Turning Into ICE

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Teaching biological ice nucleation and the global water cycle

This article describes an interdisciplinary unit in which students explore biological *ice nucleation*—by particles that cause water to freeze at temperatures above -38°C—through the lens of the microbial ice nucleator *Pseudomonas syringae*. Such microorganisms are arguably some of the most important ice nucleators at temperatures above -15°C (Murray et al. 2012). The bacterium *P. syringae* has been found in clouds, rain, and snow. It produces an outer membrane protein functioning as an ice nucleus (Wolber et al. 1986; Morris et al. 2008; and Monteil, Bardin, and Morris 2014) and can initiate precipitation (Morris et al. 2014) (Figure 1, p. 38).

This activity, which aligns with the *Next Generation Science Standards* (see box, p. 43), exposes students to the global water cycle, highlighting the importance of critical and ethical thinking at the intersection of biology and engineering. Furthermore, the activity is designed to

- introduce students to principles of ice nucleation and allow them to recognize the diversity of ice-nucleating particles;
- allow students to recognize how interdisciplinary research can address complex problems across multiple fields of study, using ice nucleation and its relationship to atmospheric processes as an example;
- have students consider principles of experimental design, including the formulation of testable hypotheses and the inclusion of appropriate positive and negative controls in developing an ice nucleation test;
- have students design and conduct an ice nucleation experiment with known and unknown samples of undetermined ice nucleation activity; and
- highlight connections between systems of different scale such as microbiology (ice nucleation) and the global water cycle (bioprecipitation).

An interdisciplinary approach

The National Science Foundation (NSF) has called for interdisciplinary research approaches to environmental education (Vincent and Focht 2011). Ice nucleation and bioprecipitation are ideal systems for an interactive interdisciplinary curriculum that ranges from the molecular scale (ice nucleation protein) to a global scale (water cycle). Students consider and discuss new research, rather than going through prescribed lab exercises. The concept of a bacterium causing water to freeze increases student interest. Most students will be surprised to learn that pure water may stay in a liquid form down to about -38°C.

Part 1: The discovery of microbial ice nucleation (~5 minutes)

A plant pathology group discovered ice nucleation by *P. syringae* in the 1970s, noticing that plants infected with northern corn leaf blight showed increased frost damage. Considerable investigation revealed the causal agent of the increased frost damage (Lee, Warren, and Gusta 1995). In a story format, walk students through the discovery of biological ice nucleation without mentioning that *P. syringae* is the freezing agent, so that students can think about this as an "unsolved mystery" (see "On the web").

Part 2: Ice nucleation demonstration (~15 minutes)

Provide a demonstration of the ice nucleation phenomenon. First, place a glass bottle containing 100 ml of sterile nanopure water into an Alpha 12 cooling bath at -8°C (see Figure 2 for a supplies list). Next, add 4 ml of room-temperature water containing a single strain of ice+ *P. syringae* into a bottle and swish it around. The suspension in the bottle instantly freezes.

Part 3: Ice nucleation and bioprecipitation (~30 minutes)

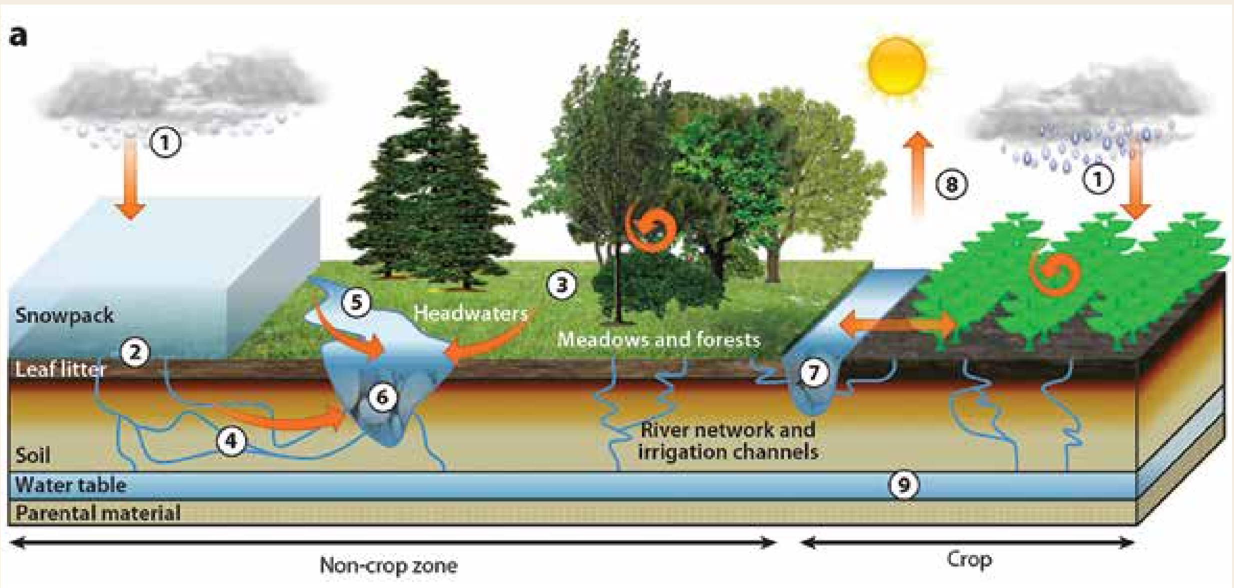
Ask students to comment on the ice nucleation demonstration and make connections to complete the story they heard in the first part of the lesson. This lecture also discusses the concepts of ice nucleation; cloud formation via ice nucleation; and *bioprecipitation*, the idea that ice+ bacteria could make rain (Sands et al. 1982).

Part 4: Guided inquiry (~75 minutes)

In the fourth part of the unit, we present experimental design concepts to students. We divide students into groups of four and conduct a droplet-freezing ice nucleation test. Students test 10 different ice+ and ice- samples from a variety of organic and inorganic sources (Figure 3, p. 40). The ice- strain

FIGURE 1

The bacterium *Pseudomonas syringae* has been found in clouds and snow (1), snowpack (2), leaf litter and grass (3), subsurface water (4), rivers and lakes (5), epilithic biofilms (6), irrigation water from rivers (7), aerosolized in the atmosphere (8), and groundwater (9).



of *P. syringae* is unable to freeze water at higher temperatures (-8°C) like the ice+ strains, likely due to lack of production of the membrane surface protein. Students also collect an environmental sample outside the classroom in 50 ml conical tubes containing 25 ml of water. (**Safety note:** Although the two strains of *P. syringae* are not known to be pathogenic to plants and do not infect humans, students should wear appropriate personal protective equipment, including gloves and safety goggles, and use appropriate hygiene practices.)

The students are given handouts to guide them through their experimental design (see "On the web"). The students select three of the 10 samples, plus sterile water as a negative control, and write hypotheses about the expected freezing temperatures of the samples. Droplets of 12 µl of each sample are loaded onto Parafilm M and placed in the cooling bath. We add a fluorescein disodium salt dye to the sample to visualize freezing, watching it change from green (liquid) to yellow (frozen) (Figure 4, p. 41). The students watch for approximately 30 minutes while the bath cools from -2° to -12°C, recording the temperature at which the droplets freeze.

Part 5: Discussion (~25 minutes)

When we conducted this activity, after entering the freezing temperatures into an Excel spreadsheet (see "On the web"), we projected them on a screen for the whole class to see. We discussed the students' ice nucleation test results, including expected and unexpected results, along with ideas for experiment improvements (see students' responses "On the web"). Students came up with new hypotheses, followed by a discussion period on ethics of human manipulations of the water cycle.

Students discussed the positive and negative impacts of human intervention, providing an opportunity to illustrate how nearly every aspect of biology requires ethical considerations. Students raised several unexpected negative consequences, including how increasing rain by cloud seeding, a form of weather modification, could affect native flora and fauna. It was also suggested that manipulations could be used with bad intentions, such as deliberately spreading a pathogenic bacterium or purposely increasing severe weather events to cause harm in certain areas of the world.

Part 6: Processing outdoor environmental samples (post lesson)

Of the two strains of *P. syringae* given to the students, one is ice+ and the other is ice-, allowing students to see both phenotypes of *P. syringae.* The students identify environmental samples that have some of the warmest freezing temperatures (Figure 5, p. 41). Five of the six samples freeze, and surprisingly, the freezing temperatures are the warmest observed, with the exception of Snomax, an ice-nucleating protein often used by ski resorts to improve snowmaking operations. Such warm temperatures suggest a potential link to microbial ice nucleation, leading to follow-up experiments in the Schmale lab at Virginia Tech, where we isolated and tested bacteria from these samples for ice+ activity. We find ice-nucleating bacteria from every outdoor sample, except

FIGURE 2

Estimated cost of the supplies for this unit.

Item	Vendor	Item #	Quantity needed	Cost for one group of students
Professional cooling bath Alternative: Ethanol/dry Ice cooling bath (metal pan with lid, 70% ethanol, and dry ice)	Lauda YBM Home	Alpha 12 2403	1 1	$2,585 $30
96 well plates (case of 100)	Sigma	CLS9018	1	$360
500 ml bottles (pack of four)	Sigma	CLS1399500	1	$55
50 ml conical tubes (case of 500)	Fisher	05-539-5	1	$250
2 ml Eppendorf tubes (pack of 500)	Fisher	05-408-138	1	$70
Flourescein, disodium salt (100 g)	Fisher Acros	173241000	1	$23
Parafilm® M (4 X 125')	Sigma	P7793	1	$60
10-100 µL pipette for each group	Cole Parmer	SC-07859-07	1	$181
Pipet tips 20-200 µl	Cole Parmer	UX-07909-19	100	$28
100-1000 µl pipette (optional)	Cole Parmer	SC-07859-11	1	$181
Pipet tips 100-1000 µl (optional) (1000)	Cole Parmer	UX-07909-21	100	$36.50

sample A (mulch), with 23% of all the bacteria tested being ice+. These results are shared with the biology teacher, co-author Cindy Bohland. The students are pleased to know that they have found ice nucleators, and there is a sense of excitement and investment in being part of science.

Reflections and assessment

Before sharing their thoughts in a group discussion, students document their learning through written responses, providing conclusions, limitations, and future improvements to the experiment (see lesson evaluation sheet "On the web"). Students not only mention ideas related directly to the material presented but also relate ideas beyond the immediate application. During the discussion on impacts of manipulating ice nucleation, for example, some students make the less-direct connection that altering soil salinity could affect species diversity.

Students see how different disciplines would approach one problem and focus on different aspects of such a large system. For example, molecular biologists would study the ice nucleation protein, computational modelers would look at predictive patterns, and atmospheric scientists would be interested in applications to weather patterns.

The most successful parts of the unit are having the students collect unknown samples outdoors and recording the data from the ice nucleation test in a dynamic spreadsheet. However, this part of the unit could still be improved upon. First, students could design their own controls for the experiment. Second, many of the students would have benefited from a short tutorial on pipetting techniques. Third, the worksheets could include a section for students to write their individual thoughts about the ethical issues surrounding biological ice nucleation.

Modifications

This unit could be modified in several ways to accommodate different classroom needs. If a cooling bath is not an option, a lower-cost method could be implemented using a stainless steel bucket or pan containing 70% ethanol and dry ice. Cover the bottom of the container with small pieces of dry ice, and carefully poor the ethanol over the dry ice. Add additional pieces of dry ice to achieve the desired temperature (around -10°C). Make sure to wear gloves and goggles. With this method, the

FIGURE 3

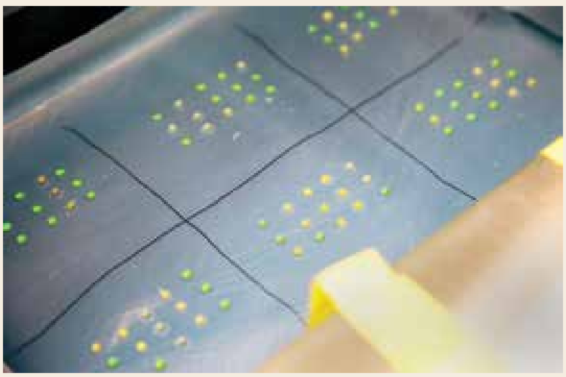
Ice+ and ice- samples.

Sample Number	Sample Name	Description	Concentration	Item #	Source
Control	Sterile deionized water				
U	Sample collected from outside				
1	Tap water				Lab
2	Mineral water				Grocery store
3	Feldspar	Inorganic, igneous rock, mineral	0.0005 g/ml		READE advanced materials
4	Montmorillonite	Inorganic, clay	0.5 mg/ml	69866	Sigma
5	*P. syringae* strain 1 (strain # 642)	Bacterium isolated in Blacksburg, Virginia	Suspension grown in Tryptic Soy Agar (TSA) for 24 hours		Vinatzer lab, Virginia Tech
6	*P. syringae* strain 2 (strain # 892)	Bacterium isolated in Blacksburg, Virginia	Suspension grown in TSA for 24 hours		Vinatzer lab, Virginia Tech
7	Snomax	Inert bacteria	0.05 mg/ml		Snomax International
8	Fennell pollen	Pollen	10 mg/ml	1245	My Spice Sage
9	Bee pollen	Pollen	0.3 mg/ml	618	Brushy Mountain Bee Farm
10	Button mushroom spores	Fungus	Unknown; spores from one mushroom cap/2 ml		Grocery store

FIGURE 4

Environmental samples collected outside the classroom by students.

Ice nucleation test showing yellow (frozen) droplets and green (liquid) droplets.



BOTH PHOTOS BY CHRISTINA O'CONNOR, RANDOM FOUND OBJECTS.

temperature will gradually rise, so the droplets must be placed on the bath as soon as the ethanol starts "boiling" at a slow and steady rate. One limitation is that the temperature can't be controlled as precisely as with a cooling bath.

Conclusion

This unit can increase students' interest in STEM fields and train them in the methods used in STEM careers. Even for students not destined for STEM careers, being aware of current research topics with global effects will make them better citizens (Jacobson and Wilensky 2006). ■

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FIGURE 5

The ice nucleation test results from students' outdoor environmental samples and from bacteria isolated from the samples.

Description of sample	Mean freezing temperature	Percentage of bacterial suspensions that froze at -9°C	Morphology of samples that froze
Mulch	-7.8°C	0.0% (0/9)	
Dandelion seed/fruit	-5.2°C	12.5% (1/8)	white
Dandelion flower	-8.4°C	37.5% (3/8)	yellow, orange
Mud	-7.7°C	0.0% (0/8)	
Tree leaf	Did not freeze	83.3% (5/6)	white, yellow
Dandelion leaves	-8.8°C	23.5% (4/17)	yellow, white, pink

On the web

Lecture on the history of microbial ice nucleation: *http://youtu.be/qPEuWrTbWbE*

Ice nucleation demonstration: *http://youtu.be/648QSyHaNII*

Ethanol bath: *http://wiki.bugwood.org/Bacterial_ice_nucleation*

Students' responses, ice nucleation handout, spreadsheet, and lesson evaluation sheet: *www.nsta.org/highschool/connections.aspx*

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Connecting to the *Next Generation Science Standards* (NGSS Lead States 2013).

Standards
HS-ESS2 Earth's Systems
HS-ETS1 Engineering Design

Performance Expectations
The chart below makes one set of connections between the instruction outlined in this article and the *NGSS*. Other valid connections are likely; however, space restrictions prevent us from listing all possibilities. The materials/lessons/activities outlined in this article are just one step toward reaching the performance expectations listed below.

HS-ESS2-2. Analyze geoscience data to make the claim that one change to Earth's surface can create feedbacks that cause changes to other Earth systems.

HS-ETS1-3. Evaluate a solution to a complex real-world problem based on prioritized criteria and trade-offs that account for a range of constraints, including cost, safety, reliability, and aesthetics, as well as possible social, cultural, and environmental impacts.

Dimension	Name and *NGSS* code/citation	Specific connection to classroom activity
Science and Engineering Practices	**Analyzing and Interpreting Data** • Analyze data using tools, technologies, and/or models in order to make valid and reliable scientific claims or determine an optimal design solution. (HS-ESS2-2)	Students perform an ice nucleation test to determine the best ice nucleator, using known and unknown samples. The experiment design is discussed to evaluate strengths and weaknesses.
	Constructing Explanations and Designing Solutions • Evaluate a solution to a complex real-world problem, based on scientific knowledge, student-generated sources of evidence, prioritized criteria, and trade-off considerations. (HS-ETS1-3)	The data the students gathered from the ice nucleation test is used in a discussion of the potential natural and manipulated impacts of of ice nucleators.
Disciplinary Core Ideas	**Earth Material and Systems** • Earth's systems, being dynamic and interacting, cause feedback effects that can increase or decrease the original changes. (HS-ESS2-2)	Students discuss the potential for ice-nucleating bacteria to be part of a positive feedback cycle generating rain.
	Developing Possible Solutions • When evaluating solutions, it is important to take into account a range of constraints, including cost, safety, reliability, and aesthetics, and to consider social, cultural, and environmental impacts. (HS-ETS1-3)	Students discuss manipulations of a bioprecipitation cycle, including negative and positive impacts to various effected groups.
Crosscutting Concepts	**Stability and Change** • Feedback (negative or positive) can stabilize or destabilize a system. (HS-ESS2-2)	Bioprecipitation hypothesis is presented illustrating how organisms as small as bacteria can have a significant part in a global scale feedback cycle.
	Influence of Science, Engineering, and Technology on Society and the Natural World • New technologies can have deep impacts on society and the environment, including some that were not anticipated. Analysis of costs and benefits is a critical aspect of decisions about technology. (HS-ETS1-3)	Human interference in the ice nucleation process is discussed, including possible consequences that were not considered and how testing could determine the probability of negative consequences.