2.0E-02	0.10	0.69	-0.06	0.81	1.95	1.2E-02	0.34	0.12	0.17	0.45	1.66	4.2E-02	-0.12	0.57	-0.16	0.47	1.85	-1.7E-02
	Mean	0.02	0.16	0.46	0.01	0.98	1.61	2.2E-02	0.16	0.52	-0.12	0.62	1.92	1.9E-02	0.41	0.06	0.17	0.45	1.79	4.8E-02	-0.08	0.71	-0.14	0.52	1.71	-1.1E-02
Sum precipitation (mm)		0.00	-0.31	0.15	-0.17	0.44	2.02	-4.7E-02	-0.10	0.67	-0.23	0.35	2.20	-1.4E-02	-0.04	0.88	0.15	0.50	2.33	-4.9E-03	-0.19	0.39	0.14	0.51	1.70	-2.8E-02
Mixed Layer Depth (m)	Min	0.00	0.10	0.64	0.21	0.34	1.73	1.0E-03	0.45	0.05	0.56	0.01	1.74	3.5E-03	0.38	0.08	0.25	0.25	1.98	3.0E-03	0.49	0.02	0.40	0.06	1.08	4.6E-03
	Max	0.84	-0.24	0.28	-0.20	0.37	2.67	-7.7E-04	-0.37	0.12	-0.24	0.31	3.44	-1.1E-03	0.09	0.68	0.08	0.73	2.01	2.8E-04	-0.23	0.30	-0.14	0.52	2.37	-7.2E-04
	Mean	0.71	-0.28	0.20	-0.20	0.36	2.98	-2.5E-03	0.00	0.99	0.03	0.90	2.14	2.2E-05	0.12	0.59	0.17	0.44	1.88	9.3E-04	0.12	0.58	0.10	0.64	1.10	1.1E-03
Relative humidity (%)	Min	0.38	0.06	0.79	0.18	0.40	1.53	5.5E-03	0.18	0.45	0.25	0.30	1.29	1.5E-02	0.16	0.47	0.25	0.27	1.60	1.3E-02	0.14	0.54	0.27	0.21	0.89	1.3E-02
	Max	0.01	-0.12	0.58	-0.05	0.83	2.98	-1.3E-02	0.00	0.99	0.10	0.69	2.18	-3.5E-04	-0.04	0.86	0.05	0.84	2.62	-3.6E-03	0.12	0.59	0.23	0.28	0.50	1.3E-02
	Mean	0.29	0.01	0.97	0.08	0.70	1.79	6.6E-04	0.14	0.57	0.21	0.38	1.26	1.3E-02	0.05	0.81	0.15	0.52	2.00	4.5E-03	0.15	0.50	0.27	0.21	0.62	1.4E-02
Terrain Height (m, agl)	Min	0.04	-0.11	0.63	-0.19	0.38	1.99	-8.3E-04	-0.28	0.25	-0.35	0.14	2.47	-1.8E-03	0.04	0.86	0.05	0.82	2.26	2.6E-04	0.07	0.74	0.00	0.99	1.49	5.5E-04
	Max	0.00	-0.07	0.76	-0.11	0.61	2.55	-1.0E-03	0.01	0.96	0.07	0.79	2.05	1.5E-04	0.11	0.63	0.20	0.37	1.34	1.4E-03	0.12	0.57	0.10	0.67	0.29	1.9E-03
	Mean	0.10	-0.15	0.50	-0.20	0.36	2.23	-1.1E-03	-0.32	0.18	-0.34	0.16	2.86	-2.0E-03	-0.05	0.82	-0.03	0.89	2.43	-3.2E-04	-0.01	0.96	-0.08	0.73	1.62	-8.9E-05
Sum of sun flux ($W.m^{-2}$)	Sum	0.60	-0.14	0.52	-0.22	0.31	2.28	-9.0E-05	-0.31	0.19	-0.45	0.05	3.05	-1.8E-04	0.09	0.68	-0.07	0.74	2.04	5.4E-05	-0.18	0.42	-0.19	0.38	2.12	-1.1E-04
	Max	0.38	-0.17	0.44	-0.19	0.39	2.44	-1.1E-03	-0.28	0.25	-0.31	0.19	3.10	-1.6E-03	0.10	0.64	0.01	0.95	1.97	6.1E-04	-0.20	0.36	-0.13	0.55	2.27	-1.2E-03
	Mean	0.60	-0.14	0.52	-0.22	0.31	2.28	-2.3E-03	-0.31	0.19	-0.45	0.05	3.05	-4.4E-03	0.09	0.68	-0.07	0.74	2.04	1.4E-03	-0.18	0.42	-0.19	0.38	2.12	-2.8E-03
Distance traveled (km)		0.00	-0.38	0.08	-0.24	0.28	2.51	-1.7E-03	0.07	0.78	-0.02	0.92	2.05	2.6E-04	-0.31	0.16	-0.22	0.32	2.79	-1.2E-03	0.06	0.80	0.10	0.65	1.49	2.6E-04
Correlations tested with backward trajectories computed at 50 m																										
Altitude (m, agl)	Min	0.00	0.12	0.59	0.17	0.44	1.80	2.0E-02	0.01	0.98	-0.15	0.53	2.15	8.8E-04	0.07	0.77	0.11	0.64	2.29	9.0E-03	0.09	0.67	0.20	0.36	1.56	1.5E-02
	Max	0.00	0.03	0.91	-0.19	0.39	1.81	4.1E-04	-0.01	0.97	-0.03	0.90	2.17	-1.4E-04	0.14	0.55	0.15	0.52	2.17	1.8E-03	0.28	0.20	0.18	0.41	1.25	4.4E-03
	Mean	0.00	0.12	0.59	-0.14	0.53	1.76	4.3E-03	-0.03	0.89	0.06	0.80	2.18	-1.0E-03	0.15	0.50	0.19	0.40	2.23	4.7E-03	0.27	0.22	-0.03	0.88	1.42	9.7E-03
Pressure (hPa)	Min	0.10	0.37	0.08	0.21	0.35	-34.96	4.0E-02	-0.04	0.86	-0.19	0.44	6.00	-4.1E-03	0.04	0.84	-0.12	0.60	-1.46	4.1E-03	-0.20	0.37	-0.16	0.47	20.72	-2.1E-02
	Max	0.24	0.37	0.08	0.42	0.05	-20.49	2.3E-02	0.13	0.61	0.14	0.58	-4.66	6.9E-03	0.11	0.62	0.09	0.68	-3.36	5.7E-03	0.08	0.70	0.13	0.54	-3.35	5.0E-03
	Mean	0.05	0.46	0.03	0.40	0.06	-28.09	3.1E-02	0.29	0.23	0.26	0.29	-14.50	1.7E-02	0.24	0.28	0.14	0.53	-11.04	1.4E-02	0.03	0.90	0.04	0.85	-0.12	1.8E-03
Potential temperature (°C)	Min	0.07	0.07	0.75	0.02	0.92	1.77	8.7E-03	0.13	0.59	-0.02	0.93	2.00	1.5E-02	0.39	0.07	0.27	0.22	1.95	4.1E-02	0.00	0.99	0.07	0.74	1.59	1.8E-04
	Max	0.23	0.06	0.79	-0.05	0.81	1.70	7.4E-03	0.00	1.00	-0.24	0.33	2.16	-1.3E-04	0.37	0.09	0.13	0.57	1.53	3.9E-02	-0.14	0.52	-0.17	0.45	1.92	-1.7E-02
	Mean	0.09	0.08	0.73	0.02	0.93	1.71	9.6E-03	0.07	0.78	-0.10	0.69	2.04	7.5E-03	0.38	0.08	0.26	0.25	1.75	4.1E-02	-0.03	0.88	0.00	1.00	1.65	-4.0E-03
Ambient temperature (°C)	Min	0.02	0.16	0.46	0.10	0.64	1.71	2.1E-02	0.18	0.47	-0.04	0.89	1.98	2.1E-02	0.44	0.04	0.29	0.19	2.00	4.9E-02	0.01	0.96	0.07	0.75	1.58	1.5E-03
	Max	0.26	0.16	0.48	0.09	0.70	1.52	1.9E-02	0.06	0.81	-0.13	0.60	2.04	6.2E-03	0.42	0.05	0.23	0.31	1.56	4.4E-02	-0.10	0.63	-0.11	0.63	1.80	-1.3E-02
	Mean	0.02	0.15	0.49	0.04	0.84	1.64	2.0E-02	0.12	0.64	-0.08	0.75	1.99	1.3E-02	0.43	0.05	0.29	0.19	1.81	4.7E-02	-0.03	0.89	0.03	0.89	1.63	-3.8E-03
Sum_Rainfall (mm)		0.00	-0.33	0.13	-0.20	0.37	2.02	-4.0E-02	0.00	0.99	-0.14	0.56	2.15	4.0E-04	-0.01	0.97	0.09	0.69	2.32	-7.6E-04	-0.17	0.43	0.17	0.43	1.69	-2.1E-02
Mixed Layer Depth (m)	Min	0.00	-0.04	0.86	0.09	0.68	1.88	-3.5E-04	0.41	0.08	0.50	0.03	1.77	2.9E-03	0.27	0.23	0.14	0.54	2.07	2.0E-03	0.46	0.03	0.35	0.11	1.10	4.0E-03
	Max	0.54	-0.14	0.53	-0.11	0.61	2.30	-4.1E-04	-0.32	0.18	-0.29	0.23	3.19	-8.8E-04	0.12	0.59	-0.04	0.88	1.94	3.3E-04	-0.26	0.23	-0.28	0.20	2.45	-7.7E-04
	Mean	0.42	-0.22	0.31	-0.16	0.47	2.74	-1.9E-03	-0.06	0.80	0.03	0.89	2.40	-4.9E-04	0.18	0.41	0.22	0.32	1.65	1.4E-03	0.08	0.73	0.13	0.55	1.29	6.3E-04
Relative humidity (%)	Min	0.39	-0.05	0.83	0.08	0.73	2.10	-4.9E-03	0.28	0.25	0.28	0.25	0.87	2.4E-02	0.02	0.93	0.05	0.81	2.23	1.7E-03	0.20	0.36	0.30	0.16	0.52	2.0E-02
	Max	0.00	-0.17	0.44	-0.05	0.82	3.51	-1.9E-02	-0.06	0.80	0.22	0.36	2.74	-6.7E-03	0.03	0.89	0.22	0.33	2.05	3.1E-03	0.06	0.78	0.27	0.21	1.00	6.8E-03
	Mean	0.12	-0.03	0.88	0.06	0.79	2.09	-3.4E-03	0.24	0.31	0.39	0.10	0.51	2.3E-02	0.08	0.71	0.19	0.39	1.81	7.1E-03	0.21	0.34	0.30	0.16	0.15	2.0E-02
Terrain Height (m, agl)	Min	0.35	-0.32	0.13	-0.37	0.09	2.52	-2.8E-03	-0.22	0.36	-0.28	0.25	2.57	-1.7E-03	-0.16	0.47	-0.16	0.49	2.61	-1.2E-03	-0.12	0.59	-0.18	0.40	1.84	-9.9E-04
	Max	0.00	-0.26	0.23	-0.33	0.13	5.18	-5.0E-03	-0.08	0.74	-0.07	0.78	3.04	-1.3E-03	-0.01	0.97	0.05	0.83	2.41	-1.5E-04	0.06	0.78	0.04	0.86	0.80	1.2E-03
	Mean	0.09	-0.43	0.04	-0.44	0.04	3.56	-3.9E-03	-0.38	0.11	-0.38	0.11	3.47	-3.0E-03	-0.28	0.21	-0.19	0.40	3.26	-2.2E-03	-0.12	0.58	-0.17	0.44	2.07	-1.1E-03
Sum of sun flux ($W.m^{-2}$)	Sum	0.53	-0.11	0.62	-0.19	0.39	2.16	-6.7E-05	-0.29	0.23	-0.44	0.06	2.91	-1.5E-04	0.11	0.62	-0.05	0.81	2.02	5.9E-05	-0.13	0.56	-0.15	0.50	1.96	-7.5E-05
	Max	0.12	-0.09	0.67	-0.17	0.44	2.14	-5.5E-04	-0.26	0.28	-0.34	0.16	2.91	-1.3E-03	0.12	0.59	0.04	0.85	1.97	6.2E-04	-0.14	0.52	-0.12	0.60	2.04	-8.3E-04
	Mean	0.53	-0.11	0.62	-0.19	0.39	2.16	-1.7E-03	-0.29	0.23	-0.44	0.06	2.91	-3.7E-03	0.11	0.62	-0.05	0.81	2.02	1.5E-03	-0.13	0.56	-0.15	0.50	1.96	-1.9E-03
Distance traveled (km)		0.14	-0.38	0.07	-0.27	0.22	2.69	-3.5E-03	-0.10	0.69	0.02	0.94	2.33	-7.2E-04	-0.24	0.27	-0.13	0.57	2.77	-1.9E-03	0.13	0.55	0.11	0.63	1.31	1.2E-03

Climatic or chemical variables (x)[b]	Biological variables (y)[a]		Concentration of Ice+ bacteria grown on R2A that had a minimal activity during the original INA characterization (%)						Concentration of Ice+ bacteria grown on TSA that had a minimal activity during the original INA characterization (%)						Concentration of Ice+ bacteria grown on TSA 10% that had a minimal activity during the original INA characterization (%)					
			Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
			r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry																				
pH		0.40	-0.26	0.34	-0.13	0.65	6.50	-6.6E-01	0.00	1.00	0.14	0.61	1.52	-3.2E-03	0.22	0.44	-0.05	0.85	-0.245	3.8E-01
Conductivity (µS.cm)		0.00	-0.36	0.19	-0.26	0.35	2.18	-2.2E-02	-0.08	0.79	0.05	0.87	1.58	-4.1E-03	0.12	0.68	-0.15	0.60	2.397	5.0E-03
Total Disolved Solids (ppm)		0.04	-0.39	0.17	-0.18	0.54	2.38	-4.7E-02	-0.06	0.85	0.22	0.45	1.47	-5.6E-03	0.29	0.31	0.14	0.63	2.023	2.5E-02
Salinity (ppm)		0.00	-0.29	0.32	-0.17	0.55	2.05	-2.6E-02	0.02	0.96	0.29	0.31	1.36	1.1E-03	0.14	0.63	-0.16	0.59	2.274	8.8E-03
Correlations tested with backward trajectories computed at 1000 m																				
Altitude (m, agl)	Min	0.05	0.08	0.72	0.15	0.49	1.98	3.3E-04	0.22	0.31	0.29	0.18	1.39	8.6E-04	0.25	0.28	0.05	0.82	1.971	8.3E-04
	Max	0.00	-0.12	0.58	-0.11	0.63	3.35	-1.1E-03	0.08	0.72	0.18	0.40	1.01	6.9E-04	0.22	0.34	0.22	0.35	0.551	1.6E-03
	Mean	0.44	0.11	0.63	0.14	0.53	1.81	4.7E-04	0.18	0.40	0.27	0.22	1.25	7.7E-04	0.27	0.24	0.16	0.48	1.712	9.4E-04
Pressure (hPa)	Min	0.00	0.34	0.11	0.19	0.38	-25.42	3.3E-02	0.04	0.87	-0.06	0.79	-1.02	3.4E-03	-0.19	0.42	-0.10	0.67	14.073	-1.4E-02
	Max	0.29	0.01	0.97	0.04	0.86	1.87	2.6E-04	-0.19	0.39	-0.22	0.31	7.32	-5.9E-03	-0.23	0.32	-0.06	0.80	8.120	-6.1E-03
	Mean	0.76	-0.01	0.95	-0.03	0.90	2.58	-5.1E-04	-0.06	0.79	-0.14	0.52	3.55	-2.0E-03	-0.21	0.37	-0.09	0.70	7.594	-5.8E-03
Potential temperature (°C)	Min	0.15	0.43	0.04	0.42	0.04	1.38	5.3E-02	0.46	0.03	0.59	0.00	1.02	5.3E-02	-0.13	0.57	-0.18	0.44	2.504	-1.3E-02
	Max	0.05	0.46	0.03	0.48	0.02	0.00	9.1E-02	0.41	0.05	0.47	0.02	-0.03	7.7E-02	-0.18	0.44	-0.08	0.73	2.971	-2.8E-02
	Mean	0.04	0.41	0.05	0.35	0.11	1.10	5.8E-02	0.42	0.04	0.48	0.02	0.76	5.6E-02	-0.11	0.65	-0.20	0.39	2.535	-1.2E-02
Ambient temperature (°C)	Min	0.17	0.54	0.01	0.48	0.02	1.59	9.2E-02	0.46	0.03	0.49	0.02	1.33	7.4E-02	-0.21	0.35	-0.13	0.57	2.500	-2.9E-02
	Max	0.40	0.43	0.04	0.35	0.10	1.32	6.4E-02	0.45	0.03	0.44	0.03	0.96	6.3E-02	-0.22	0.33	-0.03	0.91	2.672	-2.7E-02
	Mean	0.29	0.46	0.03	0.42	0.05	1.41	7.5E-02	0.46	0.03	0.49	0.02	1.09	7.1E-02	-0.21	0.37	-0.11	0.65	2.591	-2.7E-02
Sum_Rainfall (mm)		0.00	0.11	0.61	0.16	0.46	2.04	2.0E-02	0.20	0.37	0.07	0.76	1.62	3.4E-02	-0.40	0.07	-0.12	0.60	2.555	-5.6E-02
Mixed Layer Depth (m)	Min	0.00	-0.22	0.30	0.04	0.87	2.41	-2.1E-03	-0.01	0.97	0.14	0.51	1.77	-8.4E-05	0.26	0.25	0.06	0.78	2.047	1.9E-03
	Max	0.55	0.11	0.61	-0.03	0.90	1.74	3.5E-04	0.22	0.32	0.14	0.52	1.07	6.3E-04	-0.24	0.29	0.00	1.00	3.010	-6.1E-04
	Mean	0.08	-0.07	0.75	-0.03	0.90	2.44	-6.4E-04	0.09	0.67	0.13	0.54	1.36	8.0E-04	-0.01	0.96	0.08	0.72	2.362	-8.8E-05
Relative humidity (%)	Min	0.54	0.12	0.59	0.17	0.42	1.63	8.6E-03	0.01	0.98	0.11	0.61	1.73	4.4E-04	0.04	0.85	0.14	0.56	2.171	2.5E-03
	Max	0.02	0.09	0.69	0.09	0.69	1.48	7.8E-03	-0.14	0.52	-0.04	0.85	2.76	-1.2E-02	0.03	0.89	0.09	0.71	2.127	2.3E-03
	Mean	0.33	0.10	0.66	0.10	0.66	1.58	7.8E-03	-0.11	0.61	0.01	0.96	2.34	-8.4E-03	0.06	0.78	0.19	0.41	2.026	4.1E-03
Terrain Height (m, agl)	Min	0.01	-0.12	0.58	-0.19	0.39	2.30	-1.1E-03	-0.16	0.47	-0.18	0.42	1.97	-1.3E-03	0.08	0.73	0.01	0.95	2.222	5.6E-04
	Max	0.00	0.04	0.85	0.02	0.94	1.63	7.1E-04	-0.41	0.05	-0.28	0.19	6.28	-6.6E-03	0.08	0.72	0.11	0.63	1.456	1.3E-03
	Mean	0.29	-0.11	0.63	-0.12	0.59	2.40	-8.3E-04	-0.24	0.26	-0.23	0.30	2.36	-1.8E-03	-0.02	0.91	-0.02	0.95	2.369	-1.5E-04
Sum of sun flux ($W.m^{-2}$)	Sum	0.39	0.36	0.09	0.24	0.28	0.97	2.4E-04	0.40	0.06	0.37	0.08	0.56	2.5E-04	-0.30	0.18	-0.14	0.56	3.180	-1.7E-04
	Max	0.07	0.39	0.07	0.29	0.19	0.71	2.6E-03	0.46	0.03	0.40	0.06	0.19	2.9E-03	-0.35	0.12	-0.27	0.25	3.403	-1.9E-03
	Mean	0.39	0.36	0.09	0.24	0.28	0.97	6.0E-03	0.40	0.06	0.37	0.08	0.56	6.3E-03	-0.30	0.18	-0.14	0.56	3.180	-4.3E-03
Distance traveled (km)		0.00	-0.23	0.30	-0.16	0.47	2.54	-6.5E-04	-0.01	0.98	-0.25	0.25	1.77	-1.7E-05	0.02	0.93	-0.03	0.88	2.289	4.4E-05
Correlations tested with backward trajectories computed at 500 m																				
Altitude (m, agl)	Min	0.00	-0.14	0.53	-0.19	0.39	2.25	-1.4E-03	0.04	0.85	0.09	0.69	1.72	4.1E-04	0.33	0.14	0.19	0.40	2.043	2.7E-03
	Max	0.00	0.02	0.93	-0.06	0.77	2.00	2.1E-04	0.13	0.56	0.11	0.63	0.99	1.4E-03	0.10	0.67	0.15	0.51	1.817	8.8E-04
	Mean	0.00	-0.02	0.91	-0.04	0.87	2.16	-1.8E-04	0.08	0.71	0.05	0.81	1.63	5.8E-04	0.20	0.38	0.09	0.69	2.049	1.2E-03
Pressure (hPa)	Min	0.02	0.20	0.36	0.14	0.52	-15.55	2.0E-02	-0.07	0.76	-0.11	0.60	7.32	-6.3E-03	-0.01	0.96	0.05	0.82	3.110	-9.0E-04
	Max	0.03	-0.02	0.93	0.00	1.00	2.98	-8.8E-04	0.00	1.00	-0.01	0.97	1.70	5.3E-05	-0.01	0.96	0.04	0.88	2.777	-4.7E-04
	Mean	0.30	0.01	0.98	0.04	0.86	1.84	3.0E-04	0.04	0.85	0.01	0.97	0.03	1.8E-03	0.04	0.86	0.13	0.59	0.897	1.5E-03
Potential temperature (°C)	Min	0.10	0.39	0.06	0.28	0.20	1.65	4.6E-02	0.37	0.08	0.40	0.06	1.33	4.2E-02	-0.05	0.83	0.06	0.81	2.372	-4.9E-03
	Max	0.10	0.43	0.04	0.35	0.10	0.78	6.8E-02	0.41	0.05	0.42	0.05	0.55	6.1E-02	-0.24	0.30	-0.11	0.63	2.957	-3.1E-02
	Mean	0.08	0.41	0.05	0.37	0.09	1.33	5.4E-02	0.40	0.06	0.43	0.04	1.03	5.0E-02	-0.13	0.58	-0.05	0.82	2.535	-1.4E-02
Ambient temperature (°C)	Min	0.03	0.48	0.02	0.42	0.05	1.67	7.0E-02	0.43	0.04	0.49	0.02	1.38	6.0E-02	-0.13	0.59	-0.09	0.71	2.422	-1.5E-02
	Max	0.39	0.51	0.01	0.41	0.05	0.92	8.0E-02	0.53	0.01	0.54	0.01	0.59	7.8E-02	-0.20	0.39	-0.10	0.67	2.711	-2.5E-02
	Mean	0.02	0.46	0.03	0.41	0.05	1.39	7.0E-02	0.47	0.03	0.55	0.01	1.06	6.7E-02	-0.13	0.57	-0.17	0.47	2.499	-1.6E-02
Sum precipitation (mm)		0.00	0.20	0.36	0.29	0.18	1.99	3.4E-02	0.08	0.72	0.06	0.80	1.71	1.3E-02	-0.57	0.01	-0.28	0.21	2.579	-8.1E-02
Mixed Layer Depth (m)	Min	0.00	0.03	0.88	0.10	0.66	2.08	3.5E-04	0.09	0.68	0.06	0.79	1.66	9.0E-04	0.36	0.11	0.36	0.11	1.983	3.0E-03
	Max	0.84	0.15	0.48	0.04	0.87	1.52	5.5E-04	0.29	0.18	0.17	0.45	0.69	9.9E-04	-0.32	0.16	-0.03	0.89	3.400	-9.7E-04
	Mean	0.71	0.02	0.92	0.08	0.71	2.02	2.1E-04	0.15	0.49	0.22	0.31	1.10	1.4E-03	-0.06	0.80	0.06	0.80	2.536	-4.6E-04
Relative humidity (%)	Min	0.38	0.13	0.54	0.17	0.44	1.35	1.4E-02	0.14	0.52	0.31	0.15	0.98	1.4E-02	0.02	0.92	0.07	0.77	2.211	1.9E-03
	Max	0.01	0.08	0.73	0.02	0.93	1.35	9.0E-03	-0.03	0.91	0.11	0.62	2.00	-2.9E-03	-0.04	0.87	0.03	0.90	2.630	-3.6E-03
	Mean	0.29	0.15	0.49	0.16	0.46	1.01	1.6E-02	0.07	0.76	0.23	0.29	1.29	6.6E-03	-0.02	0.92	0.00	1.00	2.464	-2.1E-03
Terrain Height (m, agl)	Min	0.04	0.07	0.75	0.03	0.90	2.01	6.0E-04	-0.19	0.38	-0.21	0.33	2.04	-1.5E-03	-0.18	0.44	-0.22	0.33	2.543	-1.2E-03
	Max	0.00	0.11	0.63	-0.01	0.95	0.87	1.8E-03	-0.05	0.82	0.01	0.97	2.32	-8.3E-04	-0.07	0.76	-0.10	0.68	2.997	-1.0E-03
	Mean	0.10	0.05	0.84	-0.02	0.94	1.99	3.7E-04	-0.19	0.38	-0.22	0.32	2.29	-1.5E-03	-0.24	0.29	-0.27	0.23	2.895	-1.6E-03
Sum of sun flux ($W.m^{-2}$)	Sum	0.60	0.33	0.12	0.22	0.31	1.00	2.3E-04	0.41	0.05	0.36	0.09	0.46	2.7E-04	-0.43	0.05	-0.30	0.19	3.602	-2.6E-04
	Max	0.38	0.36	0.09	0.30	0.16	0.74	2.5E-03	0.51	0.01	0.48	0.02	-0.12	3.4E-03	-0.44	0.05	-0.25	0.28	3.832	-2.6E-03
	Mean	0.60	0.33	0.12	0.22	0.31	1.00	5.8E-03	0.41	0.05	0.36	0.09	0.46	6.7E-03	-0.43	0.05	-0.30	0.19	3.602	-6.4E-03
Distance traveled (km)		0.00	-0.34	0.11	-0.20	0.36	2.79	-1.7E-03	-0.07	0.76	-0.14	0.53	1.88	-3.2E-04	0.07	0.76	-0.02	0.93	2.204	2.8E-04
Correlations tested with backward trajectories computed at 50 m																				
Altitude (m, agl)	Min	0.00	0.10	0.65	0.07	0.74	2.08	1.8E-02	0.02	0.94	0.04	0.87	1.75	2.7E-03	0.05	0.84	-0.02	0.92	2.301	6.7E-03
	Max	0.00	0.05	0.83	-0.05	0.82	2.05	8.3E-04	0.13	0.56	0.06	0.79	1.59	2.2E-03	0.00	0.99	-0.14	0.54	2.314	3.9E-05
	Mean	0.00	0.11	0.60	-0.15	0.49	2.04	4.6E-03	0.11	0.63	0.05	0.81	1.68	4.1E-03	0.01	0.98	-0.08	0.72	2.313	2.2E-04
Pressure (hPa)	Min	0.10	0.20	0.37	0.17	0.43	-19.47	2.3E-02	0.02	0.94	0.00	1.00	0.17	1.7E-03	0.05	0.83	0.05	0.83	-1.991	4.6E-03
	Max	0.24	0.11	0.62	0.14	0.52	-5.12	7.3E-03	0.01	0.98	-0.03	0.91	1.44	3.2E-04	0.25	0.27	0.29	0.21	-10.948	1.3E-02
	Mean	0.05	0.16	0.47	0.16	0.48	-9.13	1.2E-02	0.08	0.71	0.07	0.74	-3.83	5.8E-03	0.29	0.20	0.31	0.17	-14.358	1.7E-02
Potential temperature (°C)	Min	0.07	0.40	0.06	0.29	0.19	1.65	5.5E-02	0.37	0.08	0.39	0.07	1.35	4.8E-02	-0.16	0.49	-0.02	0.92	2.484	-1.8E-02
	Max	0.23	0.41	0.05	0.32	0.14	1.05	5.5E-02	0.45	0.03	0.47	0.02	0.64	5.8E-02	-0.23	0.31	-0.20	0.37	2.860	-2.7E-02
	Mean	0.09	0.41	0.05	0.33	0.12	1.36	5.6E-02	0.40	0.06	0.44	0.03	1.06	5.2E-02	-0.18	0.44	-0.05	0.83	2.607	-2.0E-02
Ambient temperature (°C)	Min	0.02	0.46	0.03	0.46	0.03	1.71	6.6E-02	0.41	0.05	0.49	0.02	1.41	5.6E-02	-0.11	0.63	-0.07	0.77	2.407	-1.3E-02
	Max	0.26	0.46	0.03	0.41	0.05	1.09	6.2E-02	0.48	0.02	0.54	0.01	0.74	6.1E-02	-0.17	0.46	-0.10	0.65	2.645	-1.9E-02
	Mean	0.02	0.45	0.03	0.41	0.05	1.46	6.3E-02	0.43	0.04	0.48	0.02	1.16	5.7E-02	-0.13	0.57	-0.06	0.81	2.489	-1.5E-02
Sum_Rainfall (mm)		0.00	0.16	0.47	0.22	0.31	2.02	2.1E-02	0.11	0.61	-0.02	0.92	1.69	1.4E-02	-0.48	0.03	-0.17	0.47	2.540	-5.2E-02
Mixed Layer Depth (m)	Min	0.00	-0.12	0.59	0.02	0.92	2.26	-1.2E-03	-0.02	0.93	0.08	0.71	1.78	-1.8E-04	0.35	0.12	0.24	0.30	1.987	2.6E-03
	Max	0.54	0.21	0.33	0.09	0.70	1.34	7.0E-04	0.35	0.11	0.32	0.13	0.56	1.1E-03	-0.32	0.15	-0.11	0.62	3.345	-9.0E-04
	Mean	0.42	0.06	0.78	0.08	0.72	1.84	5.8E-04	0.20	0.36	0.24	0.27	0.90	1.8E-03	-0.07	0.75	0.09	0.70	2.594	-5.6E-04
Relative humidity (%)	Min	0.39	-0.01	0.95	-0.06	0.78	2.20	-1.4E-03	-0.01	0.95	0.03	0.90	1.83	-1.4E-03	0.13	0.57	0.19	0.40	1.696	1.2E-02
	Max	0.00	0.09	0.68	0.17	0.45	1.16	1.1E-02	0.00	1.00	0.21	0.33	1.77	-1.6E-04	-0.07	0.77	0.07	0.75	2.905	-6.7E-03
	Mean	0.12	0.06	0.78	0.03	0.88	1.64	6.8E-03	0.00	0.99	0.09	0.69	1.77	-1.9E-04	0.09	0.69	0.17	0.45	1.728	8.3E-03
Terrain Height (m, agl)	Min	0.35	-0.19	0.39	-0.22	0.31	2.55	-1.7E-03	-0.11	0.62	-0.19	0.39	2.00	-9.6E-04	-0.22	0.34	-0.32	0.15	2.743	-1.6E-03
	Max	0.00	-0.07	0.75	-0.14	0.52	3.13	-1.5E-03	-0.21	0.34	-0.11	0.60	4.52	-4.2E-03	-0.14	0.55	-0.24	0.29	3.891	-2.4E-03
	Mean	0.09	-0.19	0.39	-0.23	0.30	2.96	-1.9E-03	-0.18	0.41	-0.21	0.35	2.52	-1.8E-03	-0.30	0.18	-0.39	0.08	3.440	-2.5E-03
Sum of sun flux ($W.m^{-2}$)	Sum	0.53	0.41	0.05	0.29	0.18	0.80	2.7E-04	0.43	0.04	0.39	0.06	0.44	2.7E-04	-0.43	0.05	-0.31	0.17	3.497	-2.4E-04
	Max	0.12	0.45	0.03	0.36	0.09	0.54	2.9E-03	0.52	0.01	0.49	0.02	0.03	3.2E-03	-0.42	0.06	-0.29	0.20	3.558	-2.2E-03
	Mean	0.53	0.41	0.05	0.29	0.18	0.80	6.9E-03	0.43	0.04	0.39	0.06	0.44	6.8E-03	-0.43	0.05	-0.31	0.17	3.497	-5.9E-03
Distance traveled (km)		0.14	-0.24	0.27	-0.21	0.34	2.70	-2.4E-03	-0.16	0.48	-0.19	0.37	2.12	-1.5E-03	0.03	0.89	0.03	0.90	2.258	2.5E-04

a In addtion to the total number of ice nucleators, the following biological variables were statistically analyzed for each of the 6 media described in the material and methods section: concentration of total bacteria, frequency and concentration of culturable bacteria (i) INA only the first time they were tested and (ii) for which INA is reproducible and confirmed.

[b] All the correlations with physical properties listed in materials and methods section have been tested for the three elevations. Correlations are based on the analyse of N= 23 samples

Models are given only for variables for which at least one of the Pearson's or Spearman's coefficients was significantly different from zero are presented (P<0.1).

[c] The p-value for the Shapiro-Wilk test is given next to each *x* variable and below each *y* variable. When the p-value is under 0.05, normality of the data distribution is rejected.

Frequency of culturable bacteria grown on CA nucleating ice at > -8°C (%)

Climatic or chemical variables (x)[b]		Biological variables (y)[a]	Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
			r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry								
pH		0.40	-0.26	0.35	0.01	0.97	0.13	-1.6E-02
Conductivity (µS.cm)		0.00	-0.32	0.24	-0.55	0.03	0.03	-5.0E-04
Total Disolved Solids (ppm)		0.04	-0.39	0.17	-0.29	0.31	0.02	-4.5E-04
Salinity (ppm)		0.00	-0.31	0.28	-0.36	0.21	0.01	-2.7E-04
Correlations tested with backward trajectories computed at 1000 m								
Altitude (m, agl)	Min	0.05	0.01	0.96	0.20	0.36	0.02	1.0E-06
	Max	0.00	-0.13	0.57	0.01	0.98	0.04	-2.5E-05
	Mean	0.44	-0.05	0.80	0.24	0.28	0.02	-5.1E-06
Pressure (hPa)	Min	0.00	0.19	0.39	0.26	0.24	-0.30	3.9E-04
	Max	0.29	0.15	0.48	-0.01	0.96	-0.08	1.1E-04
	Mean	0.76	0.27	0.22	-0.08	0.70	-0.17	2.0E-04
Potential temperature (°C)	Min	0.15	-0.02	0.93	0.15	0.50	0.02	-5.0E-05
	Max	0.05	0.04	0.84	0.30	0.16	0.01	1.8E-04
	Mean	0.04	-0.04	0.84	0.08	0.73	0.02	-1.3E-04
Ambient temperature (°C)	Min	0.17	0.10	0.66	0.23	0.29	0.01	3.5E-04
	Max	0.40	0.02	0.94	0.13	0.56	0.02	5.4E-05
	Mean	0.29	0.06	0.80	0.15	0.50	0.01	1.9E-04
Sum_Rainfall (mm)		0.00	-0.09	0.68	0.00	0.99	0.02	-3.4E-04
Mixed Layer Depth (m)	Min	0.00	0.33	0.12	0.12	0.59	0.01	6.5E-05
	Max	0.55	-0.22	0.31	-0.23	0.30	0.03	-1.4E-05
	Mean	0.08	0.02	0.94	-0.04	0.85	0.01	3.2E-06
Relative humidity (%)	Min	0.54	0.33	0.12	0.24	0.28	-0.01	5.0E-04
	Max	0.02	0.38	0.07	0.36	0.09	-0.04	7.1E-04
	Mean	0.33	0.36	0.09	0.30	0.16	-0.03	6.0E-04
Terrain Height (m, agl)	Min	0.01	-0.26	0.22	-0.22	0.30	0.02	-5.0E-05
	Max	0.00	-0.09	0.68	-0.06	0.78	0.04	-3.1E-05
	Mean	0.29	-0.34	0.11	-0.16	0.46	0.03	-5.5E-05
Sum of sun flux (W.m^{-2})	Sum	0.39	0.16	0.46	0.10	0.65	0.01	2.3E-06
	Max	0.07	0.15	0.51	0.02	0.93	0.00	2.0E-05
	Mean	0.39	0.16	0.46	0.10	0.65	0.01	5.6E-05
Distance traveled (km)		0.00	0.05	0.83	-0.29	0.18	0.01	2.8E-06
Correlations tested with backward trajectories computed at 500 m								
Altitude (m, agl)	Min	0.00	-0.03	0.90	-0.01	0.96	0.02	-6.2E-06
	Max	0.00	-0.03	0.91	-0.06	0.79	0.02	-5.9E-06
	Mean	0.00	-0.11	0.63	-0.03	0.90	0.02	-1.6E-05
Pressure (hPa)	Min	0.02	0.20	0.36	0.32	0.13	-0.36	4.2E-04
	Max	0.03	0.29	0.17	0.40	0.06	-0.27	2.9E-04
	Mean	0.30	0.37	0.08	0.41	0.05	-0.31	3.5E-04
Potential temperature (°C)	Min	0.10	0.04	0.86	0.13	0.56	0.01	9.4E-05
	Max	0.10	0.01	0.95	0.13	0.56	0.01	4.9E-05
	Mean	0.08	0.01	0.96	0.20	0.36	0.02	3.2E-05
Ambient temperature (°C)	Min	0.03	0.14	0.54	0.27	0.22	0.01	4.2E-04
	Max	0.39	0.10	0.65	0.20	0.36	0.01	3.3E-04
	Mean	0.02	0.12	0.60	0.17	0.45	0.01	3.7E-04
Sum precipitation (mm)		0.00	-0.05	0.81	-0.04	0.85	0.02	-1.9E-04
Mixed Layer Depth (m)	Min	0.00	0.72	0.00	0.37	0.08	0.00	1.6E-04
	Max	0.84	-0.08	0.71	-0.12	0.58	0.02	-6.1E-06
	Mean	0.71	0.33	0.12	0.04	0.86	-0.02	6.8E-05
Relative humidity (%)	Min	0.38	0.31	0.15	0.28	0.20	-0.02	6.8E-04
	Max	0.01	0.33	0.13	0.24	0.26	-0.05	8.1E-04
	Mean	0.29	0.30	0.17	0.24	0.27	-0.03	6.5E-04
Terrain Height (m, agl)	Min	0.04	-0.36	0.09	-0.34	0.11	0.03	-6.4E-05
	Max	0.00	-0.21	0.34	-0.36	0.10	0.07	-7.6E-05
	Mean	0.10	-0.39	0.06	-0.40	0.06	0.04	-6.7E-05
Sum of sun flux (W.m^{-2})	Sum	0.60	0.02	0.94	0.04	0.84	0.01	2.5E-07
	Max	0.38	0.09	0.70	0.01	0.97	0.01	1.2E-05
	Mean	0.60	0.02	0.94	0.04	0.84	0.01	6.3E-06
Distance traveled (km)		0.00	-0.04	0.86	-0.31	0.15	0.02	-4.2E-06
Correlations tested with backward trajectories computed at 50 m								
Altitude (m, agl)	Min	0.00	-0.04	0.86	0.21	0.34	0.02	-1.5E-04
	Max	0.00	-0.01	0.96	-0.21	0.33	0.02	-4.0E-06
	Mean	0.00	-0.02	0.94	-0.34	0.11	0.02	-1.5E-05
Pressure (hPa)	Min	0.10	0.19	0.39	0.42	0.05	-0.42	4.6E-04
	Max	0.24	0.52	0.01	0.64	0.00	-0.70	7.3E-04
	Mean	0.05	0.52	0.01	0.62	0.00	-0.75	8.0E-04
Potential temperature (°C)	Min	0.07	0.02	0.93	0.13	0.54	0.02	5.2E-05
	Max	0.23	0.01	0.96	0.10	0.66	0.02	3.0E-05
	Mean	0.09	0.03	0.88	0.18	0.42	0.01	9.2E-05
Ambient temperature (°C)	Min	0.02	0.15	0.48	0.29	0.18	0.01	4.6E-04
	Max	0.26	0.10	0.65	0.28	0.20	0.01	2.8E-04
	Mean	0.02	0.12	0.60	0.23	0.29	0.01	3.4E-04
Sum_Rainfall (mm)		0.00	-0.03	0.88	-0.10	0.64	0.02	-9.0E-05
Mixed Layer Depth (m)	Min	0.00	0.55	0.01	0.20	0.36	0.00	1.1E-04
	Max	0.54	-0.01	0.95	0.02	0.94	0.02	-9.8E-07
	Mean	0.42	0.35	0.10	0.13	0.55	-0.02	6.7E-05
Relative humidity (%)	Min	0.39	0.16	0.46	0.02	0.93	0.00	3.8E-04
	Max	0.00	0.26	0.22	0.14	0.52	-0.04	6.8E-04
	Mean	0.12	0.23	0.29	0.07	0.74	-0.02	5.3E-04
Terrain Height (m, agl)	Min	0.35	-0.54	0.01	-0.64	0.00	0.04	-1.1E-04
	Max	0.00	-0.24	0.27	-0.57	0.00	0.09	-1.1E-04
	Mean	0.09	-0.53	0.01	-0.63	0.00	0.07	-1.1E-04
Sum of sun flux (W.m^{-2})	Sum	0.53	0.03	0.90	0.09	0.69	0.01	3.9E-07
	Max	0.12	0.14	0.53	0.12	0.58	0.01	1.9E-05
	Mean	0.53	0.03	0.90	0.09	0.69	0.01	9.7E-06
Distance traveled (km)		0.14	0.20	0.36	-0.23	0.29	0.01	4.2E-05

Frequency of culturable bacteria grown on CA 10% nucleating ice at > -8°C (%)

Climatic or chemical variables (x)[b]		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		-0.38	0.18	-0.25	0.38	0.10	-1.3E-02
Conductivity (µS.cm)		-0.32	0.27	-0.35	0.22	0.02	-2.5E-04
Total Disolved Solids (ppm)		-0.28	0.36	-0.18	0.55	0.01	-2.2E-04
Salinity (ppm)		-0.17	0.59	0.05	0.88	0.01	-1.0E-04
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	-0.15	0.54	-0.02	0.95	0.01	-7.2E-06
	Max	-0.25	0.30	-0.03	0.91	0.04	-2.5E-05
	Mean	-0.20	0.41	0.06	0.81	0.02	-1.0E-05
Pressure (hPa)	Min	0.25	0.31	0.17	0.49	-0.20	2.5E-04
	Max	0.25	0.31	-0.03	0.91	-0.07	9.0E-05
	Mean	0.35	0.15	0.06	0.81	-0.11	1.3E-04
Potential temperature (°C)	Min	-0.06	0.81	0.10	0.68	0.01	-8.2E-05
	Max	0.04	0.87	0.31	0.20	0.01	8.9E-05
	Mean	-0.03	0.90	0.11	0.64	0.01	-4.9E-05
Ambient temperature (°C)	Min	0.10	0.68	0.19	0.43	0.01	2.0E-04
	Max	0.11	0.65	0.28	0.24	0.01	1.8E-04
	Mean	0.11	0.65	0.26	0.28	0.01	2.0E-04
Sum_Rainfall (mm)		-0.15	0.53	-0.16	0.52	0.01	-2.8E-04
Mixed Layer Depth (m)	Min	0.36	0.13	0.18	0.47	0.01	3.5E-05
	Max	-0.19	0.43	0.01	0.96	0.02	-6.8E-06
	Mean	0.05	0.84	0.09	0.71	0.01	5.3E-06
Relative humidity (%)	Min	0.25	0.30	0.17	0.48	0.00	2.0E-04
	Max	0.30	0.21	0.28	0.25	-0.01	3.0E-04
	Mean	0.28	0.25	0.25	0.30	-0.01	2.5E-04
Terrain Height (m, agl)	Min	-0.21	0.39	-0.20	0.40	0.01	-2.0E-05
	Max	-0.24	0.32	-0.29	0.24	0.05	-5.9E-05
	Mean	-0.35	0.14	-0.24	0.32	0.02	-3.2E-05
Sum of sun flux (W.m^{-2})	Sum	0.19	0.44	0.16	0.51	0.01	1.4E-06
	Max	0.19	0.43	0.09	0.70	0.00	1.4E-05
	Mean	0.19	0.44	0.16	0.51	0.00	3.5E-05
Distance traveled (km)		0.06	0.82	-0.26	0.28	0.01	1.7E-06
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	-0.08	0.75	0.11	0.65	0.01	-8.4E-06
	Max	-0.19	0.43	-0.07	0.77	0.02	-2.3E-05
	Mean	-0.20	0.42	0.00	0.99	0.01	-1.6E-05
Pressure (hPa)	Min	0.27	0.27	0.22	0.37	-0.25	3.0E-04
	Max	0.34	0.16	0.25	0.31	-0.15	1.7E-04
	Mean	0.44	0.06	0.31	0.19	-0.19	2.1E-04
Potential temperature (°C)	Min	0.05	0.84	0.19	0.44	0.01	6.6E-05
	Max	0.01	0.98	0.16	0.50	0.01	1.2E-05
	Mean	0.02	0.92	0.28	0.24	0.01	3.6E-05
Ambient temperature (°C)	Min	0.14	0.57	0.25	0.30	0.01	2.3E-04
	Max	0.17	0.48	0.30	0.21	0.01	3.1E-04
	Mean	0.16	0.51	0.28	0.25	0.01	2.8E-04
Sum precipitation (mm)		-0.14	0.58	-0.26	0.28	0.01	-2.5E-04
Mixed Layer Depth (m)	Min	0.67	0.00	0.34	0.16	0.00	7.2E-05
	Max	-0.13	0.59	-0.03	0.91	0.02	-5.6E-06
	Mean	0.27	0.26	0.12	0.63	0.00	3.1E-05
Relative humidity (%)	Min	0.26	0.28	0.21	0.38	-0.01	3.0E-04
	Max	0.34	0.15	0.26	0.29	-0.03	5.2E-04
	Mean	0.25	0.31	0.15	0.53	-0.01	3.1E-04
Terrain Height (m, agl)	Min	-0.40	0.09	-0.46	0.05	0.02	-3.6E-05
	Max	-0.25	0.30	-0.34	0.15	0.04	-4.6E-05
	Mean	-0.45	0.05	-0.51	0.03	0.02	-3.9E-05
Sum of sun flux (W.m^{-2})	Sum	-0.04	0.86	0.07	0.77	0.01	-3.4E-07
	Max	0.07	0.78	0.06	0.81	0.01	5.7E-06
	Mean	-0.04	0.86	0.07	0.77	0.01	-8.4E-06
Distance traveled (km)		-0.03	0.90	-0.18	0.46	0.01	-1.6E-06
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	-0.01	0.95	0.04	0.86	0.01	-2.6E-05
	Max	-0.19	0.44	-0.28	0.25	0.01	-3.4E-05
	Mean	-0.19	0.43	-0.41	0.08	0.01	-8.0E-05
Pressure (hPa)	Min	0.33	0.17	0.45	0.05	-0.40	4.4E-04
	Max	0.51	0.03	0.45	0.05	-0.38	3.9E-04
	Mean	0.57	0.01	0.54	0.02	-0.45	4.8E-04
Potential temperature (°C)	Min	0.01	0.96	0.24	0.31	0.01	1.7E-05
	Max	0.00	0.99	0.15	0.54	0.01	3.8E-06
	Mean	0.03	0.91	0.25	0.29	0.01	4.3E-05
Ambient temperature (°C)	Min	0.15	0.54	0.28	0.25	0.01	2.5E-04
	Max	0.10	0.67	0.26	0.28	0.01	1.5E-04
	Mean	0.12	0.63	0.26	0.28	0.01	1.9E-04
Sum_Rainfall (mm)		-0.05	0.83	-0.11	0.67	0.01	-7.3E-05
Mixed Layer Depth (m)	Min	0.52	0.02	0.26	0.28	0.00	5.1E-05
	Max	-0.14	0.56	0.04	0.87	0.02	-5.5E-06
	Mean	0.25	0.30	0.19	0.45	0.00	2.7E-05
Relative humidity (%)	Min	0.26	0.27	0.17	0.48	-0.01	3.2E-04
	Max	0.34	0.16	0.31	0.19	-0.03	5.1E-04
	Mean	0.30	0.21	0.17	0.50	-0.02	3.9E-04
Terrain Height (m, agl)	Min	-0.55	0.01	-0.59	0.01	0.03	-5.8E-05
	Max	-0.33	0.16	-0.57	0.01	0.06	-7.5E-05
	Mean	-0.58	0.01	-0.58	0.01	0.04	-6.4E-05
Sum of sun flux (W.m^{-2})	Sum	-0.01	0.95	0.07	0.78	0.01	-1.0E-07
	Max	0.11	0.65	0.10	0.68	0.01	7.8E-06
	Mean	-0.01	0.95	0.07	0.78	0.01	-2.5E-06
Distance traveled (km)		0.18	0.47	-0.16	0.52	0.01	1.8E-05

Frequency of culturable bacteria grown on EMB nucleating ice at > -8°C (%)

Climatic or chemical variables (x)[b]		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		-0.27	0.33	-0.26	0.36	0.22	-2.5E-02
Conductivity (µS.cm)		-0.47	0.08	-0.60	0.02	0.06	-1.0E-03
Total Disolved Solids (ppm)		-0.48	0.08	-0.53	0.05	0.05	-1.5E-03
Salinity (ppm)		-0.43	0.13	-0.33	0.25	0.05	-9.7E-04
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	-0.19	0.40	-0.11	0.61	0.04	-2.4E-05
	Max	-0.25	0.25	-0.20	0.36	0.11	-7.2E-05
	Mean	-0.21	0.36	-0.04	0.84	0.05	-2.8E-05
Pressure (hPa)	Min	0.34	0.12	0.41	0.06	-0.80	1.0E-03
	Max	0.28	0.20	0.18	0.44	-0.24	2.9E-04
	Mean	0.31	0.16	0.13	0.56	-0.27	3.3E-04
Potential temperature (°C)	Min	-0.08	0.73	0.09	0.68	0.04	-3.0E-04
	Max	0.04	0.85	0.31	0.16	0.03	2.5E-04
	Mean	-0.04	0.86	0.15	0.50	0.03	-1.7E-04
Ambient temperature (°C)	Min	0.10	0.65	0.32	0.14	0.03	5.4E-04
	Max	0.07	0.77	0.29	0.19	0.03	3.0E-04
	Mean	0.08	0.73	0.32	0.15	0.03	3.9E-04
Sum_Rainfall (mm)		-0.18	0.43	-0.16	0.48	0.04	-9.6E-04
Mixed Layer Depth (m)	Min	0.14	0.52	-0.11	0.62	0.03	4.1E-05
	Max	-0.19	0.39	-0.02	0.92	0.05	-1.8E-05
	Mean	-0.13	0.56	0.02	0.93	0.05	-3.7E-05
Relative humidity (%)	Min	0.19	0.38	0.15	0.50	0.01	4.2E-04
	Max	0.36	0.10	0.24	0.28	-0.05	1.0E-03
	Mean	0.25	0.27	0.19	0.39	-0.01	6.0E-04
Terrain Height (m, agl)	Min	-0.04	0.84	-0.07	0.76	0.03	-1.2E-05
	Max	0.08	0.73	-0.04	0.87	0.00	4.5E-05
	Mean	-0.11	0.64	-0.04	0.85	0.04	-2.5E-05
Sum of sun flux (W.m^{-2})	Sum	0.19	0.40	0.23	0.31	0.01	4.0E-06
	Max	0.17	0.45	0.16	0.48	0.01	3.5E-05
	Mean	0.19	0.40	0.23	0.31	0.01	1.0E-04
Distance traveled (km)		-0.07	0.76	-0.24	0.28	0.04	-6.0E-06
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	-0.06	0.78	-0.05	0.81	0.03	-2.0E-05
	Max	-0.14	0.54	-0.23	0.30	0.06	-4.7E-05
	Mean	-0.15	0.50	-0.09	0.68	0.04	-3.4E-05
Pressure (hPa)	Min	0.39	0.07	0.45	0.04	-1.03	1.2E-03
	Max	0.32	0.15	0.29	0.20	-0.42	4.6E-04
	Mean	0.38	0.08	0.33	0.14	-0.46	5.2E-04
Potential temperature (°C)	Min	0.04	0.86	0.22	0.34	0.03	1.4E-04
	Max	0.01	0.96	0.26	0.24	0.03	5.4E-05
	Mean	0.01	0.96	0.32	0.14	0.03	4.1E-05
Ambient temperature (°C)	Min	0.11	0.59	0.32	0.15	0.03	5.4E-04
	Max	0.11	0.61	0.33	0.13	0.02	5.5E-04
	Mean	0.12	0.59	0.30	0.18	0.03	5.6E-04
Sum precipitation (mm)		-0.15	0.50	-0.19	0.40	0.03	-8.3E-04
Mixed Layer Depth (m)	Min	0.51	0.02	0.17	0.44	0.01	1.6E-04
	Max	-0.12	0.60	0.01	0.98	0.05	-1.4E-05
	Mean	0.12	0.59	0.06	0.80	0.01	3.7E-05
Relative humidity (%)	Min	0.25	0.26	0.16	0.47	-0.01	8.0E-04
	Max	0.32	0.15	0.14	0.55	-0.07	1.2E-03
	Mean	0.23	0.30	0.07	0.77	-0.02	7.7E-04
Terrain Height (m, agl)	Min	-0.31	0.16	-0.27	0.23	0.05	-8.0E-05
	Max	-0.29	0.19	-0.33	0.14	0.14	-1.5E-04
	Mean	-0.32	0.14	-0.33	0.13	0.06	-7.9E-05
Sum of sun flux (W.m^{-2})	Sum	-0.03	0.90	0.08	0.71	0.04	-6.3E-07
	Max	0.04	0.86	0.07	0.75	0.03	9.2E-06
	Mean	-0.03	0.90	0.08	0.71	0.04	-1.6E-05
Distance traveled (km)		-0.17	0.46	-0.36	0.10	0.04	-2.6E-05
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	-0.07	0.77	0.07	0.75	0.03	-3.5E-04
	Max	-0.10	0.66	-0.40	0.06	0.04	-5.3E-05
	Mean	-0.10	0.65	-0.41	0.06	0.03	-1.3E-04
Pressure (hPa)	Min	0.41	0.06	0.53	0.01	-1.36	1.5E-03
	Max	0.55	0.01	0.58	0.00	-1.08	1.1E-03
	Mean	0.56	0.01	0.60	0.00	-1.20	1.3E-03
Potential temperature (°C)	Min	0.01	0.97	0.24	0.28	0.03	3.0E-05
	Max	0.01	0.95	0.24	0.28	0.03	6.1E-05
	Mean	0.02	0.95	0.26	0.24	0.03	6.4E-05
Ambient temperature (°C)	Min	0.14	0.54	0.33	0.13	0.03	6.0E-04
	Max	0.11	0.61	0.35	0.11	0.02	4.8E-04
	Mean	0.11	0.64	0.28	0.21	0.03	4.6E-04
Sum_Rainfall (mm)		-0.09	0.68	-0.12	0.58	0.03	-3.9E-04
Mixed Layer Depth (m)	Min	0.34	0.12	-0.02	0.93	0.02	9.9E-05
	Max	-0.10	0.65	0.06	0.79	0.04	-1.1E-05
	Mean	0.13	0.56	0.12	0.61	0.01	3.9E-05
Relative humidity (%)	Min	0.19	0.40	0.08	0.73	0.00	6.4E-04
	Max	0.29	0.20	0.11	0.64	-0.06	1.1E-03
	Mean	0.22	0.33	0.01	0.97	-0.02	7.4E-04
Terrain Height (m, agl)	Min	-0.51	0.02	-0.55	0.01	0.07	-1.5E-04
	Max	-0.36	0.10	-0.57	0.01	0.19	-2.4E-04
	Mean	-0.50	0.02	-0.56	0.01	0.10	-1.6E-04
Sum of sun flux (W.m^{-2})	Sum	0.00	0.99	0.14	0.54	0.03	-4.5E-08
	Max	0.08	0.71	0.14	0.53	0.02	1.7E-05
	Mean	0.00	0.99	0.14	0.54	0.03	-1.1E-06
Distance traveled (km)		0.06	0.80	-0.31	0.17	0.03	1.7E-05

Frequency of culturable bacteria grown on MA nucleating ice at > -8°C (%)

Climatic or chemical variables (x)[b]		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		-0.17	0.53	-0.40	0.14	0.14	-1.6E-02
Conductivity (µS.cm)		-0.42	0.11	-0.62	0.01	0.05	-9.5E-04
Total Disolved Solids (ppm)		-0.34	0.24	-0.49	0.08	0.05	-1.3E-03
Salinity (ppm)		-0.37	0.20	-0.31	0.28	0.04	-1.1E-03
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	-0.23	0.30	-0.04	0.84	0.04	-2.8E-05
	Max	-0.21	0.32	-0.11	0.62	0.09	-5.9E-05
	Mean	-0.19	0.39	0.02	0.91	0.04	-2.5E-05
Pressure (hPa)	Min	0.30	0.16	0.30	0.17	-0.69	8.7E-04
	Max	0.25	0.25	0.01	0.97	-0.21	2.4E-04
	Mean	0.21	0.33	0.02	0.92	-0.18	2.3E-04
Potential temperature (°C)	Min	-0.16	0.48	-0.06	0.78	0.03	-5.7E-04
	Max	-0.01	0.97	0.08	0.71	0.03	-4.6E-05
	Mean	-0.08	0.71	-0.04	0.87	0.03	-3.4E-04
Ambient temperature (°C)	Min	0.01	0.97	0.10	0.65	0.02	3.8E-05
	Max	0.02	0.93	0.06	0.79	0.02	7.9E-05
	Mean	-0.01	0.97	0.05	0.81	0.03	-3.5E-05
Sum_Rainfall (mm)		-0.25	0.26	-0.17	0.43	0.03	-1.3E-03
Mixed Layer Depth (m)	Min	0.16	0.48	0.09	0.68	0.02	4.3E-05
	Max	-0.22	0.31	-0.04	0.84	0.05	-2.0E-05
	Mean	-0.15	0.50	0.19	0.39	0.04	-4.0E-05
Relative humidity (%)	Min	0.00	1.00	0.01	0.96	0.02	-1.3E-06
	Max	0.29	0.19	0.18	0.41	-0.04	7.6E-04
	Mean	0.11	0.62	0.08	0.73	0.01	2.6E-04
Terrain Height (m, agl)	Min	0.10	0.65	0.07	0.74	0.02	2.7E-05
	Max	0.17	0.45	-0.03	0.88	-0.03	8.2E-05
	Mean	0.05	0.83	0.00	0.99	0.02	1.1E-05
Sum of sun flux (W.m^{-2})	Sum	0.13	0.54	0.22	0.32	0.01	2.7E-06
	Max	0.09	0.70	0.07	0.75	0.02	1.7E-05
	Mean	0.13	0.54	0.22	0.32	0.01	6.7E-05
Distance traveled (km)		-0.11	0.61	-0.20	0.35	0.03	-9.5E-06
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	0.00	0.99	0.02	0.92	0.02	7.5E-07
	Max	-0.12	0.60	-0.03	0.87	0.05	-3.9E-05
	Mean	-0.09	0.68	-0.04	0.85	0.03	-2.0E-05
Pressure (hPa)	Min	0.38	0.07	0.24	0.26	-0.98	1.1E-03
	Max	0.24	0.28	0.14	0.53	-0.30	3.3E-04
	Mean	0.31	0.15	0.27	0.22	-0.37	4.2E-04
Potential temperature (°C)	Min	-0.03	0.88	0.07	0.76	0.03	-1.1E-04
	Max	-0.06	0.77	0.04	0.87	0.03	-3.0E-04
	Mean	-0.05	0.81	0.13	0.55	0.03	-2.1E-04
Ambient temperature (°C)	Min	0.03	0.89	0.10	0.65	0.02	1.3E-04
	Max	0.01	0.97	0.02	0.94	0.02	3.3E-05
	Mean	0.03	0.90	0.02	0.94	0.02	1.2E-04
Sum precipitation (mm)		-0.20	0.36	-0.15	0.49	0.03	-1.0E-03
Mixed Layer Depth (m)	Min	0.45	0.03	0.31	0.15	0.01	1.4E-04
	Max	-0.19	0.37	-0.02	0.94	0.05	-2.1E-05
	Mean	-0.02	0.93	0.12	0.59	0.03	-5.6E-06
Relative humidity (%)	Min	0.19	0.39	0.10	0.65	-0.01	5.8E-04
	Max	0.26	0.24	0.16	0.47	-0.05	9.1E-04
	Mean	0.12	0.59	-0.01	0.95	0.00	3.7E-04
Terrain Height (m, agl)	Min	-0.23	0.30	-0.09	0.70	0.04	-5.7E-05
	Max	-0.31	0.15	-0.27	0.20	0.13	-1.6E-04
	Mean	-0.27	0.21	-0.24	0.26	0.05	-6.6E-05
Sum of sun flux (W.m^{-2})	Sum	-0.03	0.88	0.14	0.52	0.03	-6.9E-07
	Max	0.03	0.91	0.13	0.55	0.02	5.4E-06
	Mean	-0.03	0.88	0.14	0.52	0.03	-1.7E-05
Distance traveled (km)		-0.16	0.47	-0.09	0.67	0.03	-2.4E-05
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	-0.14	0.52	0.05	0.81	0.03	-7.5E-04
	Max	-0.08	0.73	-0.12	0.58	0.03	-4.0E-05
	Mean	-0.09	0.68	-0.28	0.20	0.03	-1.1E-04
Pressure (hPa)	Min	0.36	0.10	0.19	0.39	-1.13	1.2E-03
	Max	0.44	0.04	0.32	0.13	-0.82	8.6E-04
	Mean	0.49	0.02	0.40	0.06	-1.01	1.1E-03
Potential temperature (°C)	Min	-0.06	0.78	0.08	0.70	0.03	-2.4E-04
	Max	-0.05	0.81	0.02	0.91	0.03	-2.1E-04
	Mean	-0.05	0.81	0.11	0.61	0.03	-2.1E-04
Ambient temperature (°C)	Min	0.04	0.84	0.13	0.55	0.02	1.8E-04
	Max	0.03	0.89	0.11	0.61	0.02	1.2E-04
	Mean	0.02	0.92	0.11	0.63	0.02	9.7E-05
Sum_Rainfall (mm)		-0.12	0.58	-0.06	0.79	0.03	-4.8E-04
Mixed Layer Depth (m)	Min	0.31	0.16	0.15	0.50	0.01	8.7E-05
	Max	-0.24	0.26	-0.15	0.48	0.05	-2.4E-05
	Mean	-0.05	0.83	0.12	0.59	0.03	-1.3E-05
Relative humidity (%)	Min	0.21	0.35	0.15	0.51	-0.01	6.8E-04
	Max	0.26	0.23	0.19	0.39	-0.06	9.6E-04
	Mean	0.19	0.38	0.04	0.86	-0.02	6.2E-04
Terrain Height (m, agl)	Min	-0.36	0.09	-0.32	0.13	0.05	-1.0E-04
	Max	-0.29	0.18	-0.31	0.16	0.15	-1.8E-04
	Mean	-0.41	0.05	-0.37	0.09	0.08	-1.2E-04
Sum of sun flux (W.m^{-2})	Sum	0.00	0.98	0.20	0.37	0.03	-8.9E-08
	Max	0.04	0.86	0.21	0.33	0.02	7.2E-06
	Mean	0.00	0.98	0.20	0.37	0.03	-2.2E-06
Distance traveled (km)		0.05	0.84	0.03	0.89	0.02	1.4E-05

Frequency of culturable bacteria grown on R2A nucleating ice at > -8°C (%)

Climatic or chemical variables (x)[b]		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		0.06	0.84	-0.11	0.69	0.00	1.8E-03
Conductivity (µS.cm)		-0.35	0.20	-0.46	0.09	0.02	-2.9E-04
Total Disolved Solids (ppm)		-0.33	0.25	-0.43	0.12	0.02	-5.2E-04
Salinity (ppm)		-0.25	0.39	-0.05	0.86	0.02	-2.9E-04
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	-0.08	0.72	-0.14	0.53	0.01	-3.7E-06
	Max	-0.13	0.55	-0.09	0.69	0.02	-1.4E-05
	Mean	-0.05	0.81	-0.05	0.81	0.01	-2.7E-06
Pressure (hPa)	Min	0.22	0.32	0.22	0.31	-0.19	2.4E-04
	Max	0.13	0.56	0.09	0.67	-0.03	4.7E-05
	Mean	0.11	0.62	0.08	0.72	-0.03	4.4E-05
Potential temperature (°C)	Min	0.00	0.99	0.12	0.58	0.01	2.6E-06
	Max	0.09	0.68	0.26	0.24	0.01	2.0E-04
	Mean	0.04	0.87	0.17	0.44	0.01	5.7E-05
Ambient temperature (°C)	Min	0.12	0.58	0.30	0.16	0.01	2.3E-04
	Max	0.07	0.76	0.27	0.21	0.01	1.1E-04
	Mean	0.08	0.71	0.29	0.18	0.01	1.5E-04
Sum_Rainfall (mm)		-0.02	0.92	-0.02	0.92	0.01	-4.3E-05
Mixed Layer Depth (m)	Min	0.09	0.69	-0.12	0.58	0.01	9.3E-06
	Max	-0.19	0.39	0.09	0.69	0.02	-6.4E-06
	Mean	-0.15	0.48	0.10	0.65	0.02	-1.6E-05
Relative humidity (%)	Min	0.15	0.51	0.15	0.50	0.00	1.2E-04
	Max	0.37	0.08	0.21	0.34	-0.02	3.8E-04
	Mean	0.23	0.28	0.18	0.40	0.00	2.1E-04
Terrain Height (m, agl)	Min	0.07	0.76	0.10	0.66	0.01	6.9E-06
	Max	0.12	0.60	0.04	0.87	-0.01	2.2E-05
	Mean	0.03	0.90	0.09	0.68	0.01	2.4E-06
Sum of sun flux (W.m^{-2})	Sum	0.17	0.45	0.33	0.13	0.00	1.3E-06
	Max	0.16	0.46	0.29	0.18	0.00	1.2E-05
	Mean	0.17	0.45	0.33	0.13	0.00	3.1E-05
Distance traveled (km)		-0.14	0.51	-0.21	0.32	0.01	-4.6E-06
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	-0.04	0.87	0.00	0.99	0.01	-4.2E-06
	Max	0.03	0.90	-0.05	0.83	0.01	3.5E-06
	Mean	-0.01	0.95	-0.03	0.91	0.01	-1.2E-06
Pressure (hPa)	Min	0.22	0.32	0.20	0.36	-0.20	2.4E-04
	Max	0.24	0.28	0.16	0.46	-0.11	1.3E-04
	Mean	0.22	0.32	0.14	0.52	-0.10	1.1E-04
Potential temperature (°C)	Min	0.09	0.68	0.26	0.23	0.01	1.2E-04
	Max	0.06	0.77	0.27	0.21	0.01	1.2E-04
	Mean	0.08	0.73	0.30	0.17	0.01	1.1E-04
Ambient temperature (°C)	Min	0.14	0.51	0.30	0.17	0.01	2.4E-04
	Max	0.16	0.45	0.30	0.17	0.01	2.9E-04
	Mean	0.15	0.51	0.27	0.21	0.01	2.5E-04
Sum precipitation (mm)		-0.21	0.33	-0.07	0.75	0.01	-4.1E-04
Mixed Layer Depth (m)	Min	0.37	0.08	0.12	0.59	0.00	4.4E-05
	Max	-0.12	0.58	0.07	0.77	0.02	-4.9E-06
	Mean	0.02	0.93	0.15	0.50	0.01	2.1E-06
Relative humidity (%)	Min	0.25	0.25	0.27	0.21	-0.01	2.9E-04
	Max	0.33	0.13	0.26	0.23	-0.03	4.4E-04
	Mean	0.21	0.33	0.17	0.43	-0.01	2.5E-04
Terrain Height (m, agl)	Min	-0.22	0.31	-0.14	0.52	0.01	-2.1E-05
	Max	-0.10	0.65	-0.15	0.48	0.02	-1.9E-05
	Mean	-0.20	0.36	-0.17	0.43	0.02	-1.9E-05
Sum of sun flux (W.m^{-2})	Sum	0.09	0.68	0.15	0.49	0.01	7.1E-07
	Max	0.10	0.66	0.16	0.46	0.01	7.7E-06
	Mean	0.09	0.68	0.15	0.49	0.01	1.8E-05
Distance traveled (km)		-0.20	0.35	-0.25	0.24	0.01	-1.2E-05
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	0.20	0.36	0.20	0.37	0.01	4.1E-04
	Max	-0.01	0.98	-0.18	0.42	0.01	-1.1E-06
	Mean	0.04	0.86	-0.15	0.50	0.01	1.8E-05
Pressure (hPa)	Min	0.37	0.08	0.37	0.08	-0.45	4.9E-04
	Max	0.46	0.03	0.38	0.07	-0.33	3.4E-04
	Mean	0.46	0.03	0.38	0.08	-0.36	3.9E-04
Potential temperature (°C)	Min	0.07	0.76	0.27	0.21	0.01	1.0E-04
	Max	0.09	0.69	0.26	0.24	0.01	1.3E-04
	Mean	0.08	0.71	0.27	0.21	0.01	1.3E-04
Ambient temperature (°C)	Min	0.17	0.44	0.32	0.13	0.01	2.7E-04
	Max	0.18	0.42	0.35	0.10	0.01	2.7E-04
	Mean	0.16	0.47	0.29	0.17	0.01	2.5E-04
Sum_Rainfall (mm)		-0.18	0.40	-0.07	0.75	0.01	-2.8E-04
Mixed Layer Depth (m)	Min	0.23	0.29	-0.03	0.90	0.01	2.5E-05
	Max	-0.03	0.89	0.18	0.41	0.01	-1.1E-06
	Mean	0.08	0.71	0.22	0.32	0.01	8.5E-06
Relative humidity (%)	Min	0.09	0.69	0.11	0.60	0.00	1.1E-04
	Max	0.25	0.25	0.19	0.39	-0.02	3.5E-04
	Mean	0.13	0.57	0.07	0.75	0.00	1.6E-04
Terrain Height (m, agl)	Min	-0.42	0.04	-0.40	0.06	0.02	-4.4E-05
	Max	-0.33	0.12	-0.39	0.06	0.06	-7.9E-05
	Mean	-0.41	0.05	-0.35	0.10	0.03	-4.8E-05
Sum of sun flux (W.m^{-2})	Sum	0.11	0.62	0.21	0.34	0.01	8.2E-07
	Max	0.16	0.47	0.22	0.30	0.00	1.2E-05
	Mean	0.11	0.62	0.21	0.34	0.01	2.1E-05
Distance traveled (km)		-0.06	0.79	-0.17	0.43	0.01	-6.8E-06

Frequency of culturable bacteria grown on TSA nucleating ice at > -8°C (%)

Climatic or chemical variables (x)[b]		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		0.08	0.79	-0.02	0.95	-0.01	4.7E-03
Conductivity (µS.cm)		-0.41	0.13	-0.55	0.04	0.04	-6.3E-04
Total Disolved Solids (ppm)		-0.35	0.22	-0.41	0.15	0.04	-9.8E-04
Salinity (ppm)		-0.35	0.21	-0.30	0.29	0.03	-7.4E-04
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	-0.03	0.91	0.02	0.93	0.02	-2.2E-06
	Max	-0.21	0.33	-0.14	0.51	0.06	-4.1E-05
	Mean	-0.06	0.80	0.05	0.83	0.02	-5.1E-06
Pressure (hPa)	Min	0.34	0.11	0.34	0.12	-0.55	6.9E-04
	Max	0.15	0.49	0.04	0.87	-0.08	1.0E-04
	Mean	0.17	0.44	0.05	0.82	-0.09	1.2E-04
Potential temperature (°C)	Min	-0.08	0.71	0.16	0.47	0.02	-2.1E-04
	Max	0.00	0.98	0.23	0.29	0.02	-1.9E-05
	Mean	-0.06	0.77	0.15	0.49	0.02	-1.8E-04
Ambient temperature (°C)	Min	0.04	0.85	0.27	0.21	0.02	1.5E-04
	Max	-0.04	0.87	0.20	0.36	0.02	-1.1E-04
	Mean	0.00	0.98	0.25	0.25	0.02	-1.5E-05
Sum_Rainfall (mm)		-0.16	0.47	-0.20	0.36	0.02	-5.9E-04
Mixed Layer Depth (m)	Min	0.11	0.61	0.04	0.86	0.02	2.1E-05
	Max	-0.25	0.24	-0.06	0.80	0.04	-1.6E-05
	Mean	-0.21	0.34	0.05	0.83	0.04	-3.9E-05
Relative humidity (%)	Min	0.12	0.57	0.12	0.59	0.01	1.8E-04
	Max	0.39	0.07	0.21	0.34	-0.04	7.1E-04
	Mean	0.19	0.37	0.10	0.65	0.00	3.2E-04
Terrain Height (m, agl)	Min	-0.07	0.76	-0.13	0.54	0.02	-1.2E-05
	Max	0.08	0.73	-0.11	0.61	0.00	2.6E-05
	Mean	-0.08	0.71	-0.11	0.62	0.02	-1.3E-05
Sum of sun flux (W.m^{-2})	Sum	0.10	0.65	0.24	0.27	0.01	1.4E-06
	Max	0.06	0.77	0.20	0.37	0.01	8.6E-06
	Mean	0.10	0.65	0.24	0.27	0.01	3.4E-05
Distance traveled (km)		-0.16	0.46	-0.28	0.20	0.03	-9.6E-06
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	-0.11	0.63	-0.14	0.53	0.02	-2.2E-05
	Max	-0.11	0.61	-0.17	0.43	0.03	-2.6E-05
	Mean	-0.15	0.49	-0.13	0.57	0.02	-2.3E-05
Pressure (hPa)	Min	0.38	0.07	0.30	0.16	-0.67	7.9E-04
	Max	0.37	0.09	0.30	0.16	-0.33	3.5E-04
	Mean	0.39	0.06	0.30	0.16	-0.33	3.6E-04
Potential temperature (°C)	Min	-0.02	0.92	0.15	0.50	0.02	-5.5E-05
	Max	-0.03	0.91	0.21	0.33	0.02	-8.4E-05
	Mean	-0.03	0.89	0.26	0.24	0.02	-8.0E-05
Ambient temperature (°C)	Min	0.07	0.76	0.25	0.26	0.02	2.0E-04
	Max	0.09	0.70	0.31	0.14	0.01	2.7E-04
	Mean	0.08	0.73	0.29	0.18	0.02	2.4E-04
Sum precipitation (mm)		-0.23	0.29	-0.22	0.31	0.02	-8.0E-04
Mixed Layer Depth (m)	Min	0.38	0.07	0.17	0.43	0.01	8.3E-05
	Max	-0.12	0.60	0.00	1.00	0.03	-8.6E-06
	Mean	0.04	0.87	0.10	0.67	0.02	7.1E-06
Relative humidity (%)	Min	0.16	0.46	0.08	0.73	0.00	3.4E-04
	Max	0.27	0.22	0.18	0.41	-0.04	6.5E-04
	Mean	0.15	0.49	0.01	0.96	0.00	3.2E-04
Terrain Height (m, agl)	Min	-0.31	0.14	-0.22	0.31	0.03	-5.5E-05
	Max	-0.27	0.22	-0.29	0.17	0.08	-9.5E-05
	Mean	-0.32	0.14	-0.30	0.16	0.04	-5.3E-05
Sum of sun flux (W.m^{-2})	Sum	0.05	0.82	0.17	0.43	0.02	7.1E-07
	Max	0.05	0.82	0.14	0.53	0.02	7.3E-06
	Mean	0.05	0.82	0.17	0.43	0.02	1.8E-05
Distance traveled (km)		-0.22	0.32	-0.29	0.17	0.03	-2.3E-05
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	0.01	0.95	0.08	0.70	0.02	4.9E-05
	Max	-0.09	0.69	-0.24	0.27	0.02	-3.2E-05
	Mean	-0.08	0.71	-0.32	0.13	0.02	-6.9E-05
Pressure (hPa)	Min	0.46	0.03	0.48	0.02	-1.01	1.1E-03
	Max	0.55	0.01	0.51	0.01	-0.73	7.6E-04
	Mean	0.59	0.00	0.55	0.01	-0.84	9.0E-04
Potential temperature (°C)	Min	-0.05	0.83	0.17	0.44	0.02	-1.3E-04
	Max	0.02	0.94	0.23	0.28	0.02	4.8E-05
	Mean	-0.02	0.94	0.24	0.27	0.02	-4.7E-05
Ambient temperature (°C)	Min	0.08	0.70	0.30	0.16	0.02	2.5E-04
	Max	0.11	0.60	0.38	0.07	0.01	3.2E-04
	Mean	0.08	0.73	0.29	0.18	0.02	2.3E-04
Sum_Rainfall (mm)		-0.20	0.36	-0.24	0.27	0.02	-5.6E-04
Mixed Layer Depth (m)	Min	0.23	0.29	0.05	0.80	0.01	4.6E-05
	Max	-0.04	0.87	0.13	0.56	0.02	-2.5E-06
	Mean	0.08	0.71	0.20	0.37	0.01	1.5E-05
Relative humidity (%)	Min	0.01	0.97	-0.14	0.51	0.02	1.8E-05
	Max	0.18	0.40	0.07	0.76	-0.02	4.6E-04
	Mean	0.05	0.83	-0.13	0.55	0.01	1.1E-04
Terrain Height (m, agl)	Min	-0.49	0.02	-0.52	0.01	0.04	-9.4E-05
	Max	-0.39	0.07	-0.53	0.01	0.13	-1.7E-04
	Mean	-0.51	0.01	-0.49	0.02	0.07	-1.1E-04
Sum of sun flux (W.m^{-2})	Sum	0.06	0.78	0.23	0.29	0.01	8.6E-07
	Max	0.11	0.62	0.25	0.25	0.01	1.5E-05
	Mean	0.06	0.78	0.23	0.29	0.01	2.1E-05
Distance traveled (km)		-0.04	0.85	-0.21	0.33	0.02	-8.5E-06

Frequency of culturable bacteria grown on TSA 10% nucleating ice at > -8°C (%)

Climatic or chemical variables (x)[b]		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		-0.42	0.12	-0.17	0.54	0.215	-2.7E-02
Conductivity (µS.cm)		-0.36	0.18	-0.54	0.04	0.033	-5.8E-04
Total Disolved Solids (ppm)		-0.32	0.27	-0.27	0.35	0.028	-7.7E-04
Salinity (ppm)		-0.25	0.38	-0.43	0.13	0.023	-4.5E-04
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	-0.17	0.45	-0.09	0.69	0.025	-1.6E-05
	Max	-0.24	0.30	-0.07	0.76	0.068	-4.6E-05
	Mean	-0.17	0.46	0.01	0.96	0.028	-1.6E-05
Pressure (hPa)	Min	0.25	0.28	0.24	0.30	-0.393	5.0E-04
	Max	0.24	0.30	0.10	0.66	-0.143	1.7E-04
	Mean	0.30	0.19	0.11	0.64	-0.180	2.2E-04
Potential temperature (°C)	Min	0.00	0.99	0.09	0.69	0.018	-1.1E-05
	Max	0.15	0.53	0.27	0.23	0.004	5.9E-04
	Mean	0.06	0.81	0.07	0.77	0.015	1.7E-04
Ambient temperature (°C)	Min	0.17	0.46	0.20	0.38	0.014	6.1E-04
	Max	0.21	0.36	0.26	0.26	0.009	6.6E-04
	Mean	0.19	0.42	0.23	0.31	0.012	6.4E-04
Sum_Rainfall (mm)		-0.15	0.51	-0.11	0.64	0.020	-5.7E-04
Mixed Layer Depth (m)	Min	0.22	0.33	-0.02	0.93	0.012	4.3E-05
	Max	-0.10	0.66	-0.09	0.70	0.026	-6.9E-06
	Mean	0.02	0.95	-0.12	0.61	0.017	2.9E-06
Relative humidity (%)	Min	0.17	0.46	0.20	0.39	0.003	2.6E-04
	Max	0.28	0.22	0.36	0.11	-0.026	5.4E-04
	Mean	0.24	0.29	0.31	0.17	-0.011	4.1E-04
Terrain Height (m, agl)	Min	-0.08	0.72	-0.12	0.61	0.021	-1.6E-05
	Max	-0.12	0.61	-0.15	0.52	0.050	-4.7E-05
	Mean	-0.22	0.33	-0.11	0.64	0.030	-3.6E-05
Sum of sun flux (W.m^{-2})	Sum	0.21	0.37	0.24	0.30	0.003	3.1E-06
	Max	0.17	0.47	0.07	0.76	0.004	2.4E-05
	Mean	0.21	0.37	0.24	0.30	0.003	7.7E-05
Distance traveled (km)		-0.06	0.79	-0.38	0.09	0.020	-3.7E-06
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	0.00	0.99	0.04	0.87	0.018	-8.6E-07
	Max	-0.17	0.47	-0.10	0.68	0.040	-3.9E-05
	Mean	-0.09	0.70	-0.01	0.96	0.021	-1.4E-05
Pressure (hPa)	Min	0.26	0.25	0.29	0.20	-0.474	5.6E-04
	Max	0.24	0.28	0.30	0.19	-0.219	2.4E-04
	Mean	0.33	0.14	0.33	0.14	-0.290	3.2E-04
Potential temperature (°C)	Min	0.14	0.56	0.20	0.40	0.014	3.5E-04
	Max	0.09	0.71	0.20	0.38	0.012	3.0E-04
	Mean	0.11	0.63	0.28	0.21	0.013	3.2E-04
Ambient temperature (°C)	Min	0.19	0.40	0.24	0.30	0.014	6.0E-04
	Max	0.23	0.32	0.30	0.18	0.006	7.7E-04
	Mean	0.22	0.34	0.23	0.31	0.010	7.2E-04
Sum precipitation (mm)		-0.13	0.58	-0.19	0.42	0.020	-4.9E-04
Mixed Layer Depth (m)	Min	0.52	0.02	0.31	0.17	0.005	1.1E-04
	Max	-0.13	0.56	-0.11	0.63	0.030	-1.1E-05
	Mean	0.08	0.73	-0.07	0.78	0.010	1.7E-05
Relative humidity (%)	Min	0.18	0.45	0.15	0.51	-0.004	3.9E-04
	Max	0.25	0.27	0.27	0.23	-0.039	6.6E-04
	Mean	0.16	0.49	0.14	0.55	-0.008	3.7E-04
Terrain Height (m, agl)	Min	-0.31	0.17	-0.33	0.15	0.028	-5.5E-05
	Max	-0.24	0.30	-0.40	0.07	0.077	-8.6E-05
	Mean	-0.38	0.09	-0.36	0.11	0.042	-6.5E-05
Sum of sun flux (W.m^{-2})	Sum	-0.07	0.76	0.05	0.84	0.024	-1.1E-06
	Max	0.01	0.98	-0.01	0.95	0.017	1.1E-06
	Mean	-0.07	0.76	0.05	0.84	0.024	-2.8E-05
Distance traveled (km)		-0.14	0.55	-0.37	0.10	0.024	-1.5E-05
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	-0.01	0.95	0.17	0.46	0.018	-5.2E-05
	Max	-0.16	0.50	-0.34	0.13	0.023	-5.7E-05
	Mean	-0.14	0.53	-0.37	0.10	0.020	-1.2E-04
Pressure (hPa)	Min	0.30	0.18	0.47	0.03	-0.691	7.6E-04
	Max	0.44	0.05	0.60	0.00	-0.591	6.2E-04
	Mean	0.50	0.02	0.58	0.01	-0.750	8.0E-04
Potential temperature (°C)	Min	0.09	0.69	0.18	0.44	0.015	2.7E-04
	Max	0.06	0.79	0.15	0.53	0.014	1.9E-04
	Mean	0.10	0.65	0.27	0.23	0.014	3.1E-04
Ambient temperature (°C)	Min	0.21	0.35	0.25	0.27	0.014	6.5E-04
	Max	0.16	0.49	0.31	0.17	0.010	4.6E-04
	Mean	0.19	0.42	0.27	0.24	0.012	5.6E-04
Sum_Rainfall (mm)		-0.03	0.90	-0.07	0.77	0.018	-8.0E-05
Mixed Layer Depth (m)	Min	0.39	0.08	0.13	0.56	0.008	7.7E-05
	Max	-0.21	0.35	-0.02	0.94	0.036	-1.6E-05
	Mean	0.06	0.81	0.03	0.91	0.013	1.1E-05
Relative humidity (%)	Min	0.27	0.24	0.03	0.91	-0.016	6.3E-04
	Max	0.31	0.17	0.22	0.33	-0.054	8.3E-04
	Mean	0.29	0.20	0.09	0.71	-0.031	6.9E-04
Terrain Height (m, agl)	Min	-0.47	0.03	-0.60	0.00	0.042	-9.2E-05
	Max	-0.32	0.15	-0.63	0.00	0.116	-1.5E-04
	Mean	-0.52	0.02	-0.56	0.01	0.068	-1.1E-04
Sum of sun flux (W.m^{-2})	Sum	-0.02	0.94	0.06	0.79	0.019	-2.5E-07
	Max	0.04	0.87	0.08	0.73	0.015	5.3E-06
	Mean	-0.02	0.94	0.06	0.79	0.019	-6.2E-06
Distance traveled (km)		0.03	0.89	-0.34	0.14	0.016	6.5E-06

a In addtion to the total number of ice nucleators, the following biological variables were statistically analyzed for each of the 6 media described in the material and methods section: concentration of total bacteria, frequency and concentration of culturable bacteria (i) INA only the first time they were tested and (ii) for which INA is reproducible and confirmed.

[b] All the correlations with physical properties listed in materials and methods section have been tested for the three elevations. Correlations are based on the analyse of N= 23 samples

Models are given only for variables for which at least one of the Pearson's or Spearman's coefficients was significantly different from zero are presented (P<0.1).

[c] The p-value for the Shapiro-Wilk test is given next to each x variable and below each y variable. When the p-value is under 0.05, normality of the data distribution is rejected.

Climatic or chemical variables (x)[b]		Biological variables (y)[a]	Concentration of culturable bacteria grown on CA nucleating ice at > -8°C (%)					
			Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
			r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry								
pH		0.40	0.03	0.91	0.06	0.83	0.66	7.1E-02
Conductivity (µS.cm)		0.00	-0.43	0.11	-0.45	0.09	1.67	-2.5E-02
Total Disolved Solids (ppm)		0.04	-0.29	0.31	-0.17	0.57	1.52	-3.1E-02
Salinity (ppm)		0.00	-0.43	0.13	-0.44	0.12	1.58	-3.4E-02
Correlations tested with backward trajectories computed at 1000 m								
Altitude (m, agl)	Min	0.05	0.22	0.30	0.27	0.21	0.96	9.0E-04
	Max	0.00	-0.03	0.89	0.16	0.48	1.64	-2.8E-04
	Mean	0.44	0.25	0.25	0.30	0.16	0.64	1.1E-03
Pressure (hPa)	Min	0.00	0.25	0.25	0.14	0.54	-18.15	2.4E-02
	Max	0.29	-0.08	0.73	-0.05	0.81	3.61	-2.4E-03
	Mean	0.76	-0.09	0.67	-0.18	0.42	4.24	-3.2E-03
Potential temperature (°C)	Min	0.15	0.25	0.25	0.21	0.35	0.93	3.0E-02
	Max	0.05	0.32	0.14	0.34	0.11	-0.05	6.0E-02
	Mean	0.04	0.24	0.27	0.12	0.58	0.77	3.2E-02
Ambient temperature (°C)	Min	0.17	0.28	0.20	0.25	0.26	1.08	4.6E-02
	Max	0.40	0.18	0.42	0.17	0.44	1.03	2.5E-02
	Mean	0.29	0.23	0.29	0.16	0.47	1.00	3.6E-02
Sum_Rainfall (mm)		0.00	-0.11	0.63	0.11	0.62	1.42	-1.8E-02
Mixed Layer Depth (m)	Min	0.00	-0.06	0.78	0.05	0.81	1.42	-5.5E-04
	Max	0.55	-0.17	0.43	-0.20	0.35	1.90	-5.1E-04
	Mean	0.08	-0.20	0.36	-0.10	0.64	2.19	-1.7E-03
Relative humidity (%)	Min	0.54	0.27	0.22	0.31	0.16	0.29	1.8E-02
	Max	0.02	0.44	0.04	0.32	0.14	-1.81	3.8E-02
	Mean	0.33	0.36	0.10	0.36	0.10	-0.57	2.7E-02
Terrain Height (m, agl)	Min	0.01	-0.09	0.67	-0.22	0.31	1.47	-8.0E-04
	Max	0.00	-0.17	0.44	0.04	0.86	-0.56	2.8E-03
	Mean	0.29	-0.08	0.73	-0.10	0.64	1.54	-5.7E-04
Sum of sun flux (W.m^{-2})	Sum	0.39	0.04	0.84	0.01	0.96	1.21	2.8E-05
	Max	0.07	0.00	1.00	-0.04	0.86	1.34	-1.7E-06
	Mean	0.39	0.04	0.84	0.01	0.96	1.21	7.0E-04
Distance traveled (km)		0.00	-0.30	0.16	-0.26	0.23	1.88	-8.3E-04
Correlations tested with backward trajectories computed at 500 m								
Altitude (m, agl)	Min	0.00	0.19	0.38	0.08	0.72	1.17	1.9E-03
	Max	0.00	0.15	0.50	0.06	0.78	0.44	1.6E-03
	Mean	0.00	0.17	0.45	0.07	0.76	1.09	1.2E-03
Pressure (hPa)	Min	0.02	0.31	0.14	0.25	0.25	-25.40	3.0E-02
	Max	0.03	0.15	0.50	0.33	0.12	-5.18	6.6E-03
	Mean	0.30	0.21	0.34	0.34	0.12	-7.21	9.0E-03
Potential temperature (°C)	Min	0.10	0.26	0.24	0.17	0.45	1.05	2.9E-02
	Max	0.10	0.18	0.42	0.15	0.48	0.80	2.7E-02
	Mean	0.08	0.21	0.34	0.21	0.33	0.96	2.6E-02
Ambient temperature (°C)	Min	0.03	0.34	0.12	0.29	0.18	1.04	4.8E-02
	Max	0.39	0.23	0.30	0.20	0.35	0.83	3.4E-02
	Mean	0.02	0.29	0.18	0.15	0.49	0.91	4.2E-02
Sum precipitation (mm)		0.00	-0.12	0.58	0.01	0.98	1.42	-2.0E-02
Mixed Layer Depth (m)	Min	0.00	0.28	0.20	0.31	0.15	1.03	2.8E-03
	Max	0.84	-0.12	0.60	-0.07	0.76	1.78	-4.0E-04
	Mean	0.71	-0.05	0.82	0.00	1.00	1.57	-4.9E-04
Relative humidity (%)	Min	0.38	0.32	0.14	0.29	0.19	-0.43	3.2E-02
	Max	0.01	0.19	0.38	0.13	0.57	-0.57	2.2E-02
	Mean	0.29	0.27	0.22	0.21	0.33	-0.55	2.7E-02
Terrain Height (m, agl)	Min	0.04	-0.29	0.17	-0.32	0.14	1.79	-2.4E-03
	Max	0.00	-0.19	0.37	-0.25	0.24	3.54	-3.2E-03
	Mean	0.10	-0.34	0.11	-0.35	0.10	2.32	-2.7E-03
Sum of sun flux (W.m^{-2})	Sum	0.60	-0.06	0.80	-0.04	0.84	1.53	-3.8E-05
	Max	0.38	-0.10	0.65	-0.07	0.77	1.72	-6.8E-04
	Mean	0.60	-0.06	0.80	-0.04	0.84	1.53	-9.6E-04
Distance traveled (km)		0.00	-0.40	0.06	-0.30	0.16	2.10	-2.0E-03
Correlations tested with backward trajectories computed at 50 m								
Altitude (m, agl)	Min	0.00	0.15	0.51	0.26	0.23	1.29	2.5E-02
	Max	0.00	0.11	0.61	-0.18	0.42	1.19	1.9E-03
	Mean	0.00	0.19	0.40	-0.20	0.36	1.21	7.2E-03
Pressure (hPa)	Min	0.10	0.35	0.10	0.31	0.14	-35.39	3.9E-02
	Max	0.24	0.56	0.00	0.58	0.00	-34.44	3.6E-02
	Mean	0.05	0.63	0.00	0.55	0.01	-41.57	4.5E-02
Potential temperature (°C)	Min	0.07	0.21	0.34	0.17	0.44	1.11	2.7E-02
	Max	0.23	0.17	0.45	0.10	0.64	0.92	2.2E-02
	Mean	0.09	0.20	0.35	0.18	0.40	0.98	2.7E-02
Ambient temperature (°C)	Min	0.02	0.35	0.10	0.29	0.17	1.05	4.9E-02
	Max	0.26	0.29	0.18	0.26	0.23	0.73	3.7E-02
	Mean	0.02	0.31	0.15	0.23	0.29	0.91	4.2E-02
Sum_Rainfall (mm)		0.00	-0.16	0.48	-0.10	0.67	1.44	-2.0E-02
Mixed Layer Depth (m)	Min	0.00	0.12	0.60	0.15	0.48	1.21	1.1E-03
	Max	0.54	0.00	1.00	0.07	0.74	1.34	3.0E-06
	Mean	0.42	0.05	0.84	0.10	0.65	1.15	4.1E-04
Relative humidity (%)	Min	0.39	0.09	0.69	0.01	0.96	0.84	9.5E-03
	Max	0.00	0.17	0.45	0.06	0.77	-0.37	2.0E-02
	Mean	0.12	0.20	0.36	0.09	0.67	-0.16	2.1E-02
Terrain Height (m, agl)	Min	0.35	-0.55	0.01	-0.58	0.00	2.57	-5.0E-03
	Max	0.00	-0.35	0.10	-0.47	0.02	6.09	-7.2E-03
	Mean	0.09	-0.62	0.00	-0.60	0.00	3.99	-6.1E-03
Sum of sun flux (W.m^{-2})	Sum	0.53	-0.03	0.88	-0.03	0.90	1.45	-2.1E-05
	Max	0.12	-0.02	0.94	0.00	0.99	1.40	-1.1E-04
	Mean	0.53	-0.03	0.88	-0.03	0.90	1.45	-5.3E-04
Distance traveled (km)		0.14	-0.31	0.14	-0.30	0.17	2.08	-3.1E-03

Climatic or chemical variables (x)[b]		Concentration of culturable bacteria grown on CA 10% nucleating ice at > -8°C (%)					
		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		-0.02	0.95	0.02	0.96	1.95	-4.2E-02
Conductivity (µS.cm)		0.16	0.59	0.00	0.99	1.47	8.7E-03
Total Disolved Solids (ppm)		0.29	0.33	0.38	0.19	1.05	3.1E-02
Salinity (ppm)		0.25	0.41	0.14	0.65	1.20	2.0E-02
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	0.26	0.29	0.27	0.26	1.27	9.4E-04
	Max	0.01	0.96	0.27	0.27	1.61	8.2E-05
	Mean	0.27	0.26	0.28	0.25	0.99	1.0E-03
Pressure (hPa)	Min	-0.01	0.98	-0.11	0.65	2.07	-4.4E-04
	Max	-0.20	0.40	-0.16	0.51	7.04	-5.6E-03
	Mean	-0.13	0.60	-0.11	0.64	4.96	-3.6E-03
Potential temperature (°C)	Min	0.32	0.18	0.10	0.68	1.15	3.5E-02
	Max	0.25	0.30	0.21	0.39	0.72	4.0E-02
	Mean	0.28	0.24	0.03	0.91	1.06	3.3E-02
Ambient temperature (°C)	Min	0.26	0.28	0.14	0.58	1.42	3.8E-02
	Max	0.24	0.33	0.22	0.37	1.30	2.9E-02
	Mean	0.27	0.26	0.16	0.52	1.30	3.7E-02
Sum_Rainfall (mm)		0.15	0.54	0.22	0.37	1.61	2.1E-02
Mixed Layer Depth (m)	Min	0.35	0.14	0.37	0.12	1.31	2.6E-03
	Max	0.03	0.91	0.00	0.99	1.61	7.6E-05
	Mean	0.33	0.16	0.18	0.47	0.22	2.8E-03
Relative humidity (%)	Min	0.48	0.04	0.52	0.02	-0.07	2.9E-02
	Max	0.45	0.06	0.42	0.07	-1.07	3.3E-02
	Mean	0.48	0.04	0.57	0.01	-0.69	3.3E-02
Terrain Height (m, agl)	Min	-0.15	0.53	-0.32	0.18	1.88	-1.1E-03
	Max	-0.27	0.27	-0.30	0.22	5.07	-5.0E-03
	Mean	-0.21	0.39	-0.34	0.15	2.16	-1.4E-03
Sum of sun flux (W.m^{-2})	Sum	0.05	0.84	-0.04	0.87	1.56	2.9E-05
	Max	0.07	0.77	-0.02	0.94	1.48	3.9E-04
	Mean	0.05	0.84	-0.04	0.87	1.56	7.1E-04
Distance traveled (km)		-0.15	0.54	-0.18	0.46	1.94	-3.4E-04
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	0.41	0.08	0.37	0.12	1.32	3.4E-03
	Max	0.14	0.57	0.17	0.48	0.98	1.3E-03
	Mean	0.24	0.32	0.16	0.51	1.35	1.5E-03
Pressure (hPa)	Min	0.01	0.98	-0.02	0.92	1.27	5.0E-04
	Max	-0.02	0.94	0.23	0.35	2.34	-6.5E-04
	Mean	0.13	0.59	0.26	0.29	-2.86	4.8E-03
Potential temperature (°C)	Min	0.37	0.12	0.22	0.37	1.22	3.8E-02
	Max	0.21	0.38	0.10	0.70	1.07	3.0E-02
	Mean	0.30	0.22	0.17	0.49	1.12	3.5E-02
Ambient temperature (°C)	Min	0.40	0.09	0.25	0.31	1.27	5.2E-02
	Max	0.31	0.19	0.24	0.33	1.00	4.2E-02
	Mean	0.37	0.11	0.18	0.45	1.10	4.9E-02
Sum precipitation (mm)		0.00	0.99	-0.03	0.89	1.70	5.0E-04
Mixed Layer Depth (m)	Min	0.49	0.04	0.53	0.02	1.23	4.0E-03
	Max	0.01	0.96	0.08	0.75	1.66	3.6E-05
	Mean	0.32	0.18	0.29	0.23	0.36	2.8E-03
Relative humidity (%)	Min	0.44	0.06	0.38	0.10	-0.53	3.9E-02
	Max	0.31	0.20	0.23	0.35	-1.44	3.6E-02
	Mean	0.35	0.14	0.31	0.20	-0.72	3.4E-02
Terrain Height (m, agl)	Min	-0.41	0.08	-0.54	0.02	2.21	-2.8E-03
	Max	-0.20	0.42	-0.19	0.42	3.56	-2.7E-03
	Mean	-0.49	0.03	-0.55	0.01	2.84	-3.2E-03
Sum of sun flux (W.m^{-2})	Sum	0.00	0.99	-0.07	0.78	1.69	2.0E-06
	Max	0.03	0.91	0.01	0.98	1.60	1.8E-04
	Mean	0.00	0.99	-0.07	0.78	1.69	5.1E-05
Distance traveled (km)		-0.08	0.76	-0.10	0.68	1.83	-3.0E-04
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	0.10	0.70	-0.02	0.92	1.67	1.3E-02
	Max	0.07	0.77	-0.07	0.77	1.62	9.9E-04
	Mean	0.06	0.80	-0.05	0.83	1.66	2.0E-03
Pressure (hPa)	Min	0.04	0.86	0.00	1.00	-2.32	4.3E-03
	Max	0.27	0.26	0.29	0.22	-14.06	1.6E-02
	Mean	0.44	0.06	0.41	0.08	-25.23	2.8E-02
Potential temperature (°C)	Min	0.33	0.17	0.28	0.25	1.28	3.9E-02
	Max	0.24	0.33	0.08	0.74	1.11	2.8E-02
	Mean	0.30	0.22	0.26	0.36	1.15	3.5E-02
Ambient temperature (°C)	Min	0.42	0.07	0.26	0.28	1.27	5.3E-02
	Max	0.31	0.19	0.18	0.47	1.05	3.5E-02
	Mean	0.37	0.12	0.23	0.34	1.15	4.5E-02
Sum_Rainfall (mm)		0.08	0.74	-0.02	0.92	1.66	8.9E-03
Mixed Layer Depth (m)	Min	0.40	0.09	0.46	0.05	1.30	3.0E-03
	Max	0.04	0.86	0.13	0.59	1.55	1.3E-04
	Mean	0.32	0.17	0.35	0.15	0.33	2.7E-03
Relative humidity (%)	Min	0.36	0.13	0.23	0.34	-0.07	3.3E-02
	Max	0.30	0.21	0.31	0.20	-1.35	3.5E-02
	Mean	0.40	0.09	0.38	0.11	-1.14	3.9E-02
Terrain Height (m, agl)	Min	-0.42	0.07	-0.52	0.02	2.53	-3.4E-03
	Max	-0.26	0.27	-0.33	0.16	4.77	-4.6E-03
	Mean	-0.55	0.01	-0.58	0.01	3.76	-4.7E-03
Sum of sun flux (W.m^{-2})	Sum	0.00	0.99	-0.10	0.67	1.71	-1.7E-06
	Max	0.03	0.89	-0.03	0.91	1.60	1.8E-04
	Mean	0.00	0.99	-0.10	0.67	1.71	-4.3E-05
Distance traveled (km)		-0.09	0.72	-0.04	0.86	1.87	-6.9E-04

Climatic or chemical variables (x)[b]		Concentration of culturable bacteria grown on EMB nucleating ice at > -8°C (%)					
		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		0.03	0.92	0.02	0.94	1.28	5.4E-02
Conductivity (µS.cm)		-0.51	0.05	-0.37	0.18	2.19	-2.6E-02
Total Disolved Solids (ppm)		-0.41	0.15	-0.12	0.68	2.19	-3.9E-02
Salinity (ppm)		-0.51	0.06	-0.46	0.10	2.16	-3.6E-02
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	0.10	0.66	0.06	0.80	1.59	3.6E-04
	Max	-0.19	0.41	0.01	0.96	3.36	-1.5E-03
	Mean	0.14	0.53	0.12	0.58	1.39	5.4E-04
Pressure (hPa)	Min	0.38	0.08	0.22	0.32	-24.27	3.2E-02
	Max	-0.01	0.97	0.08	0.72	1.94	-2.1E-04
	Mean	-0.04	0.85	-0.07	0.76	2.95	-1.3E-03
Potential temperature (°C)	Min	0.39	0.07	0.34	0.12	1.16	4.1E-02
	Max	0.38	0.08	0.40	0.06	0.26	6.4E-02
	Mean	0.36	0.10	0.27	0.23	0.96	4.4E-02
Ambient temperature (°C)	Min	0.44	0.04	0.44	0.04	1.36	6.4E-02
	Max	0.36	0.10	0.40	0.06	1.16	4.6E-02
	Mean	0.40	0.07	0.39	0.07	1.22	5.5E-02
Sum_Rainfall (mm)		0.06	0.80	0.12	0.59	1.71	8.9E-03
Mixed Layer Depth (m)	Min	-0.17	0.44	-0.13	0.57	1.95	-1.4E-03
	Max	-0.20	0.37	-0.18	0.42	2.35	-5.4E-04
	Mean	-0.35	0.11	-0.25	0.26	3.13	-2.8E-03
Relative humidity (%)	Min	0.23	0.30	0.32	0.15	0.94	1.4E-02
	Max	0.31	0.16	0.23	0.30	-0.22	2.4E-02
	Mean	0.28	0.21	0.33	0.13	0.45	1.9E-02
Terrain Height (m, agl)	Min	-0.05	0.83	-0.09	0.68	1.81	-3.6E-04
	Max	0.08	0.74	0.02	0.91	0.91	1.2E-03
	Mean	-0.02	0.94	0.00	1.00	1.78	-1.0E-04
Sum of sun flux (W.m^{-2})	Sum	0.18	0.43	0.20	0.38	1.23	1.1E-04
	Max	0.24	0.28	0.19	0.39	0.97	1.4E-03
	Mean	0.18	0.43	0.20	0.38	1.23	2.7E-03
Distance traveled (km)		-0.31	0.16	-0.38	0.08	2.22	-7.4E-04
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	0.15	0.49	0.15	0.50	1.62	1.4E-03
	Max	0.03	0.91	-0.04	0.86	1.61	2.4E-04
	Mean	0.12	0.61	0.10	0.65	1.59	7.3E-04
Pressure (hPa)	Min	0.36	0.10	0.23	0.31	-25.88	3.1E-02
	Max	0.11	0.63	0.21	0.35	-2.58	4.4E-03
	Mean	0.14	0.52	0.19	0.41	-3.53	5.5E-03
Potential temperature (°C)	Min	0.39	0.07	0.35	0.11	1.32	4.0E-02
	Max	0.31	0.17	0.34	0.12	0.90	4.2E-02
	Mean	0.35	0.11	0.41	0.06	1.15	4.0E-02
Ambient temperature (°C)	Min	0.48	0.03	0.46	0.03	1.35	6.0E-02
	Max	0.42	0.05	0.42	0.05	0.86	5.7E-02
	Mean	0.43	0.04	0.36	0.10	1.14	5.7E-02
Sum precipitation (mm)		0.02	0.92	0.00	1.00	1.74	3.5E-03
Mixed Layer Depth (m)	Min	0.19	0.39	0.24	0.27	1.56	1.7E-03
	Max	-0.15	0.51	-0.07	0.77	2.30	-5.0E-04
	Mean	-0.17	0.46	-0.07	0.74	2.43	-1.4E-03
Relative humidity (%)	Min	0.30	0.18	0.38	0.08	0.30	2.6E-02
	Max	0.09	0.70	0.06	0.80	0.99	8.9E-03
	Mean	0.19	0.41	0.23	0.31	0.55	1.7E-02
Terrain Height (m, agl)	Min	-0.22	0.32	-0.24	0.28	2.05	-1.6E-03
	Max	-0.10	0.64	-0.15	0.52	2.79	-1.5E-03
	Mean	-0.23	0.31	-0.23	0.30	2.32	-1.6E-03
Sum of sun flux (W.m^{-2})	Sum	0.08	0.73	0.01	0.96	1.50	5.0E-05
	Max	0.14	0.52	0.08	0.72	1.22	9.4E-04
	Mean	0.08	0.73	0.01	0.96	1.50	1.3E-03
Distance traveled (km)		-0.47	0.03	-0.54	0.01	2.54	-2.0E-03
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	0.11	0.63	0.19	0.39	1.71	1.7E-02
	Max	-0.01	0.97	-0.29	0.19	1.76	-1.3E-04
	Mean	0.08	0.71	-0.07	0.76	1.69	2.9E-03
Pressure (hPa)	Min	0.38	0.08	0.26	0.25	-34.35	3.9E-02
	Max	0.43	0.04	0.45	0.04	-22.86	2.5E-02
	Mean	0.52	0.01	0.47	0.03	-30.28	3.3E-02
Potential temperature (°C)	Min	0.35	0.11	0.35	0.11	1.38	4.1E-02
	Max	0.32	0.15	0.29	0.19	0.99	3.8E-02
	Mean	0.32	0.14	0.33	0.13	1.21	3.9E-02
Ambient temperature (°C)	Min	0.45	0.03	0.44	0.04	1.39	5.6E-02
	Max	0.42	0.05	0.41	0.06	0.89	5.0E-02
	Mean	0.41	0.06	0.36	0.10	1.20	5.1E-02
Sum_Rainfall (mm)		0.07	0.76	-0.03	0.90	1.72	8.1E-03
Mixed Layer Depth (m)	Min	-0.02	0.93	0.01	0.97	1.77	-1.7E-04
	Max	-0.02	0.94	0.02	0.93	1.80	-4.9E-05
	Mean	-0.08	0.72	-0.02	0.92	2.08	-6.7E-04
Relative humidity (%)	Min	0.07	0.76	0.14	0.53	1.40	6.6E-03
	Max	0.06	0.78	0.14	0.52	1.17	6.7E-03
	Mean	0.13	0.57	0.16	0.48	0.89	1.2E-02
Terrain Height (m, agl)	Min	-0.44	0.04	-0.45	0.04	2.64	-3.5E-03
	Max	-0.26	0.24	-0.40	0.07	4.95	-4.8E-03
	Mean	-0.50	0.02	-0.50	0.02	3.66	-4.3E-03
Sum of sun flux (W.m^{-2})	Sum	0.10	0.66	0.03	0.88	1.45	5.9E-05
	Max	0.17	0.44	0.11	0.63	1.19	1.0E-03
	Mean	0.10	0.66	0.03	0.88	1.45	1.5E-03
Distance traveled (km)		-0.48	0.02	-0.53	0.01	2.75	-4.2E-03

Climatic or chemical variables (x)[b]		Concentration of culturable bacteria grown on MA nucleating ice at > -8°C (%)					
		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		-0.26	0.36	-0.32	0.25	5.60	-6.3E-01
Conductivity (µS.cm)		-0.06	0.83	-0.46	0.08	1.16	-3.7E-03
Total Disolved Solids (ppm)		0.05	0.86	-0.27	0.35	0.86	5.8E-03
Salinity (ppm)		0.06	0.83	-0.18	0.53	0.86	5.3E-03
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	0.14	0.53	0.09	0.68	0.90	5.3E-04
	Max	0.03	0.88	0.07	0.76	0.80	3.0E-04
	Mean	0.18	0.41	0.16	0.47	0.64	7.5E-04
Pressure (hPa)	Min	0.07	0.74	0.17	0.45	-4.34	6.6E-03
	Max	-0.16	0.47	-0.11	0.60	5.77	-4.9E-03
	Mean	-0.15	0.49	-0.14	0.53	5.75	-5.1E-03
Potential temperature (°C)	Min	-0.01	0.97	-0.05	0.84	1.14	-9.2E-04
	Max	0.01	0.96	0.03	0.90	1.08	2.0E-03
	Mean	0.01	0.95	-0.07	0.76	1.10	1.7E-03
Ambient temperature (°C)	Min	0.03	0.88	0.05	0.83	1.10	5.3E-03
	Max	-0.06	0.77	0.00	1.00	1.24	-8.9E-03
	Mean	-0.05	0.83	-0.03	0.91	1.20	-7.1E-03
Sum_Rainfall (mm)		-0.21	0.34	-0.01	0.98	1.27	-3.5E-02
Mixed Layer Depth (m)	Min	0.33	0.12	0.12	0.59	0.72	2.9E-03
	Max	-0.01	0.97	-0.02	0.94	1.15	-2.4E-05
	Mean	0.21	0.33	0.22	0.32	0.24	1.8E-03
Relative humidity (%)	Min	0.17	0.43	0.16	0.46	0.47	1.2E-02
	Max	0.30	0.16	0.22	0.31	-0.97	2.5E-02
	Mean	0.21	0.33	0.19	0.39	0.02	1.6E-02
Terrain Height (m, agl)	Min	0.21	0.33	0.14	0.52	0.84	1.8E-03
	Max	0.10	0.66	0.06	0.80	0.09	1.5E-03
	Mean	0.08	0.72	0.05	0.81	0.94	5.7E-04
Sum of sun flux (W.m^{-2})	Sum	0.14	0.52	0.16	0.47	0.71	8.8E-05
	Max	0.03	0.88	0.04	0.87	1.02	2.0E-04
	Mean	0.14	0.52	0.16	0.47	0.71	2.2E-03
Distance traveled (km)		-0.23	0.29	-0.16	0.45	1.52	-6.1E-04
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	0.39	0.06	0.18	0.41	0.78	3.8E-03
	Max	0.25	0.24	0.15	0.51	-0.38	2.7E-03
	Mean	0.26	0.23	0.11	0.61	0.74	1.8E-03
Pressure (hPa)	Min	0.06	0.77	0.07	0.75	-4.12	6.0E-03
	Max	-0.19	0.38	0.00	0.99	9.41	-8.4E-03
	Mean	-0.02	0.91	0.12	0.59	2.13	-1.0E-03
Potential temperature (°C)	Min	0.09	0.67	0.12	0.59	1.02	1.0E-02
	Max	-0.02	0.94	0.01	0.97	1.17	-2.4E-03
	Mean	0.05	0.83	0.10	0.65	1.04	5.8E-03
Ambient temperature (°C)	Min	0.09	0.69	0.07	0.75	1.05	1.2E-02
	Max	-0.02	0.91	-0.07	0.73	1.18	-3.5E-03
	Mean	0.04	0.85	-0.05	0.82	1.07	5.8E-03
Sum precipitation (mm)		-0.30	0.17	-0.03	0.90	1.30	-4.7E-02
Mixed Layer Depth (m)	Min	0.52	0.01	0.33	0.12	0.56	5.1E-03
	Max	0.00	1.00	0.05	0.84	1.12	3.9E-06
	Mean	0.26	0.23	0.22	0.32	0.03	2.4E-03
Relative humidity (%)	Min	0.25	0.25	0.24	0.28	-0.22	2.4E-02
	Max	0.22	0.30	0.19	0.37	-1.01	2.5E-02
	Mean	0.13	0.57	0.10	0.64	0.26	1.2E-02
Terrain Height (m, agl)	Min	-0.04	0.87	-0.04	0.86	1.18	-2.8E-04
	Max	-0.14	0.51	-0.13	0.56	2.71	-2.3E-03
	Mean	-0.17	0.45	-0.19	0.38	1.58	-1.3E-03
Sum of sun flux (W.m^{-2})	Sum	0.06	0.80	0.07	0.74	0.95	3.7E-05
	Max	0.05	0.80	0.09	0.68	0.93	3.6E-04
	Mean	0.06	0.80	0.07	0.74	0.95	9.2E-04
Distance traveled (km)		-0.06	0.78	-0.04	0.85	1.24	-2.9E-04
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	-0.09	0.69	0.13	0.54	1.16	-1.5E-02
	Max	0.28	0.20	0.07	0.75	0.76	4.6E-03
	Mean	0.24	0.27	-0.09	0.67	0.96	9.2E-03
Pressure (hPa)	Min	-0.04	0.84	0.00	0.98	5.55	-4.8E-03
	Max	0.22	0.31	0.21	0.35	-12.40	1.4E-02
	Mean	0.24	0.28	0.25	0.24	-14.57	1.6E-02
Potential temperature (°C)	Min	0.08	0.73	0.12	0.58	1.05	9.6E-03
	Max	0.01	0.96	-0.02	0.94	1.10	1.4E-03
	Mean	0.06	0.78	0.10	0.64	1.02	7.8E-03
Ambient temperature (°C)	Min	0.13	0.56	0.12	0.59	1.02	1.7E-02
	Max	0.07	0.75	0.05	0.81	0.98	8.7E-03
	Mean	0.10	0.65	0.10	0.65	0.99	1.3E-02
Sum_Rainfall (mm)		-0.22	0.31	0.00	1.00	1.26	-2.8E-02
Mixed Layer Depth (m)	Min	0.44	0.04	0.18	0.42	0.65	3.9E-03
	Max	-0.08	0.73	-0.14	0.52	1.39	-2.4E-04
	Mean	0.22	0.31	0.24	0.28	0.20	1.9E-03
Relative humidity (%)	Min	0.24	0.28	0.24	0.27	-0.19	2.5E-02
	Max	0.23	0.30	0.24	0.27	-1.13	2.6E-02
	Mean	0.20	0.35	0.16	0.48	-0.35	2.1E-02
Terrain Height (m, agl)	Min	-0.21	0.34	-0.24	0.28	1.57	-1.8E-03
	Max	-0.03	0.91	-0.14	0.52	1.47	-5.2E-04
	Mean	-0.25	0.24	-0.28	0.20	2.18	-2.4E-03
Sum of sun flux (W.m^{-2})	Sum	0.07	0.74	0.11	0.63	0.91	4.5E-05
	Max	0.06	0.80	0.14	0.53	0.95	3.4E-04
	Mean	0.07	0.74	0.11	0.63	0.91	1.1E-03
Distance traveled (km)		0.14	0.53	0.09	0.67	0.81	1.3E-03

Climatic or chemical variables (x)[b]		Concentration of culturable bacteria grown on R2A nucleating ice at > -8°C (%)					
		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		-0.14	0.62	-0.09	0.76	3.52	-3.2E-01
Conductivity (µS.cm)		-0.43	0.11	-0.44	0.10	1.68	-2.5E-02
Total Disolved Solids (ppm)		-0.44	0.11	-0.41	0.14	1.84	-4.8E-02
Salinity (ppm)		-0.32	0.26	-0.04	0.88	1.50	-2.6E-02
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	-0.10	0.66	-0.13	0.56	1.18	-3.6E-04
	Max	-0.07	0.75	-0.03	0.89	1.69	-6.0E-04
	Mean	-0.06	0.79	-0.05	0.84	1.18	-2.4E-04
Pressure (hPa)	Min	0.24	0.27	0.19	0.39	-16.56	2.1E-02
	Max	0.05	0.81	0.09	0.67	-0.48	1.6E-03
	Mean	0.06	0.78	0.05	0.80	-0.76	2.0E-03
Potential temperature (°C)	Min	0.18	0.42	0.14	0.52	0.76	2.0E-02
	Max	0.24	0.27	0.27	0.22	0.03	4.3E-02
	Mean	0.21	0.33	0.18	0.40	0.54	2.7E-02
Ambient temperature (°C)	Min	0.30	0.16	0.31	0.15	0.76	4.7E-02
	Max	0.25	0.25	0.32	0.14	0.60	3.4E-02
	Mean	0.26	0.22	0.30	0.16	0.66	3.9E-02
Sum_Rainfall (mm)		-0.02	0.93	0.05	0.82	1.04	-3.3E-03
Mixed Layer Depth (m)	Min	-0.12	0.57	-0.19	0.38	1.18	-1.1E-03
	Max	0.13	0.56	0.15	0.50	0.64	3.6E-04
	Mean	-0.05	0.82	0.11	0.62	1.24	-4.3E-04
Relative humidity (%)	Min	0.19	0.40	0.17	0.45	0.33	1.2E-02
	Max	0.25	0.24	0.15	0.48	-0.70	2.1E-02
	Mean	0.19	0.38	0.18	0.42	0.04	1.4E-02
Terrain Height (m, agl)	Min	0.26	0.23	0.16	0.46	0.68	2.1E-03
	Max	0.03	0.91	0.06	0.77	0.75	4.0E-04
	Mean	0.15	0.51	0.15	0.49	0.68	1.0E-03
Sum of sun flux (W.m^{-2})	Sum	0.37	0.08	0.36	0.09	-0.07	2.3E-04
	Max	0.42	0.05	0.36	0.09	-0.35	2.5E-03
	Mean	0.37	0.08	0.36	0.09	-0.07	5.8E-03
Distance traveled (km)		-0.24	0.27	-0.21	0.33	1.44	-6.3E-04
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	0.18	0.42	0.08	0.73	0.88	1.7E-03
	Max	0.18	0.40	0.01	0.97	-0.05	1.9E-03
	Mean	0.17	0.43	0.02	0.93	0.78	1.2E-03
Pressure (hPa)	Min	0.20	0.36	0.17	0.44	-15.26	1.9E-02
	Max	0.00	0.98	0.08	0.71	0.85	1.8E-04
	Mean	0.01	0.95	0.08	0.71	0.53	5.3E-04
Potential temperature (°C)	Min	0.29	0.18	0.29	0.18	0.71	3.1E-02
	Max	0.25	0.25	0.30	0.17	0.31	3.6E-02
	Mean	0.26	0.22	0.30	0.16	0.56	3.2E-02
Ambient temperature (°C)	Min	0.30	0.16	0.31	0.15	0.77	4.1E-02
	Max	0.31	0.15	0.28	0.19	0.37	4.4E-02
	Mean	0.30	0.16	0.27	0.22	0.60	4.1E-02
Sum precipitation (mm)		-0.28	0.20	0.00	1.00	1.19	-4.3E-02
Mixed Layer Depth (m)	Min	0.22	0.32	0.08	0.73	0.80	2.1E-03
	Max	0.16	0.46	0.15	0.49	0.46	5.3E-04
	Mean	0.12	0.58	0.20	0.37	0.52	1.1E-03
Relative humidity (%)	Min	0.35	0.10	0.35	0.10	-0.85	3.4E-02
	Max	0.30	0.16	0.23	0.30	-1.83	3.3E-02
	Mean	0.25	0.26	0.22	0.32	-0.64	2.4E-02
Terrain Height (m, agl)	Min	-0.12	0.58	-0.12	0.60	1.21	-9.4E-04
	Max	-0.11	0.62	-0.12	0.60	2.20	-1.7E-03
	Mean	-0.10	0.67	-0.14	0.52	1.29	-7.1E-04
Sum of sun flux (W.m^{-2})	Sum	0.21	0.35	0.16	0.47	0.40	1.3E-04
	Max	0.26	0.23	0.22	0.31	0.10	1.7E-03
	Mean	0.21	0.35	0.16	0.47	0.40	3.3E-03
Distance traveled (km)		-0.27	0.22	-0.25	0.26	1.51	-1.2E-03
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	0.22	0.32	0.21	0.33	0.95	3.5E-02
	Max	0.15	0.49	-0.15	0.49	0.84	2.5E-03
	Mean	0.20	0.36	-0.07	0.75	0.90	7.4E-03
Pressure (hPa)	Min	0.31	0.15	0.29	0.18	-30.08	3.3E-02
	Max	0.36	0.09	0.33	0.12	-20.65	2.2E-02
	Mean	0.31	0.16	0.30	0.17	-18.90	2.1E-02
Potential temperature (°C)	Min	0.26	0.22	0.30	0.16	0.75	3.3E-02
	Max	0.28	0.20	0.27	0.21	0.36	3.5E-02
	Mean	0.27	0.21	0.28	0.20	0.58	3.4E-02
Ambient temperature (°C)	Min	0.33	0.12	0.33	0.13	0.76	4.4E-02
	Max	0.35	0.10	0.36	0.09	0.31	4.3E-02
	Mean	0.32	0.13	0.30	0.16	0.59	4.2E-02
Sum_Rainfall (mm)		-0.25	0.25	-0.06	0.80	1.17	-3.0E-02
Mixed Layer Depth (m)	Min	0.06	0.79	-0.07	0.76	0.97	5.2E-04
	Max	0.22	0.30	0.24	0.27	0.27	6.8E-04
	Mean	0.21	0.33	0.27	0.21	0.16	1.8E-03
Relative humidity (%)	Min	0.14	0.54	0.15	0.50	0.29	1.4E-02
	Max	0.26	0.23	0.19	0.39	-1.51	2.9E-02
	Mean	0.15	0.48	0.11	0.62	-0.07	1.5E-02
Terrain Height (m, agl)	Min	-0.34	0.11	-0.36	0.10	1.76	-2.9E-03
	Max	-0.35	0.10	-0.33	0.12	5.57	-6.8E-03
	Mean	-0.29	0.18	-0.32	0.14	2.22	-2.7E-03
Sum of sun flux (W.m^{-2})	Sum	0.21	0.33	0.21	0.35	0.40	1.3E-04
	Max	0.29	0.18	0.24	0.28	0.09	1.7E-03
	Mean	0.21	0.33	0.21	0.35	0.40	3.3E-03
Distance traveled (km)		-0.16	0.46	-0.17	0.43	1.39	-1.5E-03

Climatic or chemical variables (x)[b]		Concentration of culturable bacteria grown on TSA nucleating ice at > -8°C (%)					
		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		0.01	0.98	0.13	0.64	1.24	1.2E-02
Conductivity (µS.cm)		-0.49	0.07	-0.41	0.13	1.87	-2.8E-02
Total Disolved Solids (ppm)		-0.41	0.15	-0.23	0.42	1.91	-4.4E-02
Salinity (ppm)		-0.46	0.10	-0.29	0.32	1.81	-3.7E-02
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	0.07	0.77	0.08	0.72	1.27	2.6E-04
	Max	-0.18	0.41	-0.02	0.93	3.16	-1.6E-03
	Mean	0.08	0.73	0.11	0.63	1.17	3.3E-04
Pressure (hPa)	Min	0.29	0.18	0.17	0.43	-21.37	2.8E-02
	Max	0.00	0.99	-0.01	0.96	1.27	1.2E-04
	Mean	0.02	0.94	-0.05	0.81	0.83	6.1E-04
Potential temperature (°C)	Min	0.26	0.23	0.32	0.14	0.95	3.1E-02
	Max	0.33	0.12	0.30	0.16	-0.08	6.3E-02
	Mean	0.27	0.20	0.25	0.25	0.72	3.7E-02
Ambient temperature (°C)	Min	0.37	0.08	0.35	0.10	1.03	6.1E-02
	Max	0.29	0.19	0.27	0.22	0.87	4.1E-02
	Mean	0.32	0.14	0.30	0.16	0.91	5.0E-02
Sum_Rainfall (mm)		-0.11	0.63	-0.06	0.79	1.46	-1.9E-02
Mixed Layer Depth (m)	Min	-0.09	0.69	0.03	0.90	1.49	-7.9E-04
	Max	0.11	0.63	0.01	0.97	1.04	3.1E-04
	Mean	-0.15	0.50	0.04	0.86	2.02	-1.3E-03
Relative humidity (%)	Min	0.06	0.78	0.10	0.65	1.13	4.3E-03
	Max	0.22	0.32	0.08	0.72	-0.19	1.9E-02
	Mean	0.07	0.74	0.03	0.91	0.98	5.7E-03
Terrain Height (m, agl)	Min	0.05	0.82	-0.06	0.78	1.31	4.3E-04
	Max	0.02	0.93	0.02	0.92	1.18	2.9E-04
	Mean	0.00	0.99	0.00	0.99	1.39	-2.0E-05
Sum of sun flux (W.m^{-2})	Sum	0.32	0.14	0.28	0.20	0.39	2.1E-04
	Max	0.32	0.14	0.30	0.17	0.28	2.0E-03
	Mean	0.32	0.14	0.28	0.20	0.39	5.2E-03
Distance traveled (km)		-0.28	0.20	-0.21	0.34	1.87	-7.7E-04
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	0.02	0.93	-0.13	0.55	1.36	2.0E-04
	Max	0.12	0.58	-0.01	0.95	0.63	1.3E-03
	Mean	0.07	0.75	-0.03	0.88	1.27	5.0E-04
Pressure (hPa)	Min	0.18	0.41	0.11	0.61	-13.98	1.7E-02
	Max	0.08	0.73	0.13	0.56	-1.99	3.4E-03
	Mean	0.10	0.66	0.13	0.54	-2.68	4.3E-03
Potential temperature (°C)	Min	0.25	0.25	0.17	0.43	1.09	2.8E-02
	Max	0.30	0.17	0.27	0.21	0.47	4.6E-02
	Mean	0.28	0.20	0.28	0.20	0.86	3.6E-02
Ambient temperature (°C)	Min	0.31	0.15	0.26	0.22	1.10	4.4E-02
	Max	0.36	0.09	0.33	0.12	0.56	5.4E-02
	Mean	0.34	0.11	0.32	0.13	0.86	4.9E-02
Sum precipitation (mm)		-0.29	0.18	-0.07	0.74	1.56	-4.7E-02
Mixed Layer Depth (m)	Min	0.15	0.48	0.04	0.84	1.20	1.6E-03
	Max	0.22	0.30	0.10	0.66	0.54	7.8E-04
	Mean	0.03	0.87	0.13	0.55	1.23	3.3E-04
Relative humidity (%)	Min	0.02	0.93	0.09	0.68	1.28	1.8E-03
	Max	0.02	0.94	0.05	0.84	1.22	1.8E-03
	Mean	-0.04	0.86	-0.01	0.95	1.66	-4.1E-03
Terrain Height (m, agl)	Min	-0.08	0.72	-0.06	0.77	1.50	-6.6E-04
	Max	-0.07	0.76	-0.11	0.62	2.14	-1.1E-03
	Mean	-0.10	0.66	-0.14	0.51	1.65	-7.6E-04
Sum of sun flux (W.m^{-2})	Sum	0.25	0.25	0.20	0.35	0.57	1.7E-04
	Max	0.27	0.22	0.24	0.28	0.39	1.8E-03
	Mean	0.25	0.25	0.20	0.35	0.57	4.2E-03
Distance traveled (km)		-0.33	0.12	-0.23	0.29	2.01	-1.6E-03
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	0.10	0.64	0.11	0.62	1.34	1.8E-02
	Max	0.16	0.46	-0.05	0.84	1.16	2.8E-03
	Mean	0.17	0.43	-0.10	0.66	1.26	6.7E-03
Pressure (hPa)	Min	0.31	0.15	0.28	0.19	-31.64	3.6E-02
	Max	0.39	0.07	0.33	0.13	-23.40	2.5E-02
	Mean	0.38	0.08	0.34	0.11	-24.38	2.7E-02
Potential temperature (°C)	Min	0.25	0.26	0.20	0.36	1.10	3.2E-02
	Max	0.34	0.12	0.29	0.18	0.53	4.4E-02
	Mean	0.29	0.19	0.28	0.20	0.87	3.8E-02
Ambient temperature (°C)	Min	0.34	0.11	0.33	0.12	1.09	4.7E-02
	Max	0.42	0.05	0.44	0.04	0.47	5.5E-02
	Mean	0.35	0.10	0.34	0.12	0.88	4.8E-02
Sum_Rainfall (mm)		-0.31	0.15	-0.19	0.38	1.56	-4.0E-02
Mixed Layer Depth (m)	Min	0.03	0.90	0.00	0.99	1.35	2.7E-04
	Max	0.32	0.14	0.22	0.30	0.26	1.0E-03
	Mean	0.19	0.39	0.25	0.24	0.56	1.7E-03
Relative humidity (%)	Min	-0.24	0.27	-0.25	0.26	2.77	-2.6E-02
	Max	0.01	0.97	0.03	0.90	1.28	1.1E-03
	Mean	-0.14	0.53	-0.16	0.48	2.42	-1.5E-02
Terrain Height (m, agl)	Min	-0.33	0.12	-0.33	0.12	2.13	-3.0E-03
	Max	-0.32	0.13	-0.30	0.17	5.77	-6.6E-03
	Mean	-0.33	0.12	-0.30	0.16	2.80	-3.3E-03
Sum of sun flux (W.m^{-2})	Sum	0.27	0.22	0.25	0.25	0.55	1.7E-04
	Max	0.31	0.14	0.31	0.15	0.31	2.0E-03
	Mean	0.27	0.22	0.25	0.25	0.55	4.3E-03
Distance traveled (km)		-0.20	0.35	-0.16	0.45	1.86	-2.0E-03

Climatic or chemical variables (x)[b]		Concentration of culturable bacteria grown on TSA 10% nucleating ice at > -8°C (%)					
		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry							
pH		-0.13	0.65	-0.14	0.61	3.563	-3.1E-01
Conductivity (µS.cm)		-0.50	0.06	-0.47	0.07	1.937	-2.9E-02
Total Disolved Solids (ppm)		-0.35	0.22	-0.15	0.60	1.821	-3.8E-02
Salinity (ppm)		-0.50	0.07	-0.50	0.07	1.878	-4.0E-02
Correlations tested with backward trajectories computed at 1000 m							
Altitude (m, agl)	Min	0.02	0.93	-0.05	0.82	1.406	7.2E-05
	Max	-0.13	0.56	0.06	0.79	2.640	-1.1E-03
	Mean	0.01	0.97	0.03	0.90	1.417	2.9E-05
Pressure (hPa)	Min	0.30	0.19	0.07	0.75	-19.395	2.5E-02
	Max	0.05	0.83	0.06	0.79	0.010	1.5E-03
	Mean	0.10	0.68	0.07	0.76	-1.262	3.0E-03
Potential temperature (°C)	Min	0.15	0.51	0.14	0.54	1.198	1.6E-02
	Max	0.28	0.22	0.28	0.22	0.289	4.9E-02
	Mean	0.20	0.38	0.10	0.66	0.979	2.5E-02
Ambient temperature (°C)	Min	0.23	0.31	0.21	0.36	1.215	3.5E-02
	Max	0.27	0.24	0.33	0.15	0.960	3.6E-02
	Mean	0.26	0.25	0.24	0.29	1.050	3.8E-02
Sum_Rainfall (mm)		-0.13	0.59	0.01	0.95	1.519	-2.0E-02
Mixed Layer Depth (m)	Min	-0.13	0.58	-0.13	0.57	1.583	-1.0E-03
	Max	-0.05	0.81	0.00	0.99	1.610	-1.5E-04
	Mean	-0.18	0.42	-0.14	0.54	2.200	-1.5E-03
Relative humidity (%)	Min	0.25	0.28	0.24	0.30	0.515	1.6E-02
	Max	0.40	0.07	0.26	0.26	-1.228	3.2E-02
	Mean	0.33	0.15	0.32	0.16	-0.220	2.4E-02
Terrain Height (m, agl)	Min	0.04	0.88	-0.06	0.79	1.388	2.8E-04
	Max	-0.01	0.96	-0.09	0.69	1.586	-2.2E-04
	Mean	-0.02	0.93	-0.03	0.91	1.484	-1.4E-04
Sum of sun flux (W.m^{-2})	Sum	0.27	0.23	0.27	0.24	0.572	1.7E-04
	Max	0.21	0.37	0.11	0.63	0.717	1.3E-03
	Mean	0.27	0.23	0.27	0.24	0.572	4.3E-03
Distance traveled (km)		-0.40	0.07	-0.37	0.09	2.101	-1.0E-03
Correlations tested with backward trajectories computed at 500 m							
Altitude (m, agl)	Min	0.18	0.45	0.13	0.56	1.275	1.6E-03
	Max	0.08	0.74	0.03	0.90	1.008	7.5E-04
	Mean	0.15	0.53	0.05	0.85	1.220	9.5E-04
Pressure (hPa)	Min	0.31	0.17	0.14	0.54	-23.116	2.8E-02
	Max	0.07	0.75	0.16	0.49	-1.560	3.1E-03
	Mean	0.14	0.55	0.21	0.37	-3.922	5.6E-03
Potential temperature (°C)	Min	0.27	0.24	0.24	0.29	1.112	2.9E-02
	Max	0.24	0.30	0.26	0.26	0.716	3.5E-02
	Mean	0.26	0.26	0.30	0.19	0.943	3.1E-02
Ambient temperature (°C)	Min	0.30	0.18	0.25	0.28	1.156	3.9E-02
	Max	0.29	0.20	0.29	0.21	0.781	4.2E-02
	Mean	0.32	0.15	0.21	0.36	0.936	4.4E-02
Sum precipitation (mm)		-0.26	0.25	-0.12	0.60	1.569	-4.2E-02
Mixed Layer Depth (m)	Min	0.25	0.27	0.21	0.36	1.179	2.3E-03
	Max	-0.04	0.86	0.03	0.91	1.595	-1.4E-04
	Mean	-0.07	0.77	-0.05	0.82	1.724	-6.1E-04
Relative humidity (%)	Min	0.20	0.37	0.18	0.44	0.370	1.9E-02
	Max	0.08	0.74	0.11	0.64	0.719	8.3E-03
	Mean	0.11	0.64	0.09	0.69	0.684	1.1E-02
Terrain Height (m, agl)	Min	-0.17	0.47	-0.25	0.28	1.671	-1.2E-03
	Max	-0.15	0.50	-0.25	0.28	3.055	-2.4E-03
	Mean	-0.25	0.28	-0.27	0.24	2.084	-1.8E-03
Sum of sun flux (W.m^{-2})	Sum	0.09	0.71	0.06	0.81	1.148	5.7E-05
	Max	0.06	0.81	0.04	0.85	1.216	3.8E-04
	Mean	0.09	0.71	0.06	0.81	1.148	1.4E-03
Distance traveled (km)		-0.47	0.03	-0.35	0.12	2.274	-2.1E-03
Correlations tested with backward trajectories computed at 50 m							
Altitude (m, agl)	Min	0.22	0.33	0.23	0.31	1.350	3.5E-02
	Max	0.03	0.90	-0.27	0.23	1.400	4.4E-04
	Mean	0.14	0.53	-0.17	0.47	1.337	5.1E-03
Pressure (hPa)	Min	0.36	0.10	0.25	0.27	-34.278	3.8E-02
	Max	0.42	0.06	0.45	0.04	-23.183	2.5E-02
	Mean	0.50	0.02	0.41	0.07	-30.972	3.4E-02
Potential temperature (°C)	Min	0.21	0.35	0.22	0.33	1.186	2.6E-02
	Max	0.24	0.29	0.17	0.45	0.806	3.1E-02
	Mean	0.26	0.25	0.31	0.17	0.959	3.3E-02
Ambient temperature (°C)	Min	0.33	0.15	0.24	0.30	1.147	4.2E-02
	Max	0.33	0.14	0.32	0.16	0.727	4.0E-02
	Mean	0.35	0.12	0.29	0.21	0.939	4.4E-02
Sum_Rainfall (mm)		-0.22	0.34	-0.07	0.78	1.547	-2.6E-02
Mixed Layer Depth (m)	Min	0.09	0.69	0.06	0.80	1.339	7.8E-04
	Max	-0.01	0.97	0.10	0.68	1.464	-2.5E-05
	Mean	0.01	0.95	0.05	0.82	1.377	1.2E-04
Relative humidity (%)	Min	0.03	0.90	-0.01	0.96	1.277	3.0E-03
	Max	0.10	0.67	0.13	0.59	0.466	1.1E-02
	Mean	0.08	0.73	0.07	0.76	0.857	8.2E-03
Terrain Height (m, agl)	Min	-0.41	0.07	-0.46	0.04	2.307	-3.4E-03
	Max	-0.32	0.16	-0.45	0.04	5.480	-6.0E-03
	Mean	-0.50	0.02	-0.45	0.04	3.502	-4.6E-03
Sum of sun flux (W.m^{-2})	Sum	0.09	0.69	0.04	0.87	1.152	5.6E-05
	Max	0.09	0.68	0.06	0.81	1.121	5.6E-04
	Mean	0.09	0.69	0.04	0.87	1.152	1.4E-03
Distance traveled (km)		-0.38	0.09	-0.35	0.13	2.251	-3.4E-03

a In addtion to the total number of ice nucleators, the following biological variables were statistically analyzed for each of the 6 media described in the material and methods section: concentration of total bacteria, frequency and concentration of culturable bacteria (i) INA only the first time they were tested and (ii) for which INA is reproducible and confirmed.

[b] All the correlations with physical properties listed in materials and methods section have been tested for the three elevations. Correlations are based on the analyse of N= 23 samples

Models are given only for variables for which at least one of the Pearson's or Spearman's coefficients was significantly different from zero are presented (P<0.1).

[c] The p-value for the Shapiro-Wilk test is given next to each *x* variable and below each *y* variable. When the p-value is under 0.05, normality of the data distribution is rejected.

Climatic or chemical variables (x)[b]		Biological variables (y)[a]	Concentration of culturable bacteria grown on CA nucleating ice at > -12°C (%)						Concentration of culturable bacteria grown on CA 10% nucleating ice at > -12°C (%)					
			Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
			r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry														
pH		0.40	0.07	0.81	0.08	0.79	0.20	1.6E-01	-0.03	0.92	0.00	0.99	2.19	-6.9E-02
Conductivity (µS.cm)		0.00	-0.48	0.07	-0.32	0.25	1.93	-2.8E-02	0.19	0.51	0.04	0.88	1.47	1.1E-02
Total Disolved Solids (ppm)		0.04	-0.32	0.26	-0.05	0.86	1.81	-3.6E-02	0.32	0.28	0.36	0.22	1.01	3.5E-02
Salinity (ppm)		0.00	-0.50	0.07	-0.42	0.13	1.91	-4.1E-02	0.29	0.34	0.11	0.72	1.18	2.4E-02
Correlations tested with backward trajectories computed at 1000 m														
Altitude (m, agl)	Min	0.05	0.22	0.32	0.24	0.27	1.11	8.7E-04	0.25	0.31	0.26	0.28	1.35	9.3E-04
	Max	0.00	-0.08	0.70	0.13	0.55	2.29	-7.5E-04	0.01	0.98	0.30	0.21	1.72	5.8E-05
	Mean	0.44	0.25	0.25	0.29	0.18	0.78	1.1E-03	0.27	0.25	0.30	0.22	1.03	1.1E-03
Pressure (hPa)	Min	0.00	0.27	0.21	0.09	0.69	-19.69	2.6E-02	0.01	0.98	-0.12	0.62	1.38	4.9E-04
	Max	0.29	-0.06	0.80	-0.02	0.92	3.14	-1.7E-03	-0.22	0.38	-0.19	0.42	7.60	-6.2E-03
	Mean	0.76	-0.10	0.67	-0.18	0.42	4.44	-3.3E-03	-0.16	0.52	-0.17	0.50	5.93	-4.6E-03
Potential temperature (°C)	Min	0.15	0.24	0.28	0.22	0.32	1.09	2.8E-02	0.34	0.16	0.09	0.73	1.18	3.7E-02
	Max	0.05	0.29	0.18	0.33	0.13	0.20	5.5E-02	0.26	0.28	0.21	0.39	0.70	4.4E-02
	Mean	0.04	0.23	0.29	0.13	0.54	0.93	3.1E-02	0.30	0.21	0.03	0.91	1.08	3.6E-02
Ambient temperature (°C)	Min	0.17	0.27	0.21	0.25	0.25	1.22	4.5E-02	0.27	0.26	0.12	0.61	1.47	4.2E-02
	Max	0.40	0.17	0.45	0.19	0.40	1.18	2.4E-02	0.25	0.31	0.22	0.37	1.35	3.1E-02
	Mean	0.29	0.22	0.31	0.18	0.40	1.15	3.5E-02	0.28	0.24	0.15	0.54	1.35	4.0E-02
Sum_Rainfall (mm)		0.00	-0.15	0.50	0.03	0.90	1.58	-2.5E-02	0.17	0.49	0.21	0.38	1.67	2.5E-02
Mixed Layer Depth (m)	Min	0.00	-0.11	0.60	0.00	0.99	1.62	-1.0E-03	0.35	0.14	0.34	0.16	1.38	2.7E-03
	Max	0.55	-0.24	0.26	-0.21	0.33	2.26	-7.1E-04	0.03	0.91	0.03	0.90	1.69	7.6E-05
	Mean	0.08	-0.33	0.12	-0.19	0.39	2.89	-2.9E-03	0.34	0.15	0.15	0.54	0.21	3.0E-03
Relative humidity (%)	Min	0.54	0.22	0.31	0.26	0.23	0.60	1.5E-02	0.51	0.02	0.52	0.02	-0.18	3.2E-02
	Max	0.02	0.39	0.06	0.25	0.26	-1.32	3.4E-02	0.46	0.05	0.41	0.08	-1.15	3.5E-02
	Mean	0.33	0.32	0.14	0.30	0.16	-0.22	2.4E-02	0.51	0.03	0.57	0.01	-0.82	3.6E-02
Terrain Height (m, agl)	Min	0.01	-0.09	0.69	-0.21	0.34	1.60	-7.6E-04	-0.11	0.65	-0.24	0.32	1.92	-8.5E-04
	Max	0.00	0.21	0.33	0.06	0.80	-0.89	3.4E-03	-0.19	0.44	-0.21	0.39	4.26	-3.7E-03
	Mean	0.29	-0.06	0.79	-0.10	0.65	1.63	-4.5E-04	-0.16	0.52	-0.27	0.27	2.13	-1.1E-03
Sum of sun flux (W.m^{-2})	Sum	0.39	-0.02	0.92	-0.04	0.87	1.54	-1.4E-05	0.08	0.76	-0.05	0.85	1.56	4.5E-05
	Max	0.07	-0.05	0.83	-0.08	0.71	1.65	-3.1E-04	0.08	0.73	-0.05	0.85	1.51	4.8E-04
	Mean	0.39	-0.02	0.92	-0.04	0.87	1.54	-3.4E-04	0.08	0.76	-0.05	0.85	1.56	1.1E-03
Distance traveled (km)		0.00	-0.33	0.13	-0.26	0.23	2.05	-8.9E-04	-0.18	0.46	-0.18	0.45	2.08	-4.3E-04
Correlations tested with backward trajectories computed at 500 m														
Altitude (m, agl)	Min	0.00	0.16	0.47	0.09	0.69	1.33	1.6E-03	0.42	0.07	0.44	0.06	1.38	3.6E-03
	Max	0.00	0.10	0.64	0.01	0.98	0.85	1.1E-03	0.14	0.56	0.24	0.32	1.02	1.3E-03
	Mean	0.00	0.14	0.51	0.08	0.71	1.26	1.0E-03	0.26	0.28	0.25	0.29	1.38	1.6E-03
Pressure (hPa)	Min	0.02	0.32	0.13	0.24	0.27	-25.96	3.1E-02	-0.01	0.98	-0.07	0.77	2.19	-4.6E-04
	Max	0.03	0.14	0.52	0.30	0.17	-4.78	6.4E-03	-0.08	0.75	0.13	0.60	4.76	-3.0E-03
	Mean	0.30	0.18	0.42	0.29	0.18	-5.74	7.6E-03	0.08	0.75	0.17	0.48	-1.00	2.9E-03
Potential temperature (°C)	Min	0.10	0.25	0.25	0.18	0.42	1.19	2.8E-02	0.39	0.10	0.25	0.31	1.25	4.2E-02
	Max	0.10	0.16	0.47	0.14	0.51	1.00	2.4E-02	0.23	0.33	0.09	0.72	1.06	3.4E-02
	Mean	0.08	0.20	0.36	0.21	0.35	1.11	2.5E-02	0.32	0.18	0.17	0.49	1.13	3.9E-02
Ambient temperature (°C)	Min	0.03	0.31	0.15	0.25	0.24	1.20	4.4E-02	0.42	0.07	0.23	0.34	1.31	5.6E-02
	Max	0.39	0.22	0.30	0.23	0.30	0.98	3.4E-02	0.31	0.19	0.20	0.41	1.05	4.4E-02
	Mean	0.02	0.27	0.21	0.17	0.45	1.07	3.9E-02	0.39	0.10	0.16	0.52	1.14	5.2E-02
Sum precipitation (mm)		0.00	-0.17	0.44	-0.08	0.71	1.58	-2.7E-02	0.04	0.87	-0.03	0.90	1.76	6.1E-03
Mixed Layer Depth (m)	Min	0.00	0.24	0.27	0.27	0.21	1.21	2.4E-03	0.49	0.03	0.52	0.02	1.29	4.1E-03
	Max	0.84	-0.21	0.35	-0.11	0.62	2.24	-7.1E-04	0.00	0.99	0.07	0.77	1.80	-1.4E-05
	Mean	0.71	-0.20	0.36	-0.09	0.69	2.34	-1.9E-03	0.32	0.18	0.24	0.31	0.41	2.8E-03
Relative humidity (%)	Min	0.38	0.16	0.48	0.22	0.32	0.61	1.6E-02	0.47	0.04	0.39	0.10	-0.68	4.3E-02
	Max	0.01	0.05	0.81	0.06	0.80	0.94	6.2E-03	0.31	0.19	0.22	0.38	-1.56	3.8E-02
	Mean	0.29	0.13	0.56	0.14	0.51	0.57	1.3E-02	0.37	0.12	0.30	0.21	-0.88	3.7E-02
Terrain Height (m, agl)	Min	0.04	-0.23	0.30	-0.31	0.15	1.82	-1.8E-03	-0.34	0.16	-0.47	0.04	2.22	-2.4E-03
	Max	0.00	-0.12	0.57	-0.25	0.24	2.87	-2.0E-03	-0.14	0.58	-0.14	0.56	3.12	-1.9E-03
	Mean	0.10	-0.26	0.22	-0.34	0.11	2.22	-2.1E-03	-0.43	0.07	-0.49	0.03	2.83	-2.9E-03
Sum of sun flux (W.m^{-2})	Sum	0.60	-0.13	0.54	-0.17	0.44	1.91	-9.0E-05	0.03	0.92	-0.10	0.68	1.70	1.6E-05
	Max	0.38	-0.17	0.43	-0.16	0.46	2.12	-1.2E-03	0.04	0.88	-0.04	0.88	1.64	2.4E-04
	Mean	0.60	-0.13	0.54	-0.17	0.44	1.91	-2.3E-03	0.03	0.92	-0.10	0.68	1.70	4.0E-04
Distance traveled (km)		0.00	-0.46	0.03	-0.38	0.07	2.35	-2.3E-03	-0.09	0.73	-0.10	0.70	1.93	-3.5E-04
Correlations tested with backward trajectories computed at 50 m														
Altitude (m, agl)	Min	0.00	0.14	0.52	0.17	0.42	1.42	2.4E-02	0.11	0.64	0.00	0.99	1.74	1.6E-02
	Max	0.00	0.07	0.74	-0.26	0.23	1.38	1.3E-03	0.07	0.76	-0.01	0.98	1.69	1.1E-03
	Mean	0.00	0.15	0.48	-0.22	0.31	1.37	6.0E-03	0.08	0.74	0.01	0.97	1.73	2.7E-03
Pressure (hPa)	Min	0.10	0.40	0.06	0.35	0.11	-40.14	4.5E-02	0.00	1.00	-0.07	0.77	1.92	-1.5E-04
	Max	0.24	0.58	0.00	0.61	0.00	-35.38	3.7E-02	0.20	0.42	0.19	0.42	-9.98	1.2E-02
	Mean	0.05	0.63	0.00	0.58	0.00	-41.26	4.4E-02	0.38	0.11	0.33	0.16	-22.27	2.5E-02
Potential temperature (°C)	Min	0.07	0.18	0.40	0.15	0.49	1.27	2.4E-02	0.36	0.13	0.29	0.23	1.30	4.5E-02
	Max	0.23	0.14	0.52	0.09	0.69	1.12	1.8E-02	0.25	0.30	0.06	0.82	1.13	3.1E-02
	Mean	0.09	0.18	0.42	0.15	0.48	1.17	2.3E-02	0.32	0.17	0.22	0.36	1.16	4.0E-02
Ambient temperature (°C)	Min	0.02	0.32	0.13	0.29	0.18	1.21	4.5E-02	0.44	0.06	0.24	0.32	1.31	5.7E-02
	Max	0.26	0.27	0.21	0.26	0.23	0.90	3.5E-02	0.32	0.19	0.13	0.58	1.10	3.7E-02
	Mean	0.02	0.28	0.20	0.22	0.31	1.08	3.8E-02	0.39	0.10	0.21	0.38	1.18	4.9E-02
Sum_Rainfall (mm)		0.00	-0.20	0.37	-0.12	0.59	1.59	-2.5E-02	0.13	0.60	0.04	0.87	1.72	1.4E-02
Mixed Layer Depth (m)	Min	0.00	0.08	0.71	0.12	0.58	1.39	7.6E-04	0.40	0.09	0.44	0.06	1.37	3.1E-03
	Max	0.54	-0.08	0.72	0.01	0.98	1.76	-2.6E-04	0.01	0.98	0.04	0.88	1.76	1.7E-05
	Mean	0.42	-0.08	0.71	0.03	0.89	1.83	-7.3E-04	0.30	0.21	0.28	0.25	0.46	2.6E-03
Relative humidity (%)	Min	0.39	-0.05	0.83	-0.01	0.95	1.75	-5.0E-03	0.41	0.08	0.32	0.18	-0.31	3.9E-02
	Max	0.00	0.01	0.96	0.04	0.86	1.36	1.4E-03	0.33	0.17	0.36	0.13	-1.69	3.9E-02
	Mean	0.12	0.07	0.74	0.08	0.72	0.92	7.8E-03	0.43	0.06	0.44	0.06	-1.43	4.4E-02
Terrain Height (m, agl)	Min	0.35	-0.54	0.01	-0.58	0.00	2.67	-4.8E-03	-0.36	0.13	-0.42	0.07	2.51	-2.9E-03
	Max	0.00	-0.34	0.11	-0.50	0.01	6.06	-6.9E-03	-0.17	0.48	-0.26	0.29	3.86	-3.1E-03
	Mean	0.09	-0.59	0.00	-0.61	0.00	4.00	-5.8E-03	-0.50	0.03	-0.51	0.03	3.70	-4.3E-03
Sum of sun flux (W.m^{-2})	Sum	0.53	-0.10	0.64	-0.14	0.53	1.79	-6.5E-05	0.02	0.93	-0.13	0.58	1.72	1.3E-05
	Max	0.12	-0.09	0.70	-0.11	0.61	1.77	-5.4E-04	0.04	0.88	-0.07	0.78	1.66	2.2E-04
	Mean	0.53	-0.10	0.64	-0.14	0.53	1.79	-1.6E-03	0.02	0.93	-0.13	0.58	1.72	3.1E-04
Distance traveled (km)		0.14	-0.41	0.05	-0.40	0.06	2.43	-4.0E-03	-0.09	0.71	-0.03	0.89	1.97	-7.6E-04

Climatic or chemical variables (x)[b]		Concentration of culturable bacteria grown on EMB nucleating ice at > -12°C (%)						Concentration of culturable bacteria grown on MA nucleating ice at > -12°C (%)					
		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry													
pH		0.02	0.94	0.02	0.94	1.42	4.1E-02	-0.14	0.62	-0.18	0.52	3.72	-3.5E-01
Conductivity (µS.cm)		-0.52	0.05	-0.37	0.18	2.24	-2.7E-02	-0.03	0.93	-0.40	0.14	1.26	-1.6E-03
Total Disolved Solids (ppm)		-0.41	0.14	-0.12	0.68	2.25	-4.0E-02	0.09	0.75	-0.18	0.54	0.92	1.1E-02
Salinity (ppm)		-0.51	0.06	-0.46	0.10	2.22	-3.7E-02	0.09	0.76	-0.12	0.70	0.97	7.5E-03
Correlations tested with backward trajectories computed at 1000 m													
Altitude (m, agl)	Min	0.10	0.66	0.08	0.73	1.65	3.6E-04	0.14	0.53	0.09	0.68	1.02	5.4E-04
	Max	-0.18	0.42	0.02	0.92	3.43	-1.5E-03	0.06	0.78	0.13	0.57	0.65	5.4E-04
	Mean	0.15	0.51	0.14	0.54	1.42	5.8E-04	0.21	0.33	0.19	0.39	0.67	8.9E-04
Pressure (hPa)	Min	0.39	0.07	0.25	0.26	-25.64	3.3E-02	0.05	0.84	0.11	0.62	-2.24	4.2E-03
	Max	-0.02	0.92	0.06	0.79	2.44	-6.8E-04	-0.20	0.36	-0.15	0.49	7.13	-6.2E-03
	Mean	-0.07	0.76	-0.09	0.69	3.74	-2.1E-03	-0.22	0.32	-0.19	0.37	7.93	-7.4E-03
Potential temperature (°C)	Min	0.41	0.06	0.36	0.10	1.17	4.4E-02	0.08	0.70	0.04	0.85	1.11	9.9E-03
	Max	0.41	0.06	0.41	0.06	0.17	7.0E-02	0.11	0.63	0.11	0.62	0.78	2.0E-02
	Mean	0.39	0.07	0.28	0.21	0.94	4.8E-02	0.11	0.62	0.02	0.94	0.99	1.5E-02
Ambient temperature (°C)	Min	0.46	0.03	0.46	0.03	1.39	6.9E-02	0.12	0.59	0.12	0.59	1.14	1.9E-02
	Max	0.38	0.08	0.41	0.06	1.17	4.9E-02	0.02	0.94	0.08	0.73	1.22	2.5E-03
	Mean	0.41	0.06	0.39	0.07	1.23	5.9E-02	0.04	0.87	0.05	0.82	1.19	5.7E-03
Sum_Rainfall (mm)		0.05	0.82	0.14	0.55	1.77	8.1E-03	-0.08	0.72	0.06	0.79	1.30	-1.4E-02
Mixed Layer Depth (m)	Min	-0.19	0.40	-0.14	0.53	2.02	-1.5E-03	0.28	0.20	0.09	0.69	0.90	2.5E-03
	Max	-0.19	0.39	-0.18	0.43	2.40	-5.3E-04	-0.02	0.94	-0.02	0.93	1.30	-4.9E-05
	Mean	-0.35	0.11	-0.24	0.27	3.23	-2.8E-03	0.15	0.50	0.15	0.51	0.62	1.3E-03
Relative humidity (%)	Min	0.24	0.29	0.33	0.13	0.95	1.5E-02	0.24	0.26	0.21	0.35	0.29	1.7E-02
	Max	0.31	0.16	0.23	0.29	-0.23	2.5E-02	0.34	0.12	0.24	0.27	-1.14	2.9E-02
	Mean	0.28	0.21	0.34	0.12	0.45	2.0E-02	0.27	0.22	0.23	0.30	-0.16	2.0E-02
Terrain Height (m, agl)	Min	0.00	1.00	-0.08	0.71	1.81	-5.5E-06	0.32	0.13	0.21	0.33	0.80	2.8E-03
	Max	0.12	0.59	0.04	0.87	0.44	2.0E-03	0.15	0.50	0.10	0.63	-0.38	2.4E-03
	Mean	0.03	0.90	0.02	0.95	1.74	2.0E-04	0.17	0.44	0.12	0.59	0.83	1.3E-03
Sum of sun flux (W.m^{-2})	Sum	0.21	0.35	0.23	0.31	1.17	1.3E-04	0.19	0.38	0.20	0.35	0.66	1.2E-04
	Max	0.26	0.24	0.21	0.34	0.94	1.5E-03	0.11	0.63	0.10	0.65	0.88	6.6E-04
	Mean	0.21	0.35	0.23	0.31	1.17	3.3E-03	0.19	0.38	0.20	0.35	0.66	3.1E-03
Distance traveled (km)		-0.32	0.14	-0.39	0.07	2.32	-8.0E-04	-0.27	0.21	-0.22	0.31	1.72	-7.3E-04
Correlations tested with backward trajectories computed at 500 m													
Altitude (m, agl)	Min	0.17	0.45	0.17	0.46	1.66	1.5E-03	0.38	0.07	0.19	0.38	0.90	3.7E-03
	Max	0.05	0.84	-0.02	0.93	1.55	4.5E-04	0.33	0.13	0.22	0.32	-0.73	3.5E-03
	Mean	0.14	0.52	0.12	0.60	1.60	9.3E-04	0.33	0.12	0.16	0.46	0.75	2.3E-03
Pressure (hPa)	Min	0.36	0.10	0.22	0.32	-25.94	3.2E-02	-0.02	0.94	0.02	0.94	2.70	-1.7E-03
	Max	0.07	0.77	0.19	0.39	-0.90	2.8E-03	-0.22	0.32	-0.01	0.96	10.81	-9.7E-03
	Mean	0.10	0.65	0.18	0.43	-2.00	4.0E-03	-0.11	0.62	0.06	0.79	5.74	-4.7E-03
Potential temperature (°C)	Min	0.42	0.05	0.37	0.09	1.34	4.4E-02	0.20	0.36	0.20	0.36	1.02	2.3E-02
	Max	0.33	0.13	0.37	0.09	0.87	4.7E-02	0.07	0.75	0.07	0.74	1.04	1.0E-02
	Mean	0.38	0.08	0.43	0.05	1.14	4.4E-02	0.15	0.51	0.17	0.43	0.98	1.8E-02
Ambient temperature (°C)	Min	0.50	0.02	0.48	0.02	1.38	6.4E-02	0.17	0.43	0.14	0.53	1.09	2.4E-02
	Max	0.43	0.04	0.41	0.06	0.86	6.1E-02	0.08	0.73	0.02	0.92	1.08	1.1E-02
	Mean	0.45	0.03	0.37	0.09	1.15	6.1E-02	0.13	0.56	0.02	0.93	1.06	1.8E-02
Sum precipitation (mm)		0.01	0.96	0.03	0.90	1.80	1.8E-03	-0.32	0.14	-0.01	0.96	1.44	-5.2E-02
Mixed Layer Depth (m)	Min	0.18	0.43	0.23	0.31	1.62	1.6E-03	0.49	0.02	0.33	0.12	0.70	5.0E-03
	Max	-0.15	0.50	-0.04	0.87	2.38	-5.2E-04	-0.03	0.90	0.00	1.00	1.35	-9.3E-05
	Mean	-0.18	0.42	-0.06	0.80	2.57	-1.6E-03	0.20	0.37	0.15	0.50	0.40	1.8E-03
Relative humidity (%)	Min	0.31	0.15	0.41	0.06	0.22	2.9E-02	0.32	0.13	0.30	0.17	-0.54	3.2E-02
	Max	0.09	0.68	0.05	0.83	0.98	9.7E-03	0.27	0.21	0.24	0.27	-1.42	3.1E-02
	Mean	0.19	0.39	0.24	0.29	0.52	1.8E-02	0.19	0.38	0.15	0.49	-0.11	1.9E-02
Terrain Height (m, agl)	Min	-0.17	0.45	-0.22	0.33	2.04	-1.3E-03	0.01	0.96	-0.01	0.95	1.23	9.2E-05
	Max	-0.06	0.79	-0.12	0.58	2.42	-9.0E-04	0.00	1.00	-0.08	0.72	1.25	-4.0E-06
	Mean	-0.19	0.40	-0.21	0.34	2.29	-1.3E-03	-0.09	0.67	-0.14	0.51	1.51	-7.3E-04
Sum of sun flux (W.m^{-2})	Sum	0.11	0.64	0.06	0.80	1.46	6.9E-05	0.08	0.73	0.07	0.74	1.00	5.0E-05
	Max	0.16	0.48	0.12	0.59	1.21	1.1E-03	0.05	0.83	0.08	0.73	1.07	3.2E-04
	Mean	0.11	0.64	0.06	0.80	1.46	1.7E-03	0.08	0.73	0.07	0.74	1.00	1.3E-03
Distance traveled (km)		-0.48	0.02	-0.53	0.01	2.64	-2.1E-03	-0.11	0.61	-0.12	0.60	1.46	-5.5E-04
Correlations tested with backward trajectories computed at 50 m													
Altitude (m, agl)	Min	0.14	0.52	0.23	0.30	1.75	2.3E-02	0.16	0.46	0.28	0.20	1.18	2.8E-02
	Max	0.01	0.97	-0.25	0.25	1.80	1.1E-04	0.30	0.17	0.10	0.63	0.85	5.0E-03
	Mean	0.12	0.60	-0.04	0.85	1.73	4.1E-03	0.32	0.14	-0.04	0.87	1.03	1.2E-02
Pressure (hPa)	Min	0.36	0.10	0.22	0.33	-33.55	3.8E-02	0.01	0.96	0.08	0.71	0.05	1.3E-03
	Max	0.40	0.07	0.41	0.06	-21.28	2.3E-02	0.22	0.31	0.23	0.30	-12.55	1.4E-02
	Mean	0.49	0.02	0.43	0.05	-28.99	3.2E-02	0.23	0.30	0.26	0.23	-14.11	1.6E-02
Potential temperature (°C)	Min	0.38	0.08	0.37	0.09	1.40	4.5E-02	0.18	0.41	0.20	0.36	1.05	2.3E-02
	Max	0.34	0.13	0.31	0.17	0.98	4.1E-02	0.09	0.69	0.04	0.87	1.03	1.1E-02
	Mean	0.35	0.11	0.36	0.10	1.21	4.3E-02	0.15	0.48	0.17	0.44	0.98	2.0E-02
Ambient temperature (°C)	Min	0.47	0.03	0.44	0.04	1.42	6.0E-02	0.22	0.31	0.20	0.36	1.06	3.1E-02
	Max	0.44	0.04	0.42	0.05	0.89	5.3E-02	0.15	0.48	0.13	0.56	0.92	2.0E-02
	Mean	0.44	0.04	0.37	0.09	1.21	5.5E-02	0.19	0.38	0.18	0.42	0.98	2.6E-02
Sum_Rainfall (mm)		0.06	0.79	-0.02	0.93	1.77	7.3E-03	-0.25	0.26	0.02	0.92	1.39	-3.1E-02
Mixed Layer Depth (m)	Min	-0.04	0.88	-0.02	0.94	1.84	-2.9E-04	0.40	0.06	0.16	0.45	0.79	3.7E-03
	Max	-0.03	0.90	0.03	0.89	1.90	-8.8E-05	-0.06	0.77	-0.11	0.61	1.47	-2.0E-04
	Mean	-0.10	0.66	-0.02	0.94	2.22	-8.4E-04	0.20	0.36	0.21	0.34	0.40	1.8E-03
Relative humidity (%)	Min	0.09	0.68	0.16	0.49	1.32	9.1E-03	0.27	0.20	0.26	0.24	-0.31	2.9E-02
	Max	0.08	0.72	0.15	0.51	1.04	8.8E-03	0.27	0.21	0.28	0.20	-1.52	3.2E-02
	Mean	0.14	0.53	0.16	0.48	0.82	1.4E-02	0.24	0.26	0.18	0.41	-0.56	2.5E-02
Terrain Height (m, agl)	Min	-0.40	0.07	-0.42	0.05	2.64	-3.3E-03	-0.22	0.32	-0.26	0.23	1.72	-1.9E-03
	Max	-0.21	0.35	-0.35	0.11	4.42	-3.9E-03	-0.06	0.79	-0.20	0.35	2.02	-1.2E-03
	Mean	-0.46	0.03	-0.47	0.03	3.63	-4.1E-03	-0.25	0.25	-0.28	0.19	2.31	-2.4E-03
Sum of sun flux (W.m^{-2})	Sum	0.13	0.57	0.08	0.74	1.42	7.8E-05	0.09	0.67	0.11	0.62	0.96	5.9E-05
	Max	0.19	0.40	0.15	0.51	1.19	1.1E-03	0.07	0.76	0.13	0.55	1.02	4.2E-04
	Mean	0.13	0.57	0.08	0.74	1.42	1.9E-03	0.09	0.67	0.11	0.62	0.96	1.5E-03
Distance traveled (km)		-0.50	0.02	-0.52	0.01	2.86	-4.4E-03	0.05	0.82	0.01	0.98	1.13	4.9E-04

Climatic or chemical variables (x)[b]		Concentration of culturable bacteria grown on R2A nucleating ice at > -12°C (%)						Concentration of culturable bacteria grown on TSA nucleating ice at > -12°C (%)						Concentration of culturable bacteria grown on TSA 10% nucleating ice at > -12°C (%)					
		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables		Pearson's rank correlation test		Spearman's rank correlation test		linear model predictor variables	
		r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope	r	p-value	rho	p-value	Intercept	Slope
Correlations tested with precipitation water chemistry																			
pH		-0.12	0.66	-0.06	0.84	3.42	-3.0E-01	-0.03	0.91	0.12	0.68	1.92	-7.6E-02	-0.16	0.58	-0.18	0.52	4.615	-3.8E-01
Conductivity (µS.cm)		-0.44	0.10	-0.43	0.11	1.78	-2.6E-02	-0.50	0.06	-0.43	0.11	1.94	-2.9E-02	-0.12	0.66	-0.27	0.33	2.014	-7.6E-03
Total Disolved Solids (ppm)		-0.44	0.12	-0.40	0.16	1.94	-5.0E-02	-0.42	0.13	-0.27	0.36	2.00	-4.6E-02	0.01	0.98	0.00	0.99	1.735	1.1E-03
Salinity (ppm)		-0.33	0.24	-0.03	0.92	1.60	-2.8E-02	-0.47	0.09	-0.28	0.34	1.88	-3.8E-02	-0.05	0.86	-0.10	0.73	1.829	-4.7E-03
Correlations tested with backward trajectories computed at 1000 m																			
Altitude (m, agl)	Min	-0.14	0.51	-0.12	0.59	1.82	-5.9E-04	0.06	0.80	0.08	0.72	1.32	2.3E-04	0.01	0.97	-0.10	0.68	1.806	3.3E-05
	Max	-0.21	0.33	-0.05	0.82	3.73	-2.0E-03	-0.18	0.40	-0.02	0.93	3.26	-1.7E-03	-0.08	0.73	0.09	0.70	2.563	-6.8E-04
	Mean	-0.08	0.71	-0.06	0.80	1.81	-3.6E-04	0.07	0.76	0.11	0.63	1.23	2.9E-04	0.03	0.88	0.03	0.89	1.729	1.4E-04
Pressure (hPa)	Min	0.43	0.04	0.32	0.14	-32.92	4.2E-02	0.30	0.17	0.18	0.41	-22.08	2.8E-02	0.14	0.54	0.08	0.73	-8.654	1.3E-02
	Max	0.08	0.73	0.12	0.59	-0.83	2.5E-03	0.01	0.97	-0.01	0.96	1.14	2.9E-04	0.00	1.00	0.08	0.73	1.794	2.7E-05
	Mean	0.04	0.85	0.05	0.81	0.22	1.5E-03	0.03	0.90	-0.05	0.82	0.57	9.4E-04	0.01	0.96	0.02	0.93	1.504	3.5E-04
Potential temperature (°C)	Min	0.47	0.02	0.45	0.03	0.77	5.8E-02	0.26	0.23	0.32	0.14	0.98	3.1E-02	-0.04	0.88	-0.18	0.43	1.878	-4.0E-03
	Max	0.57	0.00	0.51	0.01	-1.00	1.1E-01	0.33	0.12	0.31	0.15	-0.07	6.4E-02	0.02	0.92	-0.04	0.87	1.717	4.4E-03
	Mean	0.49	0.02	0.39	0.07	0.36	6.8E-02	0.27	0.20	0.25	0.25	0.75	3.8E-02	0.01	0.97	-0.21	0.37	1.797	1.2E-03
Ambient temperature (°C)	Min	0.62	0.00	0.60	0.00	0.96	1.1E-01	0.37	0.08	0.35	0.10	1.05	6.3E-02	0.02	0.93	-0.04	0.86	1.801	3.0E-03
	Max	0.56	0.00	0.53	0.01	0.52	8.4E-02	0.29	0.18	0.27	0.21	0.88	4.3E-02	0.01	0.97	0.05	0.84	1.802	1.4E-03
	Mean	0.57	0.00	0.54	0.01	0.70	9.2E-02	0.32	0.13	0.30	0.16	0.93	5.1E-02	0.01	0.95	-0.02	0.92	1.799	2.0E-03
Sum_Rainfall (mm)		0.24	0.27	0.30	0.17	1.39	4.3E-02	-0.11	0.61	-0.06	0.79	1.50	-2.0E-02	-0.17	0.46	0.09	0.71	1.938	-2.8E-02
Mixed Layer Depth (m)	Min	-0.25	0.25	-0.12	0.58	1.90	-2.3E-03	-0.09	0.70	0.04	0.85	1.53	-7.9E-04	0.15	0.51	0.00	0.99	1.640	1.3E-03
	Max	0.31	0.15	0.21	0.34	0.55	9.4E-04	0.12	0.60	0.01	0.95	1.04	3.5E-04	0.04	0.85	0.10	0.67	1.674	1.3E-04
	Mean	-0.02	0.92	0.10	0.65	1.67	-2.1E-04	-0.14	0.53	0.06	0.79	2.02	-1.2E-03	0.14	0.56	0.08	0.73	1.238	1.1E-03
Relative humidity (%)	Min	0.28	0.20	0.28	0.20	0.43	2.0E-02	0.06	0.79	0.10	0.64	1.18	4.1E-03	0.38	0.09	0.35	0.12	0.358	2.5E-02
	Max	0.27	0.21	0.18	0.42	-0.45	2.4E-02	0.22	0.32	0.09	0.67	-0.17	1.9E-02	0.45	0.04	0.32	0.16	-1.317	3.8E-02
	Mean	0.21	0.33	0.21	0.33	0.39	1.7E-02	0.07	0.74	0.03	0.88	1.02	5.7E-03	0.43	0.05	0.42	0.06	-0.414	3.2E-02
Terrain Height (m, agl)	Min	0.23	0.29	0.09	0.67	1.24	2.1E-03	0.06	0.79	-0.07	0.74	1.33	5.1E-04	0.13	0.58	0.08	0.72	1.640	1.1E-03
	Max	0.11	0.63	0.08	0.73	0.35	1.8E-03	0.01	0.97	0.01	0.96	1.31	1.6E-04	0.07	0.76	0.11	0.64	0.962	1.2E-03
	Mean	0.21	0.34	0.18	0.42	1.03	1.6E-03	0.00	0.99	-0.01	0.97	1.42	-1.7E-05	0.03	0.91	0.05	0.82	1.760	1.8E-04
Sum of sun flux (W.m^{-2})	Sum	0.67	0.00	0.61	0.00	-0.56	4.5E-04	0.33	0.12	0.29	0.19	0.38	2.2E-04	0.07	0.75	0.05	0.84	1.575	4.9E-05
	Max	0.68	0.00	0.64	0.00	-0.87	4.5E-03	0.32	0.13	0.30	0.16	0.27	2.1E-03	0.01	0.97	-0.08	0.73	1.793	4.7E-05
	Mean	0.67	0.00	0.61	0.00	-0.56	1.1E-02	0.33	0.12	0.29	0.19	0.38	5.4E-03	0.07	0.75	0.05	0.84	1.575	1.2E-03
Distance traveled (km)		-0.40	0.06	-0.34	0.11	2.31	-1.1E-03	-0.28	0.20	-0.21	0.33	1.92	-7.9E-04	-0.40	0.08	-0.17	0.47	2.500	-1.0E-03
Correlations tested with backward trajectories computed at 500 m																			
Altitude (m, agl)	Min	0.02	0.94	-0.06	0.78	1.55	1.8E-04	0.03	0.90	-0.13	0.55	1.39	2.7E-04	0.31	0.17	0.19	0.40	1.521	3.0E-03
	Max	0.13	0.54	0.06	0.80	0.72	1.5E-03	0.12	0.58	-0.01	0.95	0.65	1.4E-03	0.10	0.67	0.10	0.67	1.252	1.0E-03
	Mean	0.11	0.61	0.00	0.99	1.39	8.2E-04	0.07	0.75	-0.04	0.86	1.30	5.2E-04	0.18	0.44	0.10	0.68	1.545	1.2E-03
Pressure (hPa)	Min	0.24	0.28	0.13	0.56	-19.26	2.4E-02	0.19	0.39	0.12	0.58	-14.76	1.8E-02	0.19	0.42	0.12	0.62	-13.339	1.7E-02
	Max	-0.10	0.64	0.00	0.99	6.23	-4.7E-03	0.07	0.74	0.14	0.52	-1.83	3.3E-03	-0.07	0.75	0.00	1.00	4.929	-3.2E-03
	Mean	-0.06	0.80	0.03	0.89	3.93	-2.5E-03	0.10	0.65	0.15	0.50	-2.76	4.4E-03	0.05	0.82	0.11	0.64	-0.285	2.2E-03
Potential temperature (°C)	Min	0.50	0.01	0.42	0.05	0.97	5.9E-02	0.25	0.25	0.17	0.43	1.12	2.9E-02	0.13	0.57	0.14	0.54	1.654	1.5E-02
	Max	0.56	0.01	0.52	0.01	-0.20	9.0E-02	0.30	0.16	0.27	0.21	0.49	4.7E-02	0.03	0.91	-0.01	0.97	1.738	4.0E-03
	Mean	0.52	0.01	0.47	0.02	0.57	6.9E-02	0.28	0.19	0.28	0.19	0.88	3.6E-02	0.08	0.72	0.02	0.95	1.658	1.0E-02
Ambient temperature (°C)	Min	0.58	0.00	0.54	0.01	1.03	8.5E-02	0.31	0.14	0.27	0.21	1.13	4.5E-02	0.10	0.67	-0.01	0.97	1.725	1.3E-02
	Max	0.58	0.00	0.52	0.01	0.22	9.0E-02	0.37	0.09	0.34	0.11	0.57	5.6E-02	0.05	0.84	-0.04	0.87	1.709	7.0E-03
	Mean	0.57	0.00	0.52	0.01	0.68	8.6E-02	0.34	0.11	0.33	0.13	0.89	5.1E-02	0.10	0.65	-0.08	0.73	1.653	1.5E-02
Sum precipitation (mm)		0.12	0.58	0.33	0.13	1.49	2.0E-02	-0.29	0.17	-0.07	0.74	1.60	-4.9E-02	-0.36	0.11	-0.02	0.92	2.008	-5.9E-02
Mixed Layer Depth (m)	Min	0.06	0.78	-0.01	0.97	1.50	6.6E-04	0.16	0.47	0.06	0.80	1.23	1.6E-03	0.44	0.05	0.36	0.11	1.352	4.2E-03
	Max	0.41	0.05	0.30	0.16	-0.01	1.5E-03	0.23	0.30	0.10	0.64	0.54	8.1E-04	0.02	0.95	0.08	0.72	1.760	5.4E-05
	Mean	0.20	0.35	0.33	0.13	0.65	2.0E-03	0.04	0.86	0.15	0.51	1.24	3.7E-04	0.22	0.34	0.24	0.29	0.881	2.0E-03
Relative humidity (%)	Min	0.43	0.04	0.42	0.05	-0.91	4.4E-02	0.02	0.93	0.10	0.66	1.31	2.0E-03	0.21	0.37	0.21	0.37	0.706	2.0E-02
	Max	0.24	0.26	0.18	0.42	-0.91	2.9E-02	0.02	0.93	0.06	0.79	1.21	2.4E-03	0.18	0.44	0.21	0.36	0.073	2.0E-02
	Mean	0.28	0.20	0.26	0.22	-0.45	2.9E-02	-0.04	0.85	-0.01	0.97	1.72	-4.3E-03	0.14	0.54	0.17	0.46	0.823	1.4E-02
Terrain Height (m, agl)	Min	0.09	0.70	0.05	0.81	1.44	7.2E-04	-0.08	0.71	-0.08	0.72	1.54	-6.8E-04	-0.11	0.63	-0.18	0.45	1.982	-8.4E-04
	Max	0.05	0.84	0.01	0.97	1.04	7.9E-04	-0.08	0.72	-0.12	0.58	2.31	-1.3E-03	-0.15	0.51	-0.15	0.52	3.483	-2.4E-03
	Mean	0.05	0.81	0.01	0.96	1.42	4.2E-04	-0.10	0.65	-0.16	0.47	1.70	-8.0E-04	-0.22	0.34	-0.25	0.27	2.419	-1.6E-03
Sum of sun flux (W.m^{-2})	Sum	0.56	0.01	0.48	0.02	-0.29	3.9E-04	0.25	0.25	0.22	0.32	0.58	1.7E-04	-0.11	0.63	-0.20	0.38	2.209	-7.7E-05
	Max	0.58	0.00	0.57	0.00	-0.65	4.0E-03	0.27	0.21	0.25	0.26	0.39	1.9E-03	-0.15	0.51	-0.17	0.47	2.427	-1.1E-03
	Mean	0.56	0.01	0.48	0.02	-0.29	9.7E-03	0.25	0.25	0.22	0.32	0.58	4.3E-03	-0.11	0.63	-0.20	0.38	2.209	-1.9E-03
Distance traveled (km)		-0.40	0.06	-0.29	0.18	2.36	-2.0E-03	-0.33	0.12	-0.22	0.30	2.05	-1.7E-03	-0.25	0.27	-0.12	0.60	2.279	-1.2E-03
Correlations tested with backward trajectories computed at 50 m																			
Altitude (m, agl)	Min	0.21	0.34	0.24	0.27	1.49	3.7E-02	0.10	0.67	0.11	0.62	1.38	1.7E-02	0.14	0.56	0.07	0.76	1.766	2.2E-02
	Max	0.16	0.46	0.06	0.80	1.35	2.9E-03	0.16	0.45	-0.05	0.84	1.19	2.9E-03	0.06	0.80	-0.13	0.59	1.745	9.2E-04
	Mean	0.25	0.25	0.10	0.64	1.39	1.0E-02	0.17	0.43	-0.11	0.63	1.29	6.9E-03	0.09	0.68	-0.11	0.62	1.753	3.5E-03
Pressure (hPa)	Min	0.20	0.36	0.16	0.45	-20.18	2.3E-02	0.32	0.14	0.29	0.18	-32.40	3.6E-02	0.18	0.42	0.13	0.59	-16.915	2.0E-02
	Max	0.07	0.74	0.03	0.89	-3.19	4.8E-03	0.39	0.06	0.33	0.12	-23.90	2.6E-02	0.34	0.14	0.31	0.17	-18.569	2.1E-02
	Mean	0.14	0.53	0.11	0.61	-8.25	1.0E-02	0.38	0.08	0.35	0.10	-24.86	2.7E-02	0.38	0.09	0.31	0.17	-23.664	2.6E-02
Potential temperature (°C)	Min	0.55	0.01	0.47	0.02	0.93	7.4E-02	0.25	0.25	0.20	0.35	1.13	3.3E-02	0.09	0.71	0.10	0.66	1.714	1.1E-02
	Max	0.59	0.00	0.53	0.01	0.03	8.0E-02	0.34	0.12	0.30	0.17	0.55	4.5E-02	0.04	0.86	-0.10	0.67	1.711	5.3E-03
	Mean	0.57	0.00	0.51	0.01	0.54	7.7E-02	0.29	0.18	0.29	0.18	0.90	3.9E-02	0.09	0.69	0.06	0.79	1.644	1.2E-02
Ambient temperature (°C)	Min	0.60	0.00	0.56	0.01	1.05	8.5E-02	0.34	0.11	0.34	0.11	1.12	4.9E-02	0.17	0.47	0.03	0.89	1.669	2.2E-02
	Max	0.62	0.00	0.59	0.00	0.18	8.3E-02	0.42	0.04	0.44	0.03	0.49	5.6E-02	0.11	0.64	-0.01	0.96	1.581	1.4E-02
	Mean	0.60	0.00	0.54	0.01	0.69	8.4E-02	0.35	0.10	0.34	0.11	0.90	4.9E-02	0.16	0.50	0.06	0.79	1.588	2.1E-02
Sum_Rainfall (mm)		0.14	0.52	0.18	0.41	1.48	1.9E-02	-0.31	0.15	-0.19	0.38	1.60	-4.1E-02	-0.28	0.21	0.07	0.75	1.970	-3.5E-02
Mixed Layer Depth (m)	Min	-0.12	0.59	-0.17	0.44	1.71	-1.1E-03	0.03	0.89	0.01	0.95	1.38	2.9E-04	0.36	0.11	0.17	0.45	1.434	3.1E-03
	Max	0.43	0.04	0.41	0.05	-0.01	1.4E-03	0.31	0.15	0.24	0.28	0.28	1.0E-03	-0.04	0.87	-0.04	0.87	1.958	-1.2E-04
	Mean	0.28	0.20	0.33	0.12	0.35	2.5E-03	0.19	0.38	0.27	0.22	0.58	1.7E-03	0.22	0.34	0.27	0.24	0.879	1.9E-03
Relative humidity (%)	Min	0.17	0.45	0.09	0.69	0.59	1.8E-02	-0.23	0.28	-0.24	0.26	2.78	-2.6E-02	0.18	0.42	0.26	0.25	0.818	1.9E-02
	Max	0.33	0.12	0.28	0.20	-2.00	4.1E-02	0.02	0.95	0.04	0.87	1.25	1.8E-03	0.19	0.42	0.25	0.27	-0.072	2.2E-02
	Mean	0.18	0.42	0.09	0.67	0.21	1.9E-02	-0.13	0.54	-0.15	0.49	2.44	-1.4E-02	0.19	0.40	0.28	0.22	0.399	2.0E-02
Terrain Height (m, agl)	Min	-0.11	0.60	-0.15	0.48	1.83	-1.1E-03	-0.34	0.12	-0.35	0.11	2.18	-3.1E-03	-0.36	0.11	-0.38	0.09	2.617	-3.1E-03
	Max	-0.08	0.72	-0.14	0.52	2.69	-1.7E-03	-0.33	0.12	-0.31	0.15	5.96	-6.9E-03	-0.24	0.29	-0.28	0.22	4.997	-4.8E-03
	Mean	-0.13	0.54	-0.15	0.50	2.17	-1.4E-03	-0.33	0.12	-0.32	0.14	2.87	-3.3E-03	-0.42	0.06	-0.39	0.08	3.598	-4.0E-03
Sum of sun flux (W.m^{-2})	Sum	0.58	0.00	0.52	0.01	-0.27	3.8E-04	0.27	0.21	0.27	0.22	0.55	1.8E-04	-0.11	0.64	-0.21	0.37	2.164	-6.8E-05
	Max	0.59	0.00	0.61	0.00	-0.52	3.8E-03	0.32	0.14	0.32	0.13	0.31	2.0E-03	-0.12	0.60	-0.18	0.42	2.234	-7.4E-04
	Mean	0.58	0.00	0.52	0.01	-0.27	9.5E-03	0.27	0.21	0.27	0.22	0.55	4.5E-03	-0.11	0.64	-0.21	0.37	2.164	-1.7E-03
Distance traveled (km)		-0.25	0.25	-0.17	0.43	2.17	-2.5E-03	-0.20	0.36	-0.15	0.48	1.89	-2.0E-03	-0.07	0.76	0.01	0.96	1.975	-6.4E-04

a In addtion to the total number of ice nucleators, the following biological variables were statistically analyzed for each of the 6 media described in the material and methods section: concentration of total bacteria, frequency and concentration of culturable bacteria (i) INA only the first time they were tested and (ii) for which INA is reproducible and confirmed.

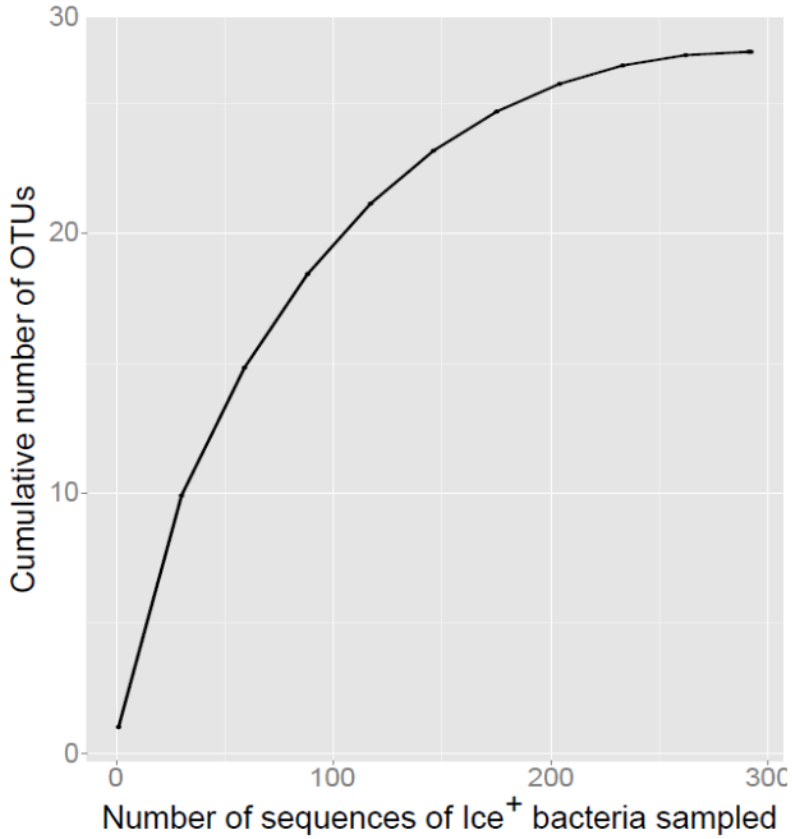
[b] All the correlations with physical properties listed in materials and methods section have been tested for the three elevations. Correlations are based on the analyse of N= 23 samples
Models are given only for variables for which at least one of the Pearson's or Spearman's coefficients was significantly different from zero are presented (P<0.1).

[c] The p-value for the Shapiro-Wilk test is given next to each *x* variable and below each *y* variable. When the p-value is under 0.05, normality of the data distribution is rejected.

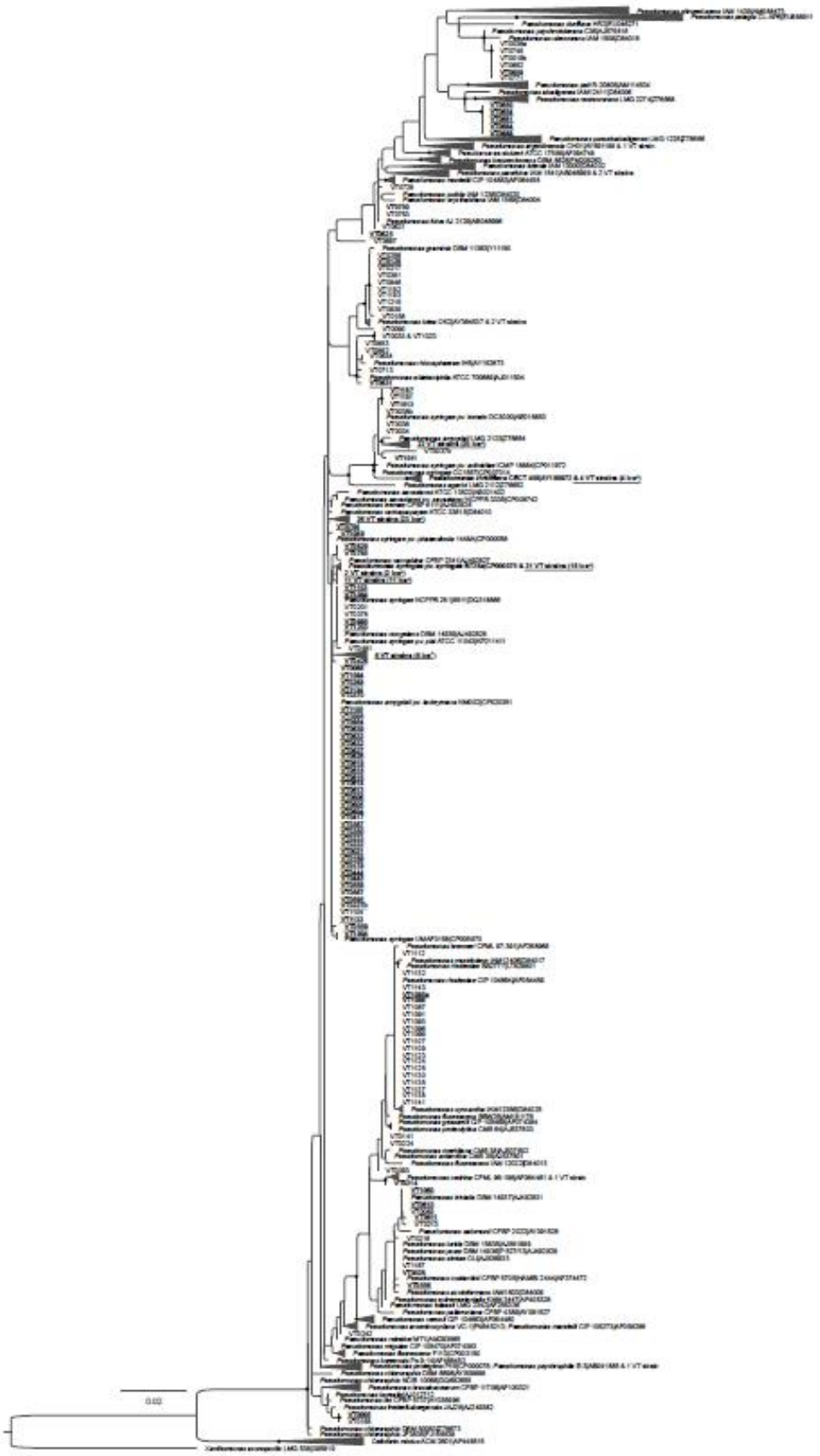
Supplemental Figures

Supplementary Figure S1. Map of sampling sites and photo of one of the Kentland Farm rain sampling sites in Blacksburg, VA, USA.

Supplementary Figure S2. Rarefaction curve plotting the cumulative species richness against the cumulative number of sequences obtained in precipitation samples collected in Virginia, USA. The species richness was obtained by affiliating each 16S rDNA sequence to an operational taxonomic unit (OTU) using the open-source bioinformatics pipeline for performing microbiome analysis QIIME (Caporaso *et al.* 2010). The curve was obtained plotting the cumulative number of OTUs against the cumulative number of sequences.

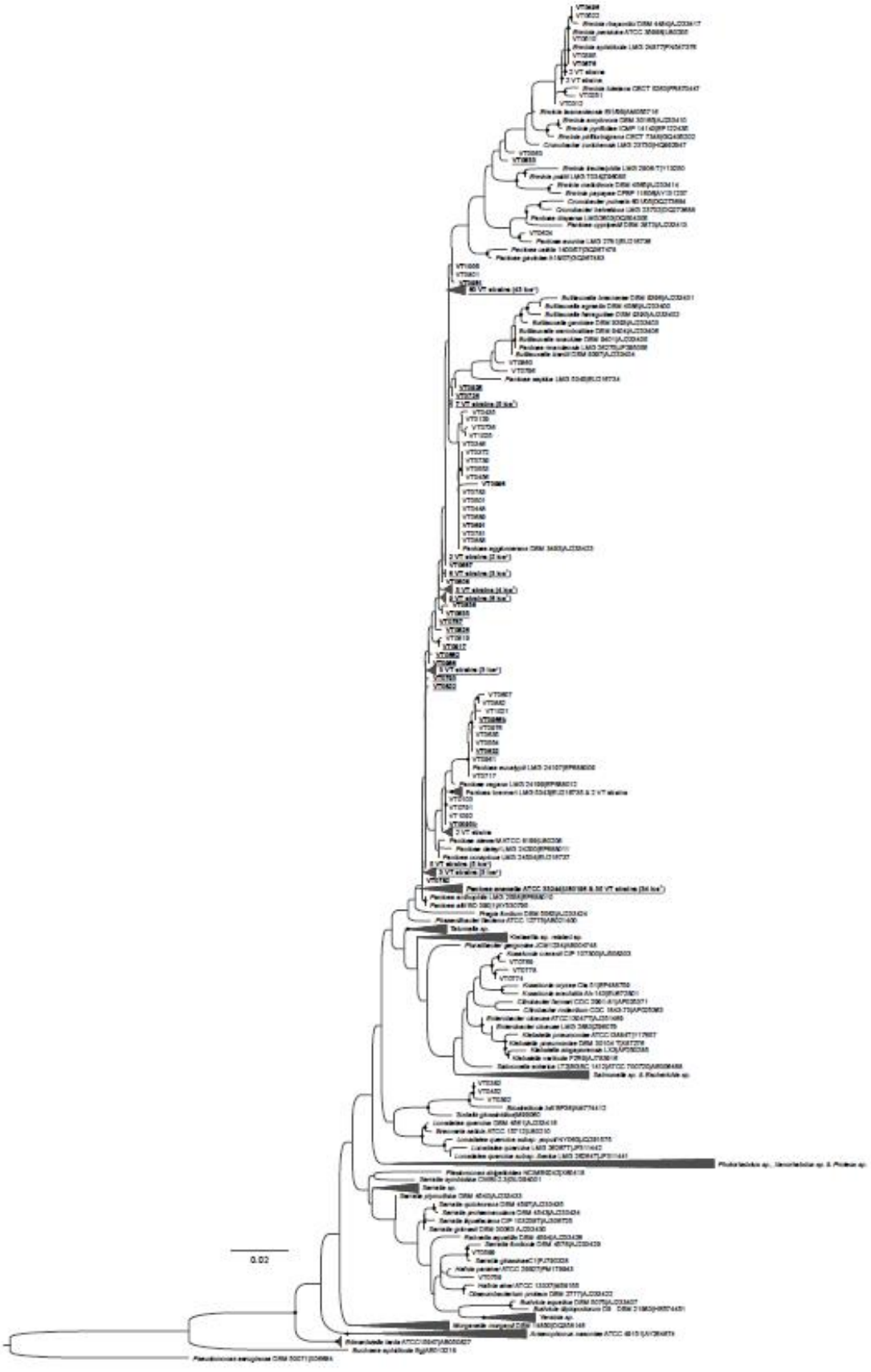


Supplementary Figure S3. Evolutionary relationships between the culturable strains isolated from precipitation and other members of the Pseudomonadaceae family based on the alignment of partial 16S rDNA sequences. A maximum likelihood tree was built from the 931pb-long sequences of 229 strains isolated in this study (labelled VT#; strains that were confirmed to be Ice^+ in repeated tests are labelled in bold), 150 type strains downloaded from the Ribosomal Database Project (RDP) (Cole *et al.* 2014) and 19 strains from NCBI, using the GTR model and 100 bootstrap replicates. Gaps were considered as missing data and were partially deleted with a site coverage cut-off of 95%. The strain *Xanthomonas axonopodis* LMG 538^T was used as a root. Only bootstrap values over 50% were included and are symbolized by black dots. Clades and leaves associated with strains that were Ice^+ in standardized re-tests at -8°C are labelled in bold and are underlined, those active at -10°C or -12°C are labelled in bold only. RDP names are composed of the species name, the strain code and the accession number in the public database NCBI. Each collapsed clade is represented by a type strain.

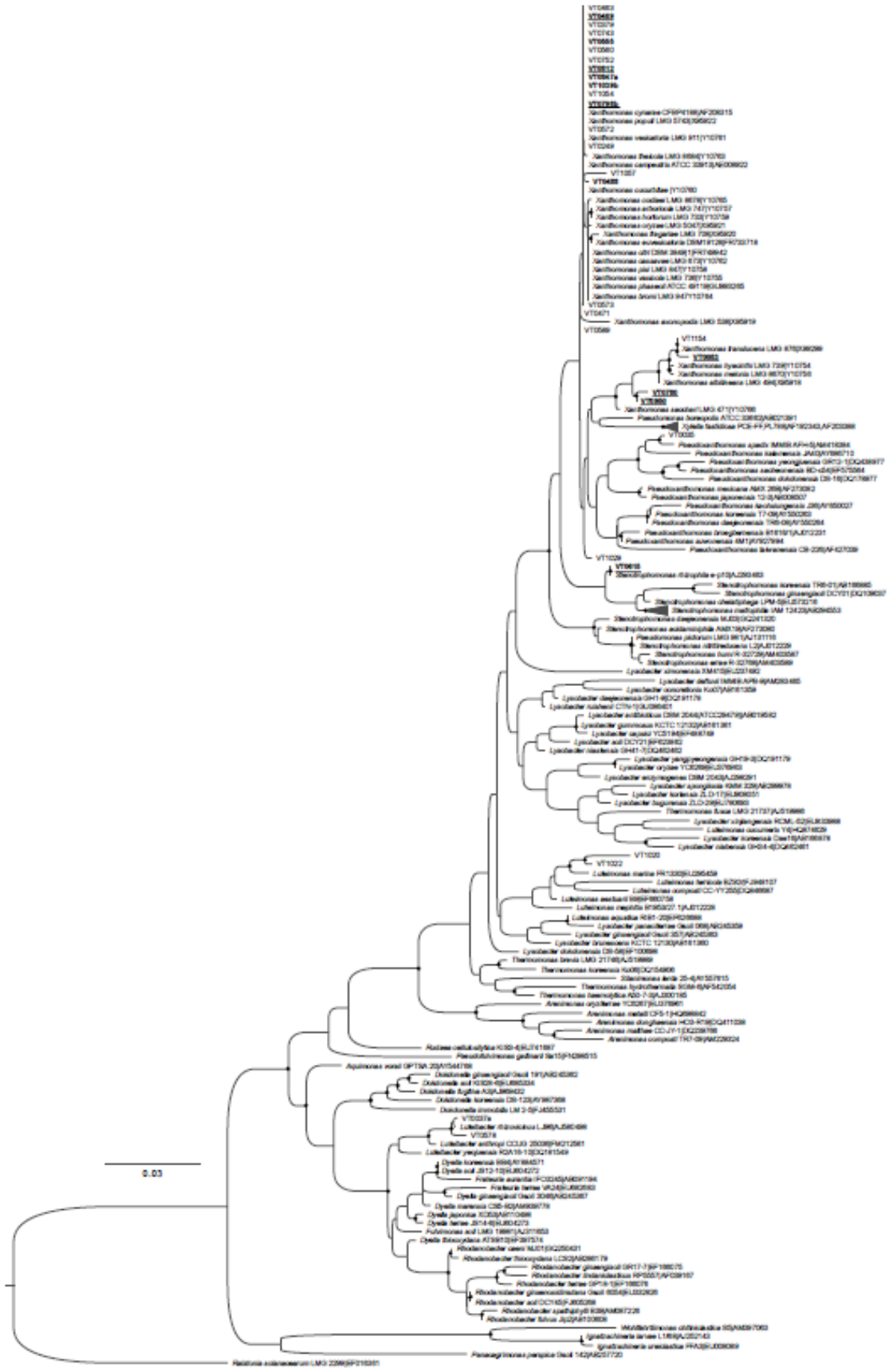


245A

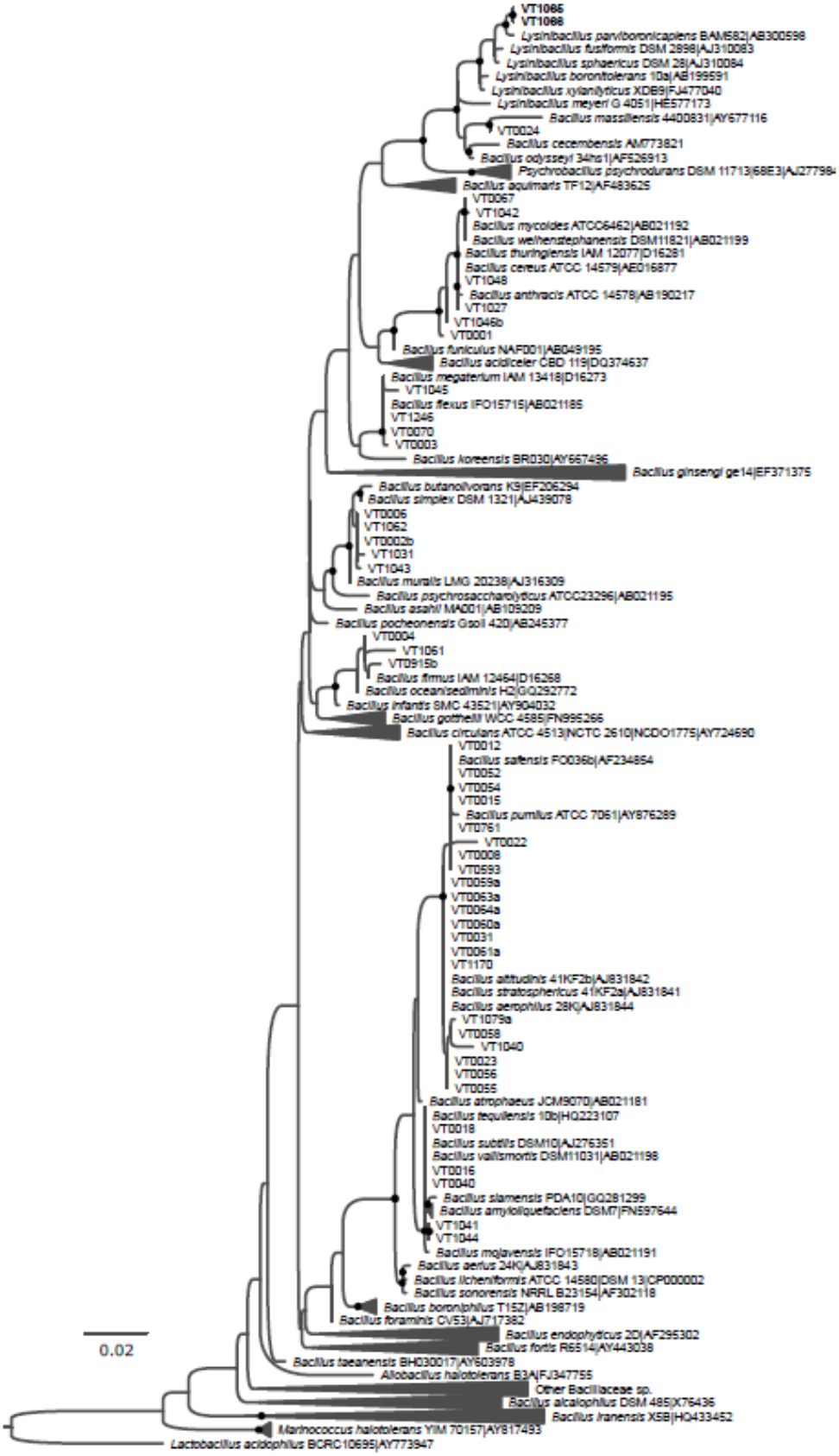
Supplementary Figure S4. Evolutionary relationships between the culturable strains isolated from precipitation and other members of the Enterobacteriaceae family based on the alignment of partial 16S rDNA sequences. The phylogenetic tree was built from 212 strains isolated in this study (labelled VT#; strains that were confirmed to be Ice^+ in repeated tests are labelled in bold) and 241 type strains downloaded from the Ribosomal Database Project (RDP) (Cole *et al.* 2014). The strain Pseudomonas aeruginosa DSM 50071 was used as a root. See Supplementary Figure S3 for details about tree construction and labels.


0.02

Supplementary Figure S5. Evolutionary relationships between the culturable strains isolated from precipitation and other members of the Xanthomonadaceae family based on the alignment of partial 16S rDNA sequences. The phylogenetic tree was built from 30 strains isolated in this study (labelled VT#, strains that were confirmed to be Ice^+ in repeated tests are labelled in bold) and 132 type strains downloaded from the Ribosomal Database Project (RDP) (Cole *et al.* 2014). The strain *Ralstonia solanacearum* LMG 2299 was used as a root. See Supplementary Figure S3 for details about tree construction and labels.


0.03

Supplementary Figure S6. Evolutionary relationships between the culturable INA strains Lysinibacillus sp. VT1065 and VT1066 isolated from precipitation and other members of the Bacillaceae family based on the alignment of 16S rDNA partial sequences. The phylogenetic tree was built from 47 strains isolated in this study (labelled VT#; strains that were confirmed to be Ice^+ in repeated tests are labelled in bold) and 353 type strains downloaded from the Ribosomal Database Project (RDP) (Cole *et al.* 2014). The strain *Lactobacillus acidophilus* BCRC10695 was used as a root. See Supplementary Figure S3 for details about tree construction and labels.


VT1065
VT1068
Lysinibacillus parviboronicapiens BAM582|AB300598
Lysinibacillus fusiformis DSM 2898|AJ310083
Lysinibacillus sphaericus DSM 28|AJ310084
Lysinibacillus boronitolerans 10a|AB199591
Lysinibacillus xylanilyticus XDB9|FJ477040
Lysinibacillus meyeri G 4051|HE577173
Bacillus massiliensis 4400831|AY677116
VT0024
Bacillus cecembensis AM773821
Bacillus odysseyi 34hs1|AF526913
Psychrobacillus psychrodurans DSM 11713|68E3|AJ27798
Bacillus aquimaris TF12|AF483625
VT0067
VT1042
Bacillus mycoides ATCC6462|AB021192
Bacillus weihenstephanensis DSM11821|AB021199
Bacillus thuringiensis IAM 12077|D16281
Bacillus cereus ATCC 14579|AE016877
VT1048
Bacillus anthracis ATCC 14578|AB190217
VT1027
VT1046b
VT0001
Bacillus funiculus NAF001|AB049195
Bacillus acidiceler CBD 119|DQ374637
Bacillus megaterium IAM 13418|D16273
VT1045
Bacillus flexus IFO15715|AB021185
VT1246
VT0070
VT0003
Bacillus koreensis BR030|AY667496
Bacillus ginsengi ge14|EF371375
Bacillus butanolivorans K9|EF206294
Bacillus simplex DSM 1321|AJ439078
VT0006
VT1062
VT0002b
VT1031
VT1043
Bacillus muralis LMG 20238|AJ316309
Bacillus psychrosaccharolyticus ATCC23296|AB021195
Bacillus asahii MA001|AB109209
Bacillus pocheonensis Gsoil 420|AB245377
VT0004
VT1061
VT0015b
Bacillus firmus IAM 12464|D16268
Bacillus oceanisediminis H2|GQ292772
Bacillus infantis SMC 43521|AY904032
Bacillus gotthelfii WCC 4585|FN995266
Bacillus circulans ATCC 4513|NCTC 2610|NCDO1775|AY724690
VT0012
Bacillus safensis FO036b|AF234854
VT0052
VT0054
VT0015
Bacillus pumilus ATCC 7061|AY876289
VT0761
VT0022
VT0008
VT0593
VT0059a
VT0063a
VT0064a
VT0060a
VT0031
VT0061a
VT1170
Bacillus altitudinis 41KF2b|AJ831842
Bacillus stratosphericus 41KF2a|AJ831841
Bacillus aerophilus 28K|AJ831844
VT1079a
VT0058
VT1040
VT0023
VT0056
VT0055
Bacillus atrophaeus JCM9070|AB021181
Bacillus tequilensis 10b|HQ223107
VT0018
Bacillus subtilis DSM10|AJ276351
Bacillus vallismortis DSM11031|AB021198
VT0016
VT0040
Bacillus siamensis PDA10|GQ281299
Bacillus amyloliquefaciens DSM7|FN597644
VT1041
VT1044
Bacillus mojavensis IFO15718|AB021191
Bacillus aerius 24K|AJ831843
Bacillus licheniformis ATCC 14580|DSM 13|CP000002
Bacillus sonorensis NRRL B23154|AF302118
Bacillus borbonhilus T15Z|AB198719
Bacillus foraminis CV53|AJ717382
Bacillus endophyticus 2D|AF295302
Bacillus fortis R6514|AY443038
0.02
Bacillus taeanensis BH030017|AY603978
Allobacillus halotolerans B3A|FJ347755
Other Bacillaceae sp.
Bacillus alcalophilus DSM 485|X76436
Bacillus iranensis X5B|HQ433452
Marinococcus halotolerans YIM 70157|AY817493
Lactobacillus acidophilus BCRC10695|AY773947

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Appendix B: Supplemental tables for Chapter Three: Lysinibacillus ice nucleation linked to a type 1 polyketide synthase – Non-ribosomal peptide synthase

Supplemental Table S1. Culture collection information for obtained Lysinibacillus type strains.

Lysinibaciilus species	Strain	Culture Collection
L. acetophenoni	KACC 18506	Korean Agricultural Culture Collection
	KCTC 13605	Korean Collection for Type Cultures
L. boronitolerans	KACC 15323	Korean Agricultural Culture Collection
	NBRC 103108	NITE Biological Research Center
L. chugkukjangi	KACC 16626	Korean Agricultural Culture Collection
L. composti	KCTC 13796	Korean Collection for Type Cultures
L. fluoroglycofenilyticus	KCTC 33183	Korean Collection for Type Cultures
L. fusiformis	DSM 2898	Bacillus Genetic Stock Center
	KACC 10903	Korean Agricultural Culture Collection
	NBRC 15717	NITE Biological Research Center
	NBRC 15718	NITE Biological Research Center
L. jejuensis	KCTC 13837	Korean Collection for Type Cultures
L. mangiferihumi	KACC 17178	Korean Agricultural Culture Collection
L. massiliensis	KACC 14317	Korean Agricultural Culture Collection
	KCTC 13178	Korean Collection for Type Cultures
L. meyeri	KACC 17179	Korean Agricultural Culture Collection
L. odysseyi	KCTC 3961	Korean Collection for Type Cultures
	NBRC 100172	NITE Biological Research Center
L. pakistanensis	KCTC 13795	Korean Collection for Type Cultures
L. parviboronicapiens	KACC 15207	Korean Agricultural Culture Collection
	KCTC 13154	Korean Collection for Type Cultures
L. sinduriensis	KACC 16611	Korean Agricultural Culture Collection
	KCTC 13296	Korean Collection for Type Cultures
L. sphaericus	DSM 28	Bacillus Genetic Stock Center
	KACC 10554	Korean Agricultural Culture Collection
	NRBC 15095	NITE Biological Research Center
L. tabacifolii	KCTC 33042	Korean Collection for Type Cultures
L. varians	NBRC 109424	NITE Biological Research Center
L. xylanilyticus	KACC 15113	Korean Agricultural Culture Collection
	KCTC 13423	Korean Collection for Type Cultures

Supplemental Table S2. List of genes present and expressed in only L. parviboronicapiens *genomes.*

Gene ID	Annotated Gene Product
4567_248975_248250	3-oxoacyl-[acyl-carrier protein] reductase (EC 1.1.1.100)
4583_13104_12403	ABC transporter ATP-binding protein
4623_11842_10157	ABC transporter ATP-binding protein uup
4583_12413_10815	ABC transporter permease protein
4595_16430_16717	Acetyltransferase
4625_28901_28470	Acyl-CoA dehydrogenase-like protein
4583_55178_54471	Alanine dehydrogenase (EC 1.4.1.1)
4567_46525_45365	Butyryl-CoA dehydrogenase (EC 1.3.8.1)
4601_49042_48191	CAAX amino terminal protease family protein
4575_14813_15640	CAAX amino terminal protease family protein
4609_11448_11248	Catalyzes the cleavage of p-aminobenzoyl-glutamate to p-aminobenzoate and glutamate, subunit B
4609_11826_11524	Catalyzes the cleavage of p-aminobenzoyl-glutamate to p-aminobenzoate and glutamate, subunit B
4571_106814_105873	CDS_ID OB2952
4571_16724_15168	CDS_ID OB2952
4609_15143_15451	Cell division protein FtsH (EC 3.4.24.-)
4567_49549_48380	Cysteine desulfurase (EC 2.8.1.7)
4645_4312_3161	D-alanyl-D-alanine carboxypeptidase (EC 3.4.16.4)
4571_83719_81920	DNA/RNA non-specific endonuclease
4567_430757_430227	DNA-directed RNA polymerase delta subunit (EC 2.7.7.6)
4573_136565_137197	Exosporium protein
4575_1615_671	Fe-bacillibactin uptake system FeuA, Fe-bacillibactin binding
4599_82211_81792	Ferrichrome-binding periplasmic protein precursor (TC 3.A.1.14.3)
4593_56262_57098	Fructosyl-amino acid oxidase (EC 1.5.3.-)
4571_2801_1776	Glutamyl endopeptidase precursor (EC 3.4.21.19), blaSE
4575_124900_125274	Glutathione peroxidase family protein
4567_350222_349098	Glycosyltransferase
4601_2089_1799	Glyoxalase family protein
4627_40486_41316	Hydrolase, alpha/beta fold family
4567_138359_138084	Hypothetical protein
4567_138958_138512	Hypothetical protein
4567_139456_139286	Hypothetical protein
4567_263095_261836	Hypothetical protein
4567_265877_264849	Hypothetical protein
4567_270364_269465	Hypothetical protein
4567_351433_350252	Hypothetical protein
4567_352624_351557	Hypothetical protein
4567_356003_355032	Hypothetical protein
4567_356856_356014	Hypothetical protein
4567_377643_377470	Hypothetical protein
4567_47397_46522	Hypothetical protein
4585_55302_55430	Hypothetical protein
4585_8861_9052	Hypothetical protein
4587_120828_121013	Hypothetical protein
4587_57796_56753	Hypothetical protein

Gene ID	Annotated Gene Product
4589_57998_58159	Hypothetical protein
4591_104025_103870	Hypothetical protein
4591_1288_785	Hypothetical protein
4591_39528_40022	Hypothetical protein
4591_99936_100172	Hypothetical protein
4593_134065_133787	Hypothetical protein
4593_4530_4895	Hypothetical protein
4595_75625_75837	Hypothetical protein
4597_66161_66021	Hypothetical protein
4597_82913_83077	Hypothetical protein
4599_30796_30653	Hypothetical protein
4601_1463_1308	Hypothetical protein
4601_70489_70316	Hypothetical protein
4603_68258_68064	Hypothetical protein
4603_96082_95486	Hypothetical protein
4569_119886_119716	Hypothetical protein
4569_79182_80312	Hypothetical protein
4609_2682_2311	Hypothetical protein
4609_59929_59345	Hypothetical protein
4609_61050_60406	Hypothetical protein
4611_71924_71685	Hypothetical protein
4571_14881_14162	Hypothetical protein
4571_255006_254851	Hypothetical protein
4571_28196_28083	Hypothetical protein
4571_63432_63022	Hypothetical protein
4571_69410_68715	Hypothetical protein
4571_84507_83836	Hypothetical protein
4629_10730_10945	Hypothetical protein
_4631_40027_39713	Hypothetical protein
4631_5971_6303	Hypothetical protein
4635_14606_14355	Hypothetical protein
4635_31855_31742	Hypothetical protein
4573_173937_175319	Hypothetical protein
4645_5081_4527	Hypothetical protein
4657_2941_2501	Hypothetical protein
4657_4915_4457	Hypothetical protein
4577_119561_120220	Hypothetical protein
4577_120354_120611	Hypothetical protein
4577_123211_123375	Hypothetical protein
4577_139630_140238	Hypothetical protein
4577_153368_153700	Hypothetical protein
4577_204565_204984	Hypothetical protein
4577_42354_42884	Hypothetical protein
4583_139964_139746	Hypothetical protein
4567_49799_49560	Iron-sulfur cluster assembly scaffold protein IscU/NifU-like

Gene ID	Annotated Gene Product
4567_353929_352676	Lipopolysaccharide biosynthesis protein
4593_131825_131259	Methyl-accepting chemotaxis protein
4569_80329_81321	Methyl-accepting chemotaxis protein
4571_143141_141486	Methyl-accepting chemotaxis protein
4567_267175_265934	Methyltransferase FkbM
4623_27557_26199	Mg/Co/Ni transporter MgtE / CBS domain
4681_827_39	Mobile element protein
4601_22564_23046	Multi antimicrobial extrusion protein (Na(+)/drug antiporter), MATE family of MDR efflux pumps
4575_32127_33338	Multidrug resistance protein B
4597_93124_93444	MutT/Nudix family protein
4571_114197_114796	Nitroreductase family protein
4567_153663_152713	Nucleoside-diphosphate-sugar epimerases
4567_41649_42590	Octaprenyl diphosphate synthase (EC 2.5.1.90) / Dimethylallyltransferase (EC 2.5.1.1) / (2E,6E)-farnesyl diphosphate synthase (EC 2.5.1.10) / Geranylgeranyl diphosphate synthase (EC 2.5.1.29)
4597_109465_108578	Permease of the drug/metabolite transporter (DMT) superfamily
4655_5043_6077	Phage tail fiber protein
4571_234383_233979	PhnB protein; putative DNA binding 3-demethylubiquinone-9 3-methyltransferase domain protein
4645_9016_8297	probable membrane protein yetF
4567_268082_267198	Protein distantly related to SAM-dependent methyltransferases
4585_96527_97009	PTS system, glucose-specific IIC component (EC 2.7.1.69) / PTS system, glucose-specific IIB component (EC 2.7.1.69) / PTS system, glucose-specific IIA component (EC 2.7.1.69)
4585_97317_98717	PTS system, N-acetylglucosamine-specific IIA component (EC 2.7.1.69) / PTS system, N-acetylglucosamine-specific IIB component (EC 2.7.1.69) / PTS system, N-acetylglucosamine-specific IIC component (EC 2.7.1.69)
4567_250445_250221	Putative acyl carrier protein
4567_264433_263105	Putative glycosyltransferase
4633_16866_17630	Putative membrane protein YfcA
4601_45781_43526	Putative secreted protein.
4573_171831_171511	Putative Zn-dependent oxidoreductase BA2113
4587_54870_55475	Rhs-family protein
4591_79722_78667	Ribosomal RNA large subunit methyltransferase N (EC 2.1.1.-)
4577_49462_49719	Ribosomal-protein-alanine acetyltransferase (EC 2.3.1.128)
4591_13606_15111	S-adenosylmethionine decarboxylase proenzyme (EC 4.1.1.50), prokaryotic class 1B
4605_75_839	Seryl-tRNA synthetase (EC 6.1.1.11)
4643_11634_10870	Seryl-tRNA synthetase (EC 6.1.1.11)
4601_65690_66841	Spore germination protein GerKB
4571_128046_129125	Spore germination protein GerXB (on pXO1)
4571_116769_116200	Substrate-specific component RibU of riboflavin ECF transporter
4575_87772_86234	Thiamin-regulated outer membrane receptor Omr1
4609_64818_65861	Transcriptional regulator, ArsR family
4619_6544_6083	Transcriptional regulator, MarR family
4589_70542_70084	Transcriptional regulator, MarR family / Acetyltransferase (GNAT)
4569_19227_19445	Transcriptional regulator, TetR family
4597_110340_109528	Transcriptional regulator, TrmB family
4625_17815_18486	Uncharacterized membrane protein Bsu2508 (YqfU)

Appendix C: Microbial ice nucleators scavenged from the atmosphere during simulated rain events

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Abstract

Rain and snow collected at ground level have been found to contain biological ice nucleators. These ice nucleators have been proposed to have originated in clouds, where they may have participated in the formation of precipitation via ice phase nucleation. We conducted a series of field experiments to test the hypothesis that at least some of the microbial ice nucleators (prokaryotes and eukaryotes) present in rain may not originate in clouds but instead be scavenged from the lower atmosphere by rainfall. Thirty-three simulated rain events were conducted over four months off the side of the Smart Road Bridge in Blacksburg, VA, USA. In each event, sterile water was dispensed over the side of the bridge and recovered in sterile containers in an open fallow agricultural field below (a distance of ~55 m). Microbes scavenged from the simulated rain events were cultured and their ice nucleation activity was examined. Putative microbial ice nucleators were cultured from 94% (31/33) of the simulated rain events, and represented 1.5% (121/8331) of the total colonies assayed. Putative ice nucleators were subjected to additional droplet freezing assays, and those confirmed through these repeated assays represented 0.4% (34/8331) of the total. Mean CFUs scavenged by simulated rain ranged from 2 to 267 CFUs/mL. Scavenged ice nucleators belong to a number of taxa including the bacterial genera *Pseudomonas*, *Pantoea*, and *Xanthomonas*, and the fungal genera *Fusarium*, *Humicola*, and *Mortierella*. An ice-nucleating strain of the fungal genus *Penicillium* was also recovered from a volumetric air sampler at the study site. This work expands our knowledge of the scavenging properties of rainfall, and suggests that at least some ice nucleators in natural precipitation events may have been scrubbed from the atmosphere during rainfall, and thus are not likely to be involved in precipitation.

Introduction

Scavenging is the process of particles being removed from the atmosphere by precipitation (Seinfeld and Pandis 2012). The dynamics of particle scavenging during rainfall are determined by a series of complex interactions among raindrop size and intensity, particle size, and relative humidity (Chate and Pranesha 2004, Chate *et al.* 2003). Larger droplets experience vortices that dissipate more quickly relative to smaller droplets, thus reducing the chance of wake collection (Engelmann 1965). Inertial impaction of particles and wake effects allow for collection of larger atmospheric particles (Engelmann 1965). Scavenging increases with rainfall intensity (Chate 2005) and particle size (Chate *et al.* 2007). Little is known about the fundamental processes of particle entrainment in droplets falling at near terminal velocities (Flossmann *et al.* 1985, Pruppacher and Klett 1997, Rodhe and Grandell 1972), and empirical data are lacking to demonstrate how these processes might contribute to precipitation dynamics across a region. Research is needed to examine raindrop dynamics during natural descent and at relevant (near terminal) velocities, and to study fundamental processes associated with the entrainment of microorganisms in raindrops during deposition.

Pure water droplets in clouds can persist in a supercooled state to temperatures as low as 38°C before freezing (Murray *et al.* 2012, Petters and Wright 2015, Rosenfeld and Woodley 2000). Metastable droplets of pure water in the upper troposphere freeze into ice crystals homogeneously below 38 C. At temperatures warmer than 38 C, ice crystals are formed by heterogeneous ice nucleation and can be initiated by an entity acting as an ice nucleating particle, or a collection of ice nucleating particles (Vali *et al.* 2015). A field study of scavenged aerosols, both natural and anthropogenic, captured various particle sizes from rainfall and confirmed the presence of particles that ranged from 0.013 to 0.75 mm (Chate and Pranesha 2004). Heterogeneous nucleation can be

catalyzed by immersion, condensation, contact, and deposition nucleation (Chou *et al.* 2011). High mineral concentrations in the atmosphere can play a significant role in shifting ice nucleation from a homogeneous to a heterogeneous event, and have implications on atmospheric conditions at the cirrus cloud level (Chou *et al.* 2011).

Natural bioaerosols, including the bacterium *Pseudomonas* syringae, cause ice nuclei formation at warmer temperatures than mineral dust (Mohler *et al.* 2008, Morris *et al.* 2013, Murray *et al.* 2012). Microbial ice nucleators in clouds have the potential to initiate heterogeneous ice nucleation and contribute to the water cycle through precipitation (Delort *et al.* 2017, Morris *et al.* 2014). At the Jungfraujoch observatory, a higher abundance of ice nucleating particles was measured prior to significant rainfall, when a moist air mass occurred concurrently with high wind speed (Stopelli *et al.* 2015). Biological ice nucleators (sensitive to heat and/or lysozyme treatment) have been found to be prevalent in rain and snow collected in locations on different continents (Christner *et al.* 2008, Monteil *et al.* 2014, Morris *et al.* 2008, Sands *et al.* 1982). *Pseudomonas* syringae represented over 80% of the culturable, ice-nucleating prokaryotes sampled in 23 precipitation events in Virginia over 15 months (Failor *et al.* 2017). The presence of multiple strains of *P. syringae* in different environments suggests that the bacterium interacts with the environment in ways that could influence atmospheric processes (Morris *et al.* 2011, Pietsch *et al.* 2017). Significant movement of *P. syringae* into the atmosphere from terrestrial sources has been demonstrated (Lindemann *et al.* 1982). A handful of fungi have also been reported to be ice nucleators, including different species of Fusarium and Mortierella (Frohlich-Nowoisky *et al.* 2015, Pouleur *et al.* 1992, Richard *et al.* 1996). Other genera of ice-nucleating microorganisms have been reported (Gurian-Sherman and Lindow 1993), but their relative abundance in the

atmosphere and their potential role in atmospheric processes is presently unknown (Morris *et al.* 2013).

We hypothesized that prokaryotic and eukaryotic microbial ice nucleators are scavenged from the atmosphere by rainfall, and thus are not exclusively involved in precipitation. To test this hypothesis, we conducted 33 Simulated Rain Events (SREs) over four different months in two calendar years off the side of the Smart Road Bridge (SRB) in Blacksburg, VA, USA. In each event, sterile water was dispensed over the side of the bridge and recovered in sterile containers following gravitational settling from the bridge to an open fallow agricultural field below (a distance of ~55 m). This work focuses on microbial ice nucleators at 8 C, and does not include non-cultured ice nucleating particles that could influence heterogeneous ice nucleation events at temperatures colder than 8 C. The specific objectives of our work were to: (1) simulate natural rainfall events off the side of a bridge to study the scavenging of microbial ice nucleators from the atmosphere, (2) culture and identify microbial ice nucleators from simulated rain events, and (3) observe the number and size of particles scavenged during simulated rainfall.

Materials and methods

Experimental site

Collection of simulated rain events was conducted at the Virginia Tech Transportation Institute (VTTI) Smart Road Bridge (SRB) in Blacksburg, VA, USA (Fig.1). The SRB extends about 700 m across Ellett Valley, and stands more than 53 m above Wilson Creek and surrounding farmland (GPS location 37.173099, 80.373299). An open, gently sloping, fallow field area below the bridge was utilized for sample collection. There were no physical objects or obstructions between the bridge pylon supports, and the simulated rain fell through the atmosphere from the top of the bridge to the ground without interference. Samples were processed and stored

immediately following collection in a mobile laboratory (an instrumented stepvan) located at the perimeter of the collection site.

Simulated rain events

Simulated Rain Event (SRE) experiments were performed on November 13, 2014, December 12, 2014, April 23, 2015, and June 9, 2015 (Table 1). Autoclaved metal 2-gallon watering cans (Home Depot #878469) were used to dispense simulated rain from the top of the SRB. Commercial spring water (Primo Water Corporation, Winston-Salem, NC, USA) was sterilized and transported in Nalgene 4 L wide mouth round bottles (Nalgene 2121-0010-4l). Six liters of sterile water was poured into autoclaved watering cans for each SRE, and was dispensed over the side of the SRB as shown in Fig. 1. Simulated rain descended from ~55 m above ground level and was collected in 31 gallon galvanized cans (Home Depot #001223296) lined with sterile polypropylene bags (Fisher #01-830E, 122 94 cm), at ~2 m above ground level. The cans were held over the heads of individuals below the bridge coordinating the collection of the simulated rain (Fig. 1). Collection liners were removed immediately following collection inside the mobile laboratory at the field site, and samples were transferred to sterile 1 L, widemouth bottles (Nalgene #2187e0032) and placed on ice.

Control collection events

Control collection events were performed on three of the four collection campaign days. Rain cans with lids were lined with sterile polypropylene bags (identical to the experimental design for the scavenging experiments), and were placed at ground level about 50 m from the perimeter of the SRE collection area. At the start of the pour of an SRE (on top of the bridge), the lid was

removed from control can (at ground level) and exposed to the atmosphere for the duration of the pour (less than 1 min). The lid was placed on the control can once the SRE was complete. The control can was then taken inside the mobile laboratory located at the perimeter of the collection site. A sterile metal 2-gallon watering can (identical to the can used to disperse water at the top of the bridge for the SREs) was used to dispense about 400 mL of sterile water into the control can. This water was dispensed around the interior walls of the lined can in an effort to capture particles that may have landed on the sterile bag of the can, as well as particles that could have been drawn toward the wall of the lined can. The collection liner was immediately removed, and the resulting collection was processed as described previously for the samples from the SREs.

Six control SREs were conducted to determine the average size of the droplets recovered at ground level (data not shown). These control SREs were conducted during two of the four sampling days. Droplets were collected in a liquid nitrogen bath consisting of a 10gallon insulated cooler, a 12-quart steel pail, and a stainless-steel mesh colander with mesh size of 2 mm 2 mm. The simulated raindrops were subsequently recovered as frozen spheres and placed in individual microcentrifuge tubes. These spheres were thawed at room temperature, and droplet volumes (sizes) were determined by pipetting. A total of six control SRE collections were conducted, and the mean droplet size for 203 droplets recovered in the liquid nitrogen bath was 9.42 ± 0.47 mL (standard error of the mean, data not shown). A droplet volume of 9.42 mL is equivalent to a droplet diameter of about 2.6 mm (based on calculations of the volume of a sphere).

Culturing of biological ice nucleators

SRE sample collection volumes were vacuum-filtered in 47 mm diameter filter holder and receiver units (Thomas #300e4100) with 0.22 mm GSWP nitrocellulose membranes (#9004-70-0,

Millipore). Membranes were then placed in 100 mL bottles with stir bars and placed on a stir plate for 10 min with 20filtrate volume to resuspend microbes. Each SRE was plated in triplicate at 20 and 2 concentration on six types of agar (TSA, R2A, and CA plates, each with and without 50 mg/L cycloheximide). Plates were incubated for 3e4 days at room temperature and moved to 4 C during screening for the ice nucleation phenotype. Colony forming units (CFUs) were counted, and photographed at 3e5 days after incubation.

Particle measurements

Particle sizes and counts were measured with a LUNA-II TM Automated Cell Counter Model L40001 with specific volume constant 2.295082 (cat #L4001, Logos Biosystems, Inc. 7700 Little River Turnpike, Annandale, VA 22003, USA). Machine parameters were set to allow for irregular shaped particles. Load volume on individual cell counting slides (cat #L12002, 500 slides) was 10 mL with a 1.5 mL read volume. Particle size data were collected for 26 SREs. Prior to particle measurements, samples were dried down and resuspended in sterile water at 1/2,1/4, or 1/8 of the stored volume. Duplicate reads were recorded for at least three replicate suspensions from each SRE.

Ice nucleation assays

Cultured microbes were transferred with sterile toothpicks to 140 mL of DI water in 96-well plates. Plates were vortexed for 30 s and incubated at 4 C for 1hr. Droplet freezing ice nucleation assays were performed on a PARAFILM® M (Sigma P6543, 20 in. 50 ft) float assembly in a Lauda Alpha RA 12 (LCKD 4908) cooling bath (LAUDA-Brinkmann, LP, Delran, NJ, 08 075) with ethylene glycol coolant fluid (Air gas RAD64000246). Twelve microliter droplets were

loaded in duplicate and the temperature was decreased stepwise every 2 min from 2 C to 12 C, or until frost spontaneously formed on the boat assembly. Microbes in duplicate droplets that froze at 8 C were considered to be putatively ice nucleation active (INA). These putative INA strains identified from the original test were grown individually on their respective media type of original isolation (CA, R2A, or TSA) and stored at 80 C in 20% glycerol. Droplet freezing assays were then repeated for positive strains (duplicate droplets, following the same protocol as the initial test) after re-plating cultures from frozen glycerol stocks (Table 1). Finally, the INA phenotype of positive strains was tested a third time at a standardized concentration 0.2, based on optical density readings measured at a wavelength of 600 nm (OD_{600}), using six droplets held at 8 C for 10 min.

Identification of culturable biological ice nucleators

To identify microorganisms that still displayed the INA phenotype after the rigorous testing described above, samples were grown on plates or in liquid culture and DNA was extracted using components from the Puregene Yeast/Bact. Kit B (Qiagen #1042607) and a modified BioSprint15 DNA Plant Kit (Qiagen #941517) protocol. For prokaryotes, portions of 16S rDNA were amplified using an Eppendorf Mastercycler epGradient machine with 16S primers: 518 forward (50 CCA GCA GCC GCG GTA ATA CG 30) and 1491 reverse (50 ATC GGY TAC CTT GTT ACG ACT TC 30) (Turner *et al.* 1999 and http://www.earthmicrobiome.org/empstandard-protocols/16s/). PCR was performed with GoTaq® Green Master Mix (Promega M712) under the following parameters: 1 cycle denaturation 95 C for 10 min, 5 cycle denaturation 95 for 1 min, annealing 53 C for 30 s, extension 72 C 1min 40 s, 25 cycle 94 C 30 s, 53 C 20 s, 72 C 1 min,1 cycle 72 C 10 min, hold 4 C). For eukaryotes, portions of ITS were amplified using the ITS forward (50 TCC TCC GCT TAT TGA TAT GC 30) and ITS reverse (50 GGA AGT AAA AGT CGT

AAC AAG G 3') primers. Reactions were visualized on a 1% TBE Ethidium Bromide gel and ExoI/rSAP cleanup was performed prior to sequencing (AB1 3730 1 DNA Sequencer, Eton Biosciences, 104 T.W. Alexander Drive, Bldg 4A, RTP, NC 27709, USA). Resulting sequences were trimmed using 4Peaks (Version 1.7) to a QC30 value or higher. Each sequence served as an individual BLAST query against GenBank.

Results

Simulated rain events

Thirty-three simulated rain events (SREs) were conducted at the SRB as shown in Table 1. Fig.1 shows the descent and collection of a calm SRE through an undisturbed column of air. The mean length of time for simulated rain events was 37 s (based on 28 SREs) from the beginning to the end of the pour. The mean recovered volume was 5.8% (350 mL/6000 mL) of the initial pour volume, with a range of 0.4% (23 mL/6000 mL) to 22.0% (1320 mL/6000 mL) (55 me2 m AGL) (Table 1).

CFUs from different types of culture media

Colony forming units (CFUs) from six types of agar plates (CA, R2A, TSA: þ/ cycloheximide, R2A (Reasoner and Geldreich 1985)) were determined after incubation at 22 C for 3e4 days (Fig. 2). Mean CFUs for the original collection ranged from 47 to 87 CFUs/mL across the six media types (Fig. 2). CFUs scavenged across all SREs, ranged from 2 to 267 CFUs/mL per event (Fig. 3). Seven SREs had greater than or equal to 100 CFUs/mL, and these higher concentrations occurred in the late Fall (Nov. 2014) and winter events (Dec. 2014) (Fig. 3).

Particle measurements

Particle measurements to determine particle number and size were performed on a subset of 26 SREs. Scavenged particles were less than or equal to 18.8 mm for all samples tested (Fig. 3), with a manufacturer-stated limit of detection from 3 to 59 mm. Mean particle counts/mL ranged from 187 to 6435 particles/mL (Fig. 3). The particle size distribution range for each measured SRE is shown in Fig. 4. These data are included as a confirmation that simulated rain was sufficient to scavenge a range of particles of various sizes from the air column, including natural bioaerosols that could also function as putative ice nucleating particles at 8 C.

Ice nucleation activity of scavenged microbes

Ice nucleation activity was assessed for a random selection of scavenged microbes using a droplet freezing method. Microbes in duplicate droplets that froze at 8 C were considered to be putatively ice nucleation active (INA). Strains putatively demonstrating the INA phenotype were recovered from 94% (31/33) of the SREs following the initial test, and represented about 1.5% (121/ 8331) of the microbes assayed from the SREs (Table 1). For SREs that contained at least one microbe with the putative INA phenotype during the original screen, the total number of INA strains ranged from 1 to 18 (0.4%e6.7% of number screened) (Table 1). The SREs with the highest number of putative INA strains were SRE 23 (10), SRE 26 (11), and SRE 31 (18).

Putative ice nucleators were subjected to additional droplet freezing assays, and those confirmed through these repeated assays represented 0.4% (34/8331) of the total (Table 1). For SREs that contained at least one microbe with the confirmed INA phenotype during the original screen, the total number of INA strains ranged from 1 to 11 (0.4%e4.1% of number screened) (Table 1). Confirmed ice nucleators were recovered from 45% (15/33) of the SREs (Table 1). The

SREs with the highest number of confirmed INA strains were SRE 26 (4) and SRE 31 (11) (Table 1).

Identification of microbial ice nucleators

Identification of INA strains to the level of genus was determined by amplifying and sequencing portions of prokaryotic (16S) or eukaryotic (ITS) rDNA (Table 2). These included 35 strains; 28 bacteria and 6 fungi (Table 2). An INA fungal strain (E5BVS) was also identified on June 9, 2014 from a Burkard volumetric sampler operating on the ground upwind of the collection site with a collection orifice about 1 m AGL (data not shown). Genera were assigned for each strain based on top hits in Genbank, with the exception of strain B423 (*Pantoea*). The top hit in Genbank for B423 was a strain of *Curtobacterium*, with other top hits matching *Pantoea*. Additional sequence alignments with known *Curtobacterium* and *Pantoea* strains indicated strain B423 was of the genus *Pantoea* (data not shown).

Bacteria listed in Table 2 were confirmed using a standardized dilution and a series of droplet freezing assays with repeat tests of six droplets. Fungi listed in Table 2 were confirmed with repeat tests of eight droplets containing mixtures of fungal tissue (hyphae, spores, etc.). For the six INA fungi scavenged from the atmosphere, four were strains of the genus Fusarium, and two were strains of the genera *Humicola* and *Mortierella* (Table 2). An ice-nucleating strain of Penicillium was also recovered from a volumetric air sampler at the study site (Table 2, E5BVS). The genus *Pseudomonas* was the top hit with 79% (22/28) of the confirmed bacteria. Four strains of INA bacteria were of the genus *Pantoea*, and one was identified as *Xanthomonas* (Table 2).

Discussion

Samples of precipitation collected at or near the surface of the Earth represent a cumulative sample of abiotic (not derived from living organisms) and biotic (derived from living organisms) constituents both in and below clouds (Christner *et al.* 2008). The bioprecipitation hypothesis (Sands *et al.* 1982) implies that biological ice nucleators in rain collections are active drivers of precipitation processes in clouds. Here, we show that at least some ice nucleators in natural precipitation events may be scavenged from the atmosphere during rainfall, and thus are not likely to be involved in precipitation. To our knowledge, this is the first detailed study of scavenging of biological ice nucleators from the atmosphere. This work revisits observations of variability in concentrations of ice nucleating particles in precipitation samples (reviewed by Petters and Wright 2015), and raises new questions about the scavenging of biological ice nucleators during precipitation.

We screened over 8300 microbes that were scavenged from the atmosphere in 33 SREs and assayed for the ice nucleation active (INA) phenotype at 8 C. The ability to repeat SRE collections over four unique calendar dates at the same location allowed for a consistent spatial sampling set with a variable time frame. CFUs from the SREs ranged from 2 to 267 CFUs/mL, with a mean of 47e87 CFUs/mL. CA and TSA media types have been used previously to compare concentrations of microbes in soil at various incubation temperatures (Vieira and Nahas 2005). Microbial counts were reported as CFUs x 10^6/g of dry soil (Vieira and Nahas 2005), supporting the viability of environmentally collected samples on similar media types. Our experimental values of 2e267 CFUs/mL are similar to CFUs collected near undeveloped (10 CFUs/mL) and developed (186 CFUs/mL) watersheds along coastal waters in California (Dwight *et al.* 2011). Seven SREs had mean CFUs/mL greater than or equal to 100 (21%). Interestingly, these higher values only

occurred in the late Fall (Nov. 2014) and winter events (Dec. 2014). Since local sources of inoculum were not likely to be operative during these cold months, we speculate that microbes scavenged during these time periods may have come from distant sources (Lin *et al.* 2014).

Concentrations of scavenged particles from the SREs ranged from 187 to 6435 particles/mL. Our observed particle size distribution (PSD) range of 4 mme18.8 mm is dominated by particles that are larger than the "Greenfield gap"; the range described in cloud processes to explain increased scavenging efficiency for aerosols with a diameter >1 mm (Greenfield 1957, Loosmore and Cederwall 2004). For particle sizes of 4.3 mm, 9.0 mm,15 mm, and 19 mm, rainfall rate was shown to have more of an effect on scavenging coefficient than particle size (Nicholson *et al.* 1991). Observed particle sizes in that study were correlated with theoretical scavenging coefficients (Nicholson *et al.* 1991). Scavenging efficiency rapidly increases for particle sizes above 10 mm; for particles greater than 13 mm, the scavenging efficiency is greater than 1 (Engelmann 1965). Our observed PSD range of 4 mme18.8 mm (within the limit of detection of our particle counter) is similar to the particle sizes studied by Nicholson *et al.* (1991) in 27 rain experiments, suggesting that the SREs were good proxies for particles scavenged during natural rain events. Nicholsan *et al.* (1991) note that the incorporation of particles into precipitation in-cloud or below-cloud will be linked to the degree of atmospheric mixing and the proximity to the source of particles. Below-cloud ice nucleating particles in a region could be stripped from the atmosphere in a short amount of time, after which in-cloud particles may have a greater contribution to the particle load in rainfall samples collected at the surface of the earth.

Putative ice nucleation active (INA) microorganisms were recovered from 94% (31/33) of the simulated rain events, and represented about 1.5% (121/8331) of the total number of colonies assayed from each event. Those confirmed through these repeated assays represented 0.4%

(34/8331) of the total. Confirmed ice nucleators were recovered from 45% (15/33) of the SREs. The recovery of multiple INA strains of the genus *Pseudomonas* in the atmosphere supports the observation that these bacteria (e.g., *P. syringae*) are ubiquitous in the environment (Monteil *et al.* 2014, Morris *et al.* 2011, Morris *et al.* 2013, Morris *et al.* 2008, Sands *et al.* 1982). The recovery of INA strains of the genera *Fusarium* and *Mortierella* from the atmosphere support previous laboratory and field observations of the ice nucleating potential of these genera (Frohlich-Nowoisky *et al.* 2015, Pouleur *et al.* 1992, Richard *et al.* 1996). The recovery of an INA strain of the genus Humicola was unexpected; members of this genus have been reported in thermophilic environments (Maheshwari *et al.* 2000, Mehta *et al.* 2016, Rahavaram *et al.* 2010), and to our knowledge have not yet been reported as ice nucleators. Additional efforts to examine the distribution and frequency of these INA taxa before, during, and after rainfall, and in different seasons (temporal), could help elucidate the path, life span, and sink for atmospheric microbes. Though the scavenging of microbial ice nucleators from the atmosphere implies a potential connection to precipitation processes, these microbes were not recovered from clouds, and future work is needed to determine their role as active or passive players in precipitation processes.

The discrepancy between the number and taxa of microbes that were INA-positive at the first test and the number and taxa of microbes that were confirmed to be INA-positive in repeated tests was probably the result of a certain rate of false positives during the first test and/or culture conditions that were not conducive to reinduction of the INA phenotype. Additional tests will need to be performed in the future using several different culture conditions (including different types of growth media and temperature). Such tests may reveal an INA-positive phenotype for some of the microbes that had a INA-negative phenotype in our repeated tests here.

This work informs future efforts to address the ice-nucleating constituents (abiotic and biotic) of individual raindrops. This study has shown that microbial ice nucleators in the atmosphere can be scavenged via rainfall. Microbial ice nucleators recovered in rain samples collected at or near the surface of the earth could have catalyzed heterogeneous ice nucleation in clouds, or they could have simply been scavenged out of the atmosphere. Studies on scavenging are complicated by the fact that heavy rainfall can influence sources of ice nuclei (Bigg *et al.* 2015, Hirano *et al.* 1996) and the generation of aerosols at the surface of the earth (Joung and Buie 2015), in an intricate feedback system operating across broad geographical regions (Morris *et al.* 2014, Morris *et al.* 2017). Future collections could include scavenging during high winds or in the presence of a moist air mass movement, to compare data sets and establish models that could predict the abundance of ice-nucleating particles across a range of freezing temperatures. Additional information on abundance and of local sources of ice-nucleating particles in terrestrial and aquatic environments surrounding our sampling area could help elucidate potential source sink relationships over space and time and during a variety of different atmospheric conditions. Moreover, scavenging studies conducted before, during, and after natural rainfall events could help increase our knowledge of potential sources and sinks of atmospheric ice nuclei linked to precipitation (Petters and Wright 2015). Future work should also include detailed measurements of aerosol concentrations before, during, and after rain to determine scavenging efficiencies of different particle sizes (Chate and Pranesha 2004). Future sampling campaigns should consider simultaneous sampling of precipitation in clouds, in the air column beneath the cloud, and at ground level. Additional work to collect individual raindrops from natural (or simulated) rain events can help us understand the sizes and constituents of precipitation impacting the surface of the earth.

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Tables

Table 1. Scavenging of microbial ice nucleators for 33 simulated rain events (SREs) ~55mabove ground level in Blacksburg, VA, USA. Collections were conducted on November 13, 2014, December 12, 2014, April 23, 2015, and June 9, 2015. Six liters of sterile spring water were dispensed from 55mabove ground level (AGL). Microbial growth counts ranged from 2 to 267 CFUs per milliliter. Particles in the range of 4 mM to 18.8 mM were counted for a subset of SREs (#6e11, #14e33). Culturable microbes scavenged from the atmosphere were screened for ice nucleation activity by a droplet freezing method. A total of 8331 microbes was screened, and in the first (orig) test,121 (1.4%) strains froze droplets at -8C. Putative ice nucleators were subjected to additional droplet freezing assays, and those confirmed through these repeated assays represented 0.4% (34/8331) of the total (rep).

Simulated Rain Event #	Date	Start Time	Pour Time (s)	Volume (mL)	% Recovered	CFU/mL	Particles/10 mLs	Particle Size uM	# Strains Tested	# IN + (orig)	% IN + (orig)	# IN + (rep)	% IN + (rep)
1	13-Nov-2014	14:35	–	500	8.3	76	–	–	267	2	0.7	2	0.7
2	13-Nov-2014	14:48	–	75	1.3	76	–	–	267	1	0.4	0	0.0
3	13-Nov-2014	15:05	–	205	3.4	94	–	–	267	4	1.5	2	0.7
4	13-Nov-2014	15:35	–	220	3.7	267	–	–	267	3	1.1	0	0.0
5	13-Nov-2014	15:47	–	220	3.7	81	–	–	267	3	1.1	0	0.0
6	12-Dec-2014	13:49	32	338	5.6	100	19	8.4	267	3	1.1	0	0.0
7	12-Dec-2014	14:11	33	250	4.2	153	56	8.5	267	2	0.7	0	0.0
8	12-Dec-2014	14:17	31	390	6.5	109	67	8.3	267	1	0.4	0	0.0
9	12-Dec-2014	14:31	30	260	4.3	231	71	7.4	267	0	0.0	0	0.0
10	12-Dec-2014	14:41	28	452	7.5	53	19	9.6	267	2	0.7	1	0.4
11	12-Dec-2014	14:51	30	468	7.8	24	27	8.9	267	0	0.0	0	0.0
12	12-Dec-2014	15:00	31	23	0.4	105	–	–	267	1	0.4	0	0.0
13	12-Dec-2014	15:09	36	48	0.8	182	–	–	267	2	0.7	2	0.7
14	23-Apr-2015	12:40	32	130	2.2	21	71	11.9	270	1	0.4	0	0.0
15	23-Apr-2015	12:53	34	330	5.5	14	177	6.9	270	7	2.6	1	0.4
16	23-Apr-2015	13:04	31	50	0.8	88	384	9.8	270	1	0.4	0	0.0
17	23-Apr-2015	13:11	31	135	2.3	28	180	11.5	270	3	1.1	1	0.4
18	23-Apr-2015	13:19	44	300	5.0	17	226	8.1	270	2	0.7	0	0.0
19	23-Apr-2015	13:43	49	26	0.4	10	156	8.8	270	1	0.4	0	0.0
20	23-Apr-2015	13:50	31	215	3.6	43	644	8.0	270	3	1.1	0	0.0
21	23-Apr-2015	13:58	32	240	4.0	12	170	7.1	270	1	0.4	0	0.0
22	23-Apr-2015	14:11	33	475	7.9	20	311	7.4	270	2	0.7	1	0.4
23	23-Apr-2015	14:20	39	128	2.1	8	179	8.2	270	10	3.7	1	0.4
24	9-June-2015	13:09	37	168	2.8	9	44	9.1	270	3	1.1	1	0.4
25	9-June-2015	13:17	43	605	10.1	20	39	9.1	270	7	2.6	2	0.7
26	9-June-2015	13:26	35	320	5.3	33	76	7.8	270	11	4.1	4	1.5
27	9-June-2015	13:38	41	610	10.2	5	406	7.6	270	9	3.3	2	0.7
28	9-June-2015	13:56	41	680	11.3	18	21	5.9	270	4	1.5	2	0.7
29	9-June-2015	14:29	45	1320	22.0	5	29	6.3	270	3	1.1	1	0.4
30	9-June-2015	14:38	45	570	9.5	12	60	7.4	270	5	1.9	0	0.0
31	9-June-2015	14:47	47	202	3.4	41	42	7.8	270	18	6.7	11	4.1
32	9-June-2015	14:55	49	930	15.5	6	56	8.7	270	4	1.5	0	0.0
33	9-June-2015	15:04	35	690	11.5	2	106	10.9	270	2	0.7	0	0.0
Mean			**37**	**351**	**5.8**	**59**	**140**	**8.4**		**4**	**1.4**	**1**	**0.4**
Total										**121**	–	**34**	–

Simulated Rain Event #	Date	Start Time	Pour Time (s)	Volume (mL)	% Recovered	CFU/mL	Particles/10 mLs	Particle Size uM	# Strains Tested	# IN + (orig)	% IN + (orig)	# IN + (rep)	% IN + (rep)
Control 1	12-Dec-2014	13:49	32	360	6.0	1	–	–	26	0	0.0	0	0.0
Control 2	23-Apr-2015	12:53	34	300	5.0	0	–	–	10	0	0.0	0	0.0
Control 3	23-Apr-2015	14:20	39	300	5.0	0	–	–	10	0	0.0	0	0.0
Control 4	9-June-2015	13:38	41	300	5.0	0	–	–	11	0	0.0	0	0.0
Control 5	9-June-2015	14:55	49	300	5.0	24	–	–	270	0	0.0	0	0.0

Table 2. Identification of confirmed ice-nucleating microorganisms (28 bacteria, and 7 fungi) recovered from simulated rain events (SREs) and a ground-based air sampler (E5BVS). Bacteria listed were confirmed using a standardized dilution and a series of droplet freezing assays. Fungi listed were confirmed with repeat tests of eight droplets containing mixtures of fungal tissue (hyphae, spores, etc.). Sequence-assisted identification was determined using 16S (prokaryotes) or ITS (eukaryotes) primers.

Strain ID	Genus	SRE	Collection	Genbank ID
B101	*Pantoea*	SRE01	13-Nov-2014	KX874614
B102	*Pantoea*	SRE03	13-Nov-2014	KX874615
B120	*Xanthomonas*	SRE03	13-Nov-2014	KX874616
B206	*Pseudomonas*	SRE10	12-Dec-2014	KX874617
B208	*Pantoea*	SRE13	12-Dec-2014	KX874618
B209	*Pseudomonas*	SRE13	12-Dec-2014	KX874619
B321	*Pantoea*	SRE22	23-Apr-2015	KX874620
B406	*Pseudomonas*	SRE25	9-Jun-2015	KX874621
B411	*Pseudomonas*	SRE26	9-Jun-2015	KX874622
B417	*Pseudomonas*	SRE26	9-Jun-2015	KX874623
B419	*Pseudomonas*	SRE26	9-Jun-2015	KX874624
B421	*Pseudomonas*	SRE26	9-Jun-2015	KX874625
B423	*Pantoea*	SRE27	9-Jun-2015	KX874626
B424	*Pseudomonas*	SRE27	9-Jun-2015	KX874627
B431	*Pseudomonas*	SRE28	9-Jun-2015	KX874628
B434	*Pseudomonas*	SRE28	9-Jun-2015	KX874629
B437	*Pseudomonas*	SRE29	9-Jun-2015	KX874630
B444	*Pseudomonas*	SRE31	9-Jun-2015	KX874631
B445	*Pseudomonas*	SRE31	9-Jun-2015	KX874632
B449	*Pseudomonas*	SRE31	9-Jun-2015	KX874633
B450	*Pseudomonas*	SRE31	9-Jun-2015	KX874634
B453	*Pseudomonas*	SRE31	9-Jun-2015	KX874635
B455	*Pseudomonas*	SRE31	9-Jun-2015	KX874636
B456	*Pseudomonas*	SRE31	9-Jun-2015	KX874637
B457	*Pseudomonas*	SRE31	9-Jun-2015	KX874638
B458	*Pseudomonas*	SRE31	9-Jun-2015	KX874639
B459	*Pseudomonas*	SRE31	9-Jun-2015	KX874640
B460	*Pseudomonas*	SRE31	9-Jun-2015	KX874641
B124	*Fusarium*	SRE01	13-Nov-2014	KX874642
B304	*Mortierella*	SRE15	23-Apr-2015	KX874643
B312	*Humicola*	SRE17	23-Apr-2015	KX874644
B323	*Fusarium*	SRE23	23-Apr-2015	KX874645
B402	*Fusarium*	SRE24	9-Jun-2015	KX874646
B410	*Fusarium*	SRE25	9-Jun-2015	KX874647
E5BVS	*Penicillium*	SRE31B	9-Jun-2015	KX874648

Figures

Figure 1. Images of a Simulated Rain Event (SRE) dispensed over the side of the Smart Road Bridge (SRB) at the Virginia Tech Transportation Institute (VTTI), in Blacksburg VA. Six litersof sterile water were dispensed for each SRE from a height of 55 m. Top panel shows an autoclaved rain can in the process of dispensing sterile water over the edge of the bridge. Bottom left shows the entire SRE as a 55 m column. Bottom right shows the collection of the SRE in sterile bags inside rain cans.

Figure 2. Colony forming units (CFUs) on six different types of media (CA, R2A, and TSA, with and without 50 mg/L cycloheximide). For the 33 simulated rain events (SREs), mean CFUs were determined for each of the six types of media and ranged from 46 to 87 CFUs/mL. Low CFUs for each of the six different media types was 2e3 CFUs/mL, while the highest means for a single SRE were 341 on R2A +cyc, and 403 on R2A -cyc, respectively.

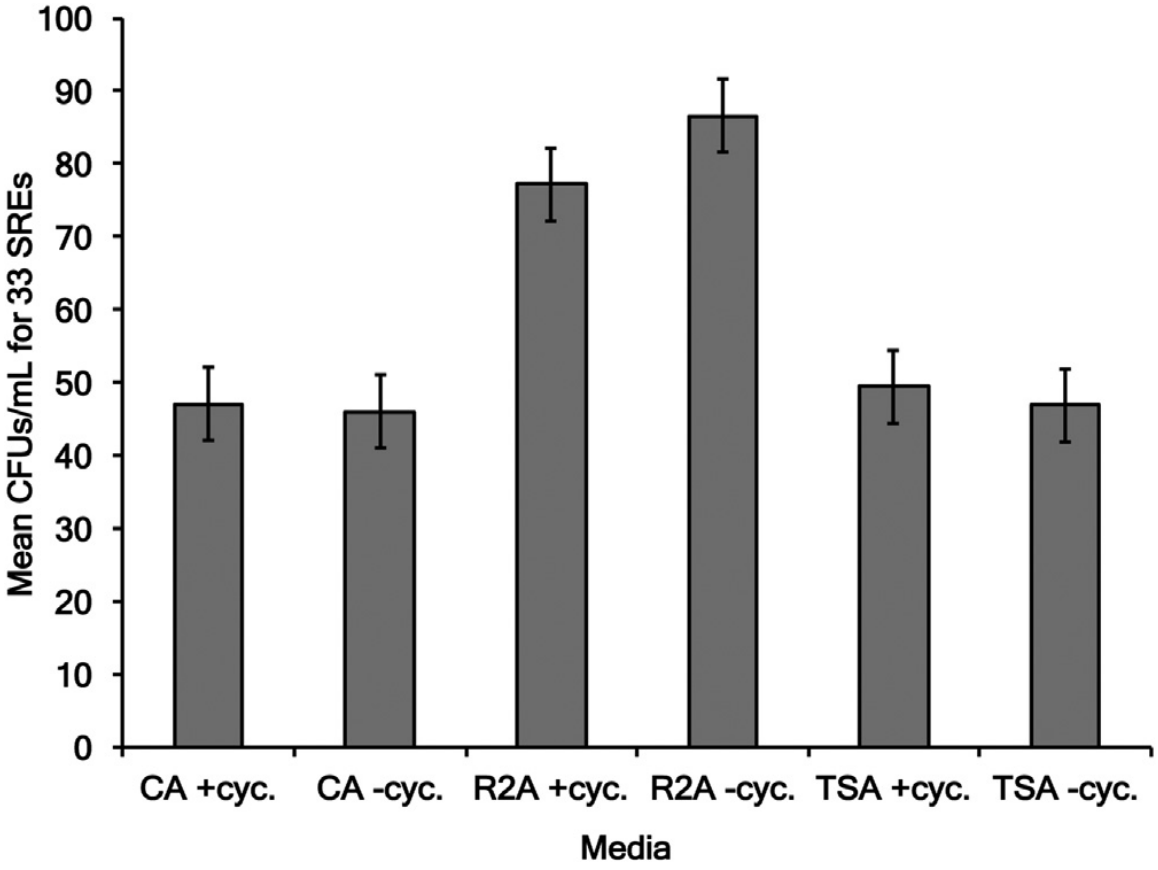


Figure 3. Mean colony forming units (CFUs) for six media types for 33 simulated rain events (SREs). CFUs ranged from 2 to 267. The number of particles/10 mL for the corresponding SREs ranged from 19 to 644. Standard error bars show high variability in cultured CFUs, compared to particle counts measured with the LUNA-II particle counter (range 3 mMe59 mM).

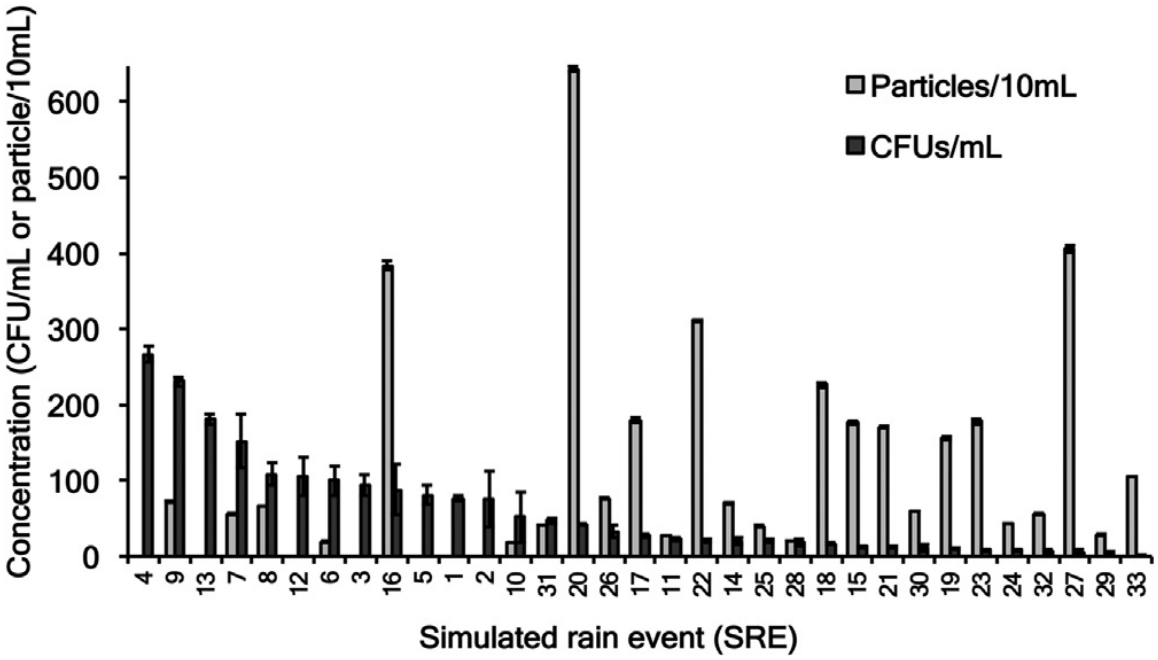
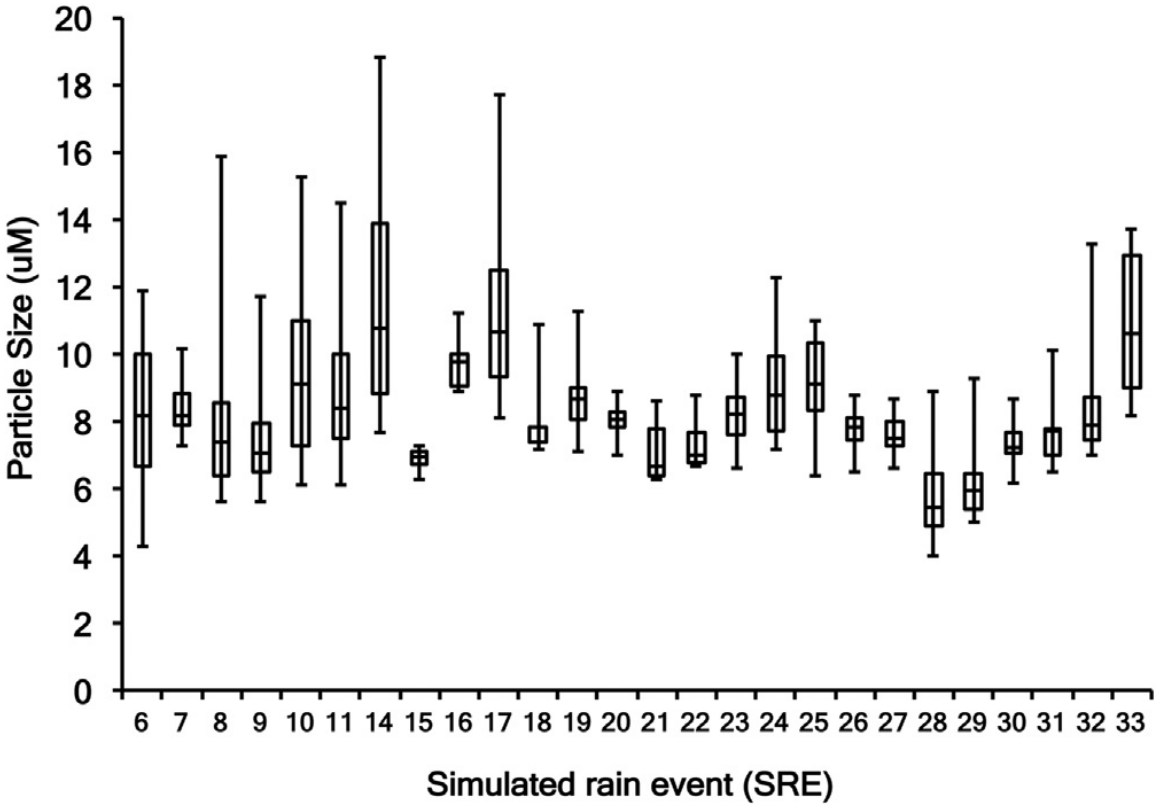
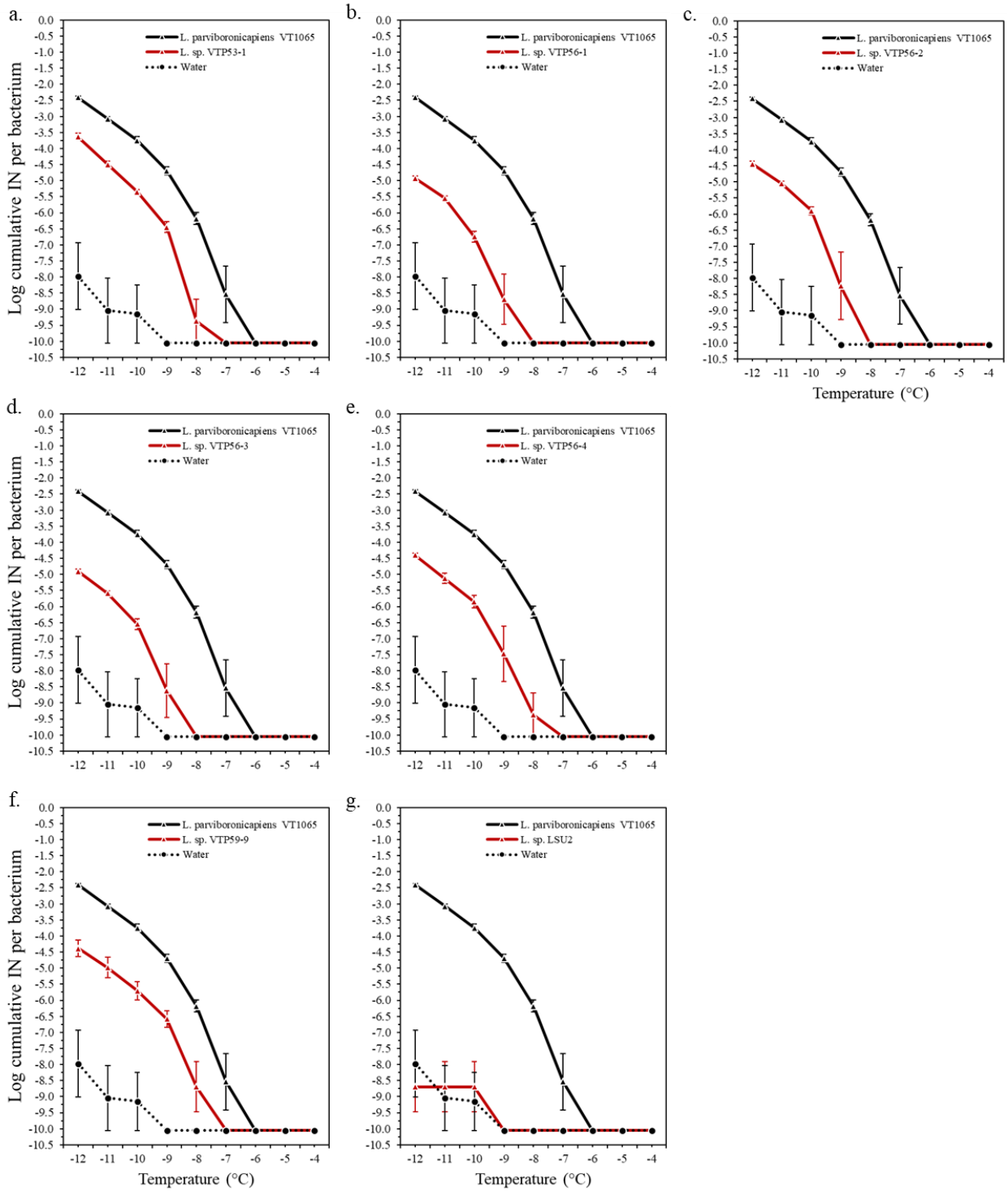


Figure 4. Mean particle sizes scavenged from 26 simulated rain events (SREs). Particle sizes ranged from 4 mM to 18.8 mM. SREs 8, 9, 18, 19, 29, 31, 32 had outliers above the box plot, and SRE20 had an outlier below the box plot.



Appendix D: Ice nucleation activity of environmentally isolated *Lysinibacillus* strains

Figure 1. Ice nucleation spectra of Lysinibacillus strains isolated from the environment and grown on R2A for 48hr at 28°C. For each temperature, average values were calculated from at four independent cultures and bars correspond to standard errors. The minimum detection threshold obtained for Lysinibacillus spp. was 10−10.05 IN per bacterium. Each strain ▲ was compared to Lysinibacillus parviboronicapiens VT1065 ▲ and a pure water control ●. (a) Ice nucleation spectra of Lysinibacillus acetophenone JC23 and L. sp. VTP53-1 10a, (b) L. VTP56-1, (c) L. sp. VTP56-2, (d) L. VTP56-3, (e) L. sp. VTP56-4, (f) L. sp. VTP59.9, and (g) L. sp. LSU2.


a.
b.
c.
d.
e.
f.
g.
Log cumulative IN per bacterium
Temperature (°C)
L. parviboronicapiens VT1065
L. sp. VTP53-1
L. sp. VTP56-1
L. sp. VTP56-2
L. sp. VTP56-3
L. sp. VTP56-4
L. sp. VTP59-9
L. sp. LSU2
Water