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Food Protection Trends, Vol. 32, No. 7, Pages 384–390

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International Association for Food Protection

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Inhibition of *Listeria monocytogenes* by *Carnobacterium piscicola* in Fresh and Pasteurized Crab Meat

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ABSTRACT

The ability of *Carnobacterium piscicola* to inhibit *Listeria monocytogenes* at different concentration levels, storage times and temperatures was examined. Twenty-five g samples of fresh and previously pasteurized blue crab meat were placed into separate sterile 59 ml soufflé cups, inoculated with 0.1 ml *C. piscicola* at three different concentration levels (2, 4 and 6 log CFU/g) and 0.1 ml (log 2.0 CFU) of *L. monocytogenes* (ratios of 1:1, 2:1 and 3:1 of *C. piscicola* to *L. monocytogenes*). Fresh crab meat and previously pasteurized crab meat was stored at 4°C or 10°C and sampled until aerobic plate count reached ≥ 7 log CFU/g, which was considered spoilage. At each sampling time, crab meat inoculated with both organisms was plated on modified *Lactobacillius* deMan Rogosa Sharpe (pH 7) and modified oxford agar (MOX). Uninoculated crab meat was plated on trypticase soy agar and meat inoculated with only *L. monocytogenes* was plated on MOX. In both fresh and pasteurized crab meat, regardless of the inoculation levels, *L. monocytogenes* and *C. piscicola* followed similar growth trends. *C. piscicola* reduced growth of *L. monocytogenes* in pasteurized crab meat after 14 day at 4°C and 10 day at 10°C ($P < 0.05$).

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INTRODUCTION

Fresh crab meat is a fully cooked product, where the crab meat is "picked" from cooked crabs, packed into plastic containers and sold refrigerated. Bacteria found in fresh crab meat are primarily the result of environmental contamination during the picking and packaging process. In coastal areas, it is not uncommon, or illegal as long as the containers do not state "Fresh Crab Meat," for pasteurized crab meat to be repacked into cups for refrigerated sales to maintain a steady supply of crab meat products throughout the year *(13)*. Crab processors are aware of possible contamination of crab meat by pathogens, preventing contamination by *Listeria monocytogenes* in fresh crab meat (ready to eat; RTE) a high priority. *L. monocytogenes* is a psychrotroph capable of growth at refrigeration temperatures *(22)*. The pathogen is found in a wide variety of fish and shellfish products and can be isolated from different water sources *(18)*. The ability of *Listeria* spp. to persist in the marine environment and fresh water streams, indicates that fish and other aquatic animals can be contaminated in their natural habitat *(14)*. There is a higher incidence of *Listeria* spp. present in chi-

tinous seafood including shrimp, crab and lobster over other seafood varieties due to their ability to uptake the pathogen *(6, 14)*.

According to the Food and Drug Administration's (FDA) Enforcement Reports there were approximately 126 Class I recalls issued in the U.S. from 1987 – 2005 for domestic and domestic/imported RTE seafood products *(14)*. Crab meat or imitation crab meat accounted for 46 of these recalls *(14)* for possible *L. monocytogenes* contamination *(3)*. Moreover, *L. monocytogenes* has been isolated as a contaminate from smoked fish and seafood salad products and dip spreads *(3)*.

Americans want safe, wholesome products with minimal processing or food additives *(5, 7, 20)*. Biopreservation is the use of non-pathogenic microorganisms, and/or metabolites to ensure food safety and extend shelf-life of food *(1, 4, 5, 7, 20)*. Some lactic acid bacteria (LAB) have the ability to produce bacteriocins which are inhibitory towards other bacteria *(5)*. Bacteriocins specifically inhibit Gram-positive bacteria, therefore the addition of LAB that produce bacteriocins to foods may be an attractive option for companies to consider using in order to improve food safety *(1, 19, 20)*.

Bacteriocins are safe additives and a natural alternative to chemical preservation methods *(12, 19)*. The prospect of using a bacteriocin from a LAB producing strain to improve food safety and food quality is a novel approach *(21)*. Products formulated with beneficial bacteria to control undesirable bacteria may appeal to more consumers compared with products containing chemical inhibitors *(5)*. Bacteriocins have been used in fishery products *(2, 8, 17, 21, 23)*, but there is little to no information on bacteriocin use in crab meat products. The purpose of this study was to examine the effect of *Carnobacterium piscicola*, a lactic acid bacterium that produces bacteriocin, on the growth of *L. monocytogenes* in fresh and pasteurized crab meat over time at 4 and 10°C.

MATERIALS AND METHODS

Culture preparation and maintenance of *Carnobacterium piscicola.* Preliminary studies were performed on different strains of *C. piscicola* testing their inhibitory capabilities against *L. monocytogenes*. The strain showing the greatest inhibitory capability was chosen for this study. The *C. piscicola* strain was subjected to an API CH 50 *Lactobacillus* test (bioMérieux, Marcy l'Etoile, France) for strain confirmation. Once verification of the strain was complete, colonies were placed in 100 ml of *Lactobacillus* deMan, Regosa, Sharpe broth (MRS; Acumedia, Lansing, MI) modified with 1 N NaOH to bring the pH to 7.0 ± 0.2 (mMRS). Preliminary studies showed agreement with the literature that growth and bacteriocin production are pH dependent, and therefore modification of pH would yield the best results *(9–11, 15, 16)*. The *C. piscicola* strain was streaked in two successive transfers onto mMRS agar, with incubations at 28°C for 24 h. A colony from the plate was randomly selected and subjected to an API CH 50 *Lactobacillus* test, and purified colonies were then transferred to mMRS broth (Acumedia, Lansing, MI). Ten ml of an overnight culture in mMRS broth was transferred into a solution of 20% glycerol (MP Biomedicals, LLC, Solon, OH) and 80% mMRS broth medium and stored at -70°C until use.

Bacteriocin confirmation. Bacteriocin activity was determined using a spot on lawn technique *(17)*. *Carnobacterium piscicola* was grown in 100 ml of modified mMRS broth for 24 h at 28°C. Following incubation, the culture was stirred and six stab inoculations were made on mMRS plates in a hexagonal pattern and incubated at 28°C for up to 72 h under anaerobic conditions to prevent hydrogen peroxide production. Once growth was visible, the plates were overlaid with approximately 6 ml of Brain Heart Infusion agar (BHI; BBL, Sparks, MD) seeded with approximately 2 log (CFU/ml) of *L. monocytogenes*, and further incubated at 28°C for 24 h. The plates were examined for zones of inhibition against growth of *L. monocytogenes*. Measurements were taken in mm (ruler #15-100-100, Manostat Corp, Switzerland) from the edge to edge of the inhibited zone.

Culture preparation and maintenance for *Listeria monocytogenes*. The *L. monocytogenes* strain used in this study was isolated from raw crabs by a commercial crab meat processing plant on Maryland's Eastern Shore. It was shipped overnight on a TSA slant to the FST department in Blacksburg, VA. Upon arrival, the strain was transferred into 10 ml of tryptic soy broth supplemented with 0.6% yeast extract (TSBYE; BBL, Sparks, MD) and incubated at 10°C for 60–72 h. The culture was streaked onto multiple modified oxford agar plates (MOX; Difco, Sparks, MD) supplemented with Modified Oxford antimicrobial supplement agent (Difco, Sparks, MD) and incubated at 35°C for 48 h. Positive *L. monocytogenes* colonies, established as a grey to green colony surrounded by a brown-black halo, were randomly selected and confirmed using *Listeria* API strips (bioMérieux, Marcy l'Etoile, France). Following confirmation, 10 ml of an overnight culture in TSBYE was transferred into a solution of 60% nutrient broth (Difco, Sparks, MD); 40% glycerol and stored at -70°C until use.

Concentration ratio preparation. For each replication of this study, one cryo-vial of *L. monocytogenes* and one cryo-vial of *C. piscicola* were removed from the -70°C freezer and thawed to room temperature. A 10 µl aliquot from each culture was transferred into 10 ml of broth. The *L. monocytogenes* culture was transferred into TSBYE and incubated at 10°C for 60–72 h; and the *C. piscicola* culture was transferred into mMRS broth and incubated for 24 h at 28°C.

After incubation, each respective culture was dispensed into a separate, sterile 15-ml polypropylene centrifuge tube (Becton Dickinson, Franklin Lakes, NJ). The tubes were centrifuged at 3000 RPM for 10 min in an IEC HN-S centrifuge (Damon/IEC Division, Needham Heights, MA). Following centrifugation, the supernatant was removed and the pellet was washed with 10 ml of sterile 0.1% peptone, and centrifuged for an additional 10 min. The peptone wash was performed a total of 3 times. At the end of the third wash, the centrifuge tube was hand shaken until the pellet was resuspended and subsequent dilutions were made. Three separate suspensions of *C. piscicola* were prepared from the original by performing serial dilutions with 0.1% peptone solution. *Listeria monocytogenes* was washed in the same manner as the *C. piscicola*, but using TSBYE instead of peptone.

Product preparation for fresh and pasteurized crab meat. Fresh and pasteurized crab meat was obtained from a commercial processor and shipped overnight to the FST department in Blacks-

TABLE 1. Growth of *Carnobacterium piscicola* and *Listeria monocytogenes* over time when co-inoculated in fresh crab meat at different ratios (*C. piscicola: L. monocytogenes*; 0:1, 1:1, 2:1, 3:1) and stored at 4°C (n = 9)

Storage time (days)	Mean growth of *C. piscicola* (log CFU/g)[1,2]			Mean growth of *L. monocytogenes* (log CFU/g)[1,2]			
	1:1	2:1	3:1	0:1	1:1	2:1	3:1
0	4.79[a] ± 0.76	4.89[a] ± 0.49	5.58[a] ± 0.26	2.19[a] ± 0.38	2.41[a] ± 0.33	2.18[a] ± 0.25	2.27[a] ± 0.24
2	6.50[a] ± 0.34	6.61[a] ± 0.42	6.82[a] ± 0.14	2.95[a] ± 0.61	3.04[a] ± 0.35	3.08[a] ± 0.30	3.11[a] ± 0.20
4	8.20[a] ± 0.25	7.81[a] ± 0.47	8.16[a] ± 0.85	3.86[a] ± 0.74	3.86[a] ± 0.44	3.48[a] ± 0.37	3.20[a] ± 0.52
6	8.63[a] ± 0.36	8.44[a] ± 0.53	8.47[a] ± 0.64	4.22[a] ± 0.41	4.15[a] ± 0.38	4.18[a] ± 0.53	3.55[a] ± 0.77

[1]Inoculum levels were at ratios of 1:1, 2:1 and 3:1 log CFU of *C. piscicola: L. monocytogenes* (CFU/g). Ratio example = 1:1 was 2 log CFU/ml of both microorganisms; 2:1 was 4 log CFU/ml of *C. piscicola* and 2 log CFU/m of *L. monocytogenes.*
[2a]Means sharing the same lowercase superscript letter within the same row for growth of *C. piscicola* or *L. monocytogenes* are not significantly different ($P > 0.05$)

burg, VA. Three replicate trials of fresh crab meat and two replicate trials of pasteurized were studied, originating from different seasons. Fresh crab meat arrived in FDA approved prime-grade polypropylene plastic tubs with snap lids (Berry Plastics, Evansville, IN). Pasteurized crab meat arrived in 454 g metal cans. In both cases, 25 g of crab meat was transferred from the original packaging into smaller, sterile 59-ml plastic soufflé containers (Monogram, Dixie, Atlanta, GA) and immediately refrigerated at 3°C. Soufflé containers were sterilized prior to use by placing them under UV-C light (30W Germicidal lamp) for 10 min. After all of the crab meat from the original containers was placed into the soufflé containers, they were removed from the refrigerator and placed on the lab bench in no particular order. The soufflé containers were then arbitrarily labeled coinciding with the ratios of *L. monocytogenes* and *C. piscicola* that they would receive and randomly assigned either 4°C or 10°C incubator. Each incubator (4°C and 10°C) had a random array of where the tubs were placed. This was done to minimize variation within the product due to possible temperature difference within the incubator.

Product inoculation. Five different ratios of the *Carnobacterium:Listeria* were tested (0:1, 1:1, 2:1, and 3:1). Inoculation of the two cultures was performed separately. The concentration of *L. monocytogenes* inoculated into the crab was held constant at 2-log CFU/g because it is believed that environmental contamination of *L. monocytogenes* would be a low concentration. *Carnobacterium piscicola* levels varied from 2 – 6 logs. The negative control was uninoculated crab meat and the positive control was inoculated with *L. monocytogenes* and received no *C. piscicola* inoculum. From the prepared inoculum, a total volume of 0.1 ml of each bacterium was dispensed directly over the 25-g portions of crab meat in several different places and mixed thoroughly using a sterile instrument. This was done to achieve concentration of *C. piscicola* of approximately 2, 4, 6, log CFU/g. Each individual container of crab meat was treated, covered and placed into the incubator (either 4°C or 10°C).

Product incubation and sampling. Testing days were designated from the time that the crab meat arrived at Virginia Tech's FST building. Due to the quick spoilage of the fresh crab meat and the lack of bacterial growth in the pasteurized crab meat in preliminary studies, sampling days were different for the fresh compared with pasteurized crab meat. Fresh crab meat held at 4°C was tested at 0, 2, 4, 6, 8, and 10 days or until spoilage (10^7 CFU/g). Fresh crab meat held at 10°C was tested on day 0, 1, 3, 5, 7, and 9 or until spoilage. Pasteurized crab meat held at 4°C, was tested on day 0, 6, 10 14, 18, 22, 26 and 28 or until spoilage. Pasteurized crab meat held at 10°C, was tested on day 0, 6, 10, 12, 14, 18, 22 and 24 or until spoilage. Three samples of each inoculation ratio, at each temperature (4°C and 10°C) were evaluated on each sampling day. Each 25-g sample of crab meat was placed into a sterile 1538 ml stomacher bag (Nasco, Ft. Atkinson, WI) with 225 ml of 0.1% peptone. The sample was homogenized in a Stomacher Lab Blender 400 (Tekmar Co., Cincinnati, OH) for 30 s. Using a sterile, prepackaged pipette (1 ml in 1/100 ml, Fisher Scientific, Pittsburgh, PA), subsequent dilutions were made using 9 ml of 0.1% peptone dilution blanks. Dilutions of the homogenate were taken and spread plated onto corresponding media. MOX was used to enumerate *L. monocytogenes*, TSA for the total plate count and mMRS agar to enumerate *C. piscicola*. MOX and TSA were incubated at 35°C for 24–48 h and mMRS was incubated at 28°C for 24 h. Randomly, colony growth from the mMRS was subjected to API CH 50 tests and colonies from the MOX plates were subjected to *Listeria* API tests.

The pH (Accumet model 15, Denver Instrument Co., Denver, CO) of uninoculated fresh and pasteurized crab meat was taken on the day of arrival to

TABLE 2. Growth of *Carnobacterium piscicola* and *Listeria monocytogenes* over time when co-inoculated in fresh crab meat at different ratios (*C. piscicola: L. monocytogenes*; 0:1, 1:1, 2:1, 3:1) and stored at 10°C (n = 9)

Storage time (days)	Mean growth of *C. piscicola* (log CFU/g)[1,2]			Mean growth of *L. monocytogenes* (log CFU/g)[1,2]			
	1:1	2:1	3:1	0:1	1:1	2:1	3:1
0	4.27[a] ± 1.1	4.69[a] ± 0.67	5.81[a] ± 0.17	2.34[a] ± 0.51	2.36[a] ± 0.51	2.51[a] ± 0.62	2.58[a] ± 0.59
1	5.82[a] ± 0.38	5.68[a] ± 0.47	6.11[a] ± 0.25	3.07[a] ± 1.0	2.82[a] ± 0.71	2.87[a] ± 0.53	2.95[a] ± 0.48
3	7.66[a] ± 0.43	7.69[a] ± 0.52	8.04[a] ± 0.43	3.94[a] ± 0.73	4.02[a] ± 0.52	3.82[a] ± 0.41	3.55[a] ± 0.33
5	8.91[a] ± 0.74	8.66[a] ± 0.51	8.46[a] ± 0.38	4.41[a] ± 0.71	4.15[a] ± 0.41	4.01[a] ± 0.44	3.96[a] ± 0.24

[1]Inoculum levels were at ratios of 1:1, 2:1 and 3:1 log CFU of *C. piscicola: L. monocytogenes* (CFU/g). Ratio example = 1:1 was 2 log CFU/ml of both microorganisms; 2:1 was 4 log CFU/ml of *C. piscicola* and 2 log CFU/m of *L. monocytogenes.*
[2a]Means sharing the same lowercase superscript letter within the same row for growth of *C. piscicola* or *L. monocytogenes* are not significantly different ($P > 0.05$)

VT's FST building. The meter electrode was submerged into one 25-g sample every sampling day per type of crab meat. A pH of 7.0 is optimum for bacteriocin production *(15)*, however the pH of the crab meat was not adjusted to obtain this pH. The pH of MRS broth and MRS agar was modified to pH of 7.0 by using 1 N NaOH to stimulate bacteriocin production.

Statistical analysis. Statistical analyses were conducted using SAS version 9.1 (SAS Institute, Cary, NC). This study was analyzed using a general linear model procedure with *C. piscicola* and *L. monocytogenes* as independent variables. The mean log survival of *L. monocytogenes* and *C. piscicola* were determined using standard plate counts from MOX and mMRS plates, respectively. Whenever the overall F-value was significant Tukey's HSD grouping was used to determine treatment differences

RESULTS

On every sample day, an aerobic plate count on TSA was performed to estimate spoilage of the crab meat. Spoilage was considered to be >7 log CFU/g. Fresh crab meat stored at 4°C or 10°C spoiled in 7 days or less. Pasteurized crab meat stored at 4°C or 10°C didn't spoil for over 24 days. Sampling ceased at 28 days due to lack of bacterial growth on the TSA samples and indicating no crab meat spoilage. For all replications (fresh and pasteurized), the uninoculated crab meat (negative control) was never positive for *C. piscicola* or *L. monocytogenes*.

Effect of product inoculation

Fresh crab meat. The mean inoculation level of *L. monocytogenes* in fresh crab meat at 4°C and 10°C on day 0 was 2.26 log (std ± 0.11) CFU/g and 2.45 log (std ± 0.12) CFU/g, respectively. The growth of *L. monocytogenes* in control treatments (crab meat that was not co-inoculated with *C. piscicola*) throughout the study in the fresh crab meat ranged from 2.19 log (std ± 0.38) CFU/g to a final concentration of 4.22 log (std ± 0.41) CFU/g on day 5 at 4°C; and 2.34 log (std ± 0.51) CFU/g to a final concentration of 4.41 log (std ± 0.71) CFU/g at 10°C on day 6 (Table 1 and 2).

Pasteurized crab meat. The mean inoculation level of *L. monocytogenes* in pasteurized crab meat at 4°C and 10°C on day 0 was 2.12 log (std ± 0.07) CFU/g and 2.20 log (std ± 0.09) CFU/g, respectively. Throughout the study, *L. monocytogenes* concentrations in pasteurized crab meat controls ranged from 2.13 log (std ± 0.14) CFU/g to a final concentration of 9.16 log (std ± 0.18) CFU/g at 4°C on day 28; and from 2.23 log (std ± 0.14) CFU/g to a final concentration of 10.1 (std ± 0.55) CFU/g at 10°C on day 28 (Tables 3 and 4).

Effect of treatments on *L. monocytogenes* growth

It was confirmed that *C. piscicola* strain used in this study was inhibitory against *L. monocytogenes*, with a zone of inhibition of 35.2 (std ± 4.22). In both fresh and pasteurized crab meat, regardless of the inoculation ratios, growth of *C. piscicola* and *L. monocytogenes* followed similar trends.

Fresh crab meat. There was no significant difference between growth of *L. monocytogenes* at 4°C or 10°C *(P > 0.05)*. The co-inoculation of *C. piscicola* did not affect the overall growth rate of *L. monocytogenes (P > 0.05)*. The mMRS agar used to determine *C. piscicola*, initial inoculation levels indicated that *C. piscicola* levels were higher than the proposed ratios. This may have been due to indigenous LAB in the crab meat. Mean bacterial concentrations (CFU/g), day and temperature data are shown in Tables 1 and 2.

Pasteurized crab meat. The growth of *L. monocytogenes* in inoculated pasteurized crab meat, was significantly affected by the different concentrations of added *C. piscicola (P < 0.05)*. At 4°C the growth

TABLE 3. Growth of *Carnobacterium piscicola* and *Listeria monocytogenes* over time when co-inoculated in pasteurized crab meat at different ratios (*C. piscicola: L. monocytogenes*; 0:1, 1:1, 2:1, 3:1) and stored at 4°C (n = 6)

Storage time (days)	Mean growth of *C. piscicola* (log CFU/g)[1,2]			Mean growth of *L. monocytogenes* (log CFU/g)[1,2]			
	1:1	2:1	3:1	0:1	1:1	2:1	3:1
0	$2.11^{a} \pm 0.45$	$3.68^{a} \pm 0.26$	$5.54^{b} \pm 0.11$	$2.13^{a} \pm 0.14$	$2.02^{a} \pm 0.37$	$2.16^{a} \pm 0.23$	$2.17^{a} \pm 0.16$
6	$5.40^{a} \pm 0.0$	$6.40^{b} \pm 0.0$	$7.40^{c} \pm 0.0$	$4.51^{a} \pm 0.13$	$5.07^{a} \pm 0.15$	$5.13^{a} \pm 0.18$	$4.33^{a} \pm 0.65$
10	$8.98^{a} \pm 0.64$	$9.84^{a} \pm 0.44$	$10.0^{a} \pm 0.09$	$6.42^{a} \pm 0.75$	$6.62^{a} \pm 1.1$	$6.83^{a} \pm 0.33$	$5.94^{a} \pm 0.59$
14	$9.93^{a} \pm 0.17$	$10.0^{a} \pm 0.14$	$10.5^{a} \pm 0.48$	$9.16^{a} \pm 0.18$	$8.63^{ab} \pm 0.11$	$7.63^{ab} \pm 0.24$	$7.12^{b} \pm 0.41$
18	$9.70^{a} \pm 0.09$	$9.77^{a} \pm 0.15$	$9.87^{a} \pm 0.08$	$8.90^{a} \pm 0.18$	$8.64^{a} \pm 0.15$	$7.34^{b} \pm 0.16$	$6.77^{b} \pm 0.17$
22	$9.37^{a} \pm 0.13$	$9.33^{a} \pm 0.26$	$9.43^{a} \pm 0.12$	$8.94^{a} \pm 0.11$	$8.62^{a} \pm 0.07$	$7.48^{b} \pm 0.11$	$6.39^{c} \pm 0.19$
26	$9.43^{a} \pm 0.13$	$9.56^{a} \pm 0.18$	$9.43^{a} \pm 0.16$	$9.04^{a} \pm 0.13$	$8.54^{a} \pm 0.14$	$7.35^{b} \pm 0.32$	$6.75^{b} \pm 0.22$
28	$9.51^{a} \pm 0.10$	$9.53^{a} \pm 0.16$	$9.67^{a} \pm 0.12$	$9.02^{a} \pm 0.13$	$8.30^{b} \pm 0.11$	$7.14^{c} \pm 0.17$	$6.53^{d} \pm 0.18$

[1]Inoculum levels were at ratios of 1:1, 2:1 and 3:1 log CFU of *C. piscicola: L. monocytogenes* (CFU/g). Ratio example = 1:1 was 2 log CFU/ml of both microorganisms; 2:1 was 4 log CFU/ml of *C. piscicola* and 2 log CFU/m of *L. monocytogenes*.
[2a, b, c, d] Means sharing the same lowercase superscript letter within the same row for growth of *C. piscicola* or *L. monocytogenes* are not significantly different ($P > 0.05$)

of *L. monocytogenes* was significantly reduced in the 3:1 *C. piscicola/L. monocytogenes* ratio after 14 days and in the 2:1 ratio after 18 d *(P < 0.05)*. At 10°C the growth of *L. monocytogenes* was significantly reduced in the 3:1 *C. piscicola/L. monocytogenes* ratio after 10 d. At 12 days, the 2:1 *C. piscicola/L. monocytogenes* ratio had significant differences in *L. monocytogenes* growth compared with the 1:1 *C. piscicola/L. monocytogenes* ratio *(P < 0.05)*.

There was a significant difference in growth of *L. monocytogenes* and *C. piscicola* between 4°C and 10°C *(P < 0.05)*. Mean growth for both *L. monocytogenes* and *C. piscicola* was greatest at 10°C. Growth of *L. monocytogenes (P < 0.05)*, was lowest in the 3:1 *C. piscicola/L. monocytogenes* ratio and highest in the 0:1 ratio. Initial inoculation levels of *C. piscicola* were not significant after 6 days for crab meat held at 4°C and after 14 days for crab meat held at 10°C *(P > 0.05)*. Mean bacterial concentrations (CFU/g) by day and temperature are shown in Tables 3 and 4.

pH of crab meat. The pH values for fresh crab meat ranged from 7.74–7.89, with the mean being 7.79 (std ± 0.8). The pH for pasteurized crab meat ranged from 7.73–7.80, with the mean being 7.70 (std ± 0.1).

DISCUSSION

Bacteriocins are appealing as a hurdle in minimally processed, refrigerated food products by reducing bacterial loads if employed under sanitary handling and storage conditions *(20, 21)*. Duffes et al. reports a bacteriocidal effect on *L. monocytogenes* by *C. piscicola* at 4°C in vacuum-packed cold smoked salmon and a delayed growth of the pathogen at 8°C *(8)*. Schobitz et al. had similar results of a bacteriostatic effect on *L. monocytogenes* by *C. piscicola* on vacuum-packaged salmon at 4°C after 15 days *(21)*. It is presumed that no significant reduction of *L. monocytogenes* occurred in fresh crab meat due to the higher background flora. Since the crabs were harvested and processed during different seasons, the changes in water temperatures in the Chesapeake Bay may account for the array of different microflora in the crab meat competing with the added *Listeria*.

Spoilage (7.0 log CFU/g) was reached in less than 7 days for fresh crab meat and 26 days or more for pasteurized crab meat. The inoculation levels of *C. piscicola* contributed to the spoilage of the crab meat, suggesting that the shelf-life of the food product may be determined by the concentration of a bacteriocin and perhaps the effectiveness of the bacteriocin. Yamazaki et al. *(23)* found that in cold smoked salmon at 4°C in a 3:1 ratio, *C. piscicola* inhibited *L. monocytogenes* after 7 days and after 21 days in a 1.3:1 ratio. The author contends that inhibition of *L. monocytogenes* depends on the initial inoculum level of *C. piscicola (23)*. Himelbloom et al. *(10)* suggests that although the addition of bacteriocin producing *Carnobacterium* spp. showed success in

TABLE 4. Growth of *Carnobacterium piscicola* and *Listeria monocytogenes* over time when co-inoculated in pasteurized crab meat at different ratios (*C. piscicola: L. monocytogenes*; 0:1, 1:1, 2:1, 3:1) and stored at 10°C (n = 6)

Storage time (days)	Mean growth of *C. piscicola* (log CFU/g)[1,2]			Mean growth of *L. monocytogenes* (log CFU/g)[1,2]			
	1:1	2:1	3:1	0:1	1:1	2:1	3:1
0	2.67[a] ± 0.42	3.85[a] ± 0.11	5.74[b] ± 0.06	2.23[a] ± 0.14	2.24[a] ± 0.12	2.06[a] ± 0.40	2.25[a] ± 0.22
6	5.40[a] ± 0.0	6.40[b] ± 0.0	8.40[c] ± 0.0	5.90[a] ± 0.55	5.90[a] ± 0.55	5.33[a] ± 0.44	4.20[a] ± 0.39
10	9.40[a] ± 1.1	9.92[a] ± 0.35	10.4[a] ± 0.45	8.95[a] ± 0.50	8.80[a] ± 0.34	7.56[ab] ± 0.44	6.70[b] ± 0.67
12	10.26[a] ± 0.52	10.42[a] ± 0.53	10.31[a] ± 0.38	10.08[a] ± 0.55	9.20[b] ± 0.43	7.95[c] ± 0.53	7.17[c] ± 0.30
14	9.78[a] ± 0.47	10.28[b] ± 0.47	10.30[b] ± 0.44	9.13[a] ± 0.14	9.04[a] ± 0.65	8.11[ab] ± 0.59	7.18[b] ± 0.42
18	9.45[a] ± 0.12	9.72[a] ± 0.23	9.76[a] ± 0.11	9.20[a] ± 0.20	8.68[a] ± 0.15	7.53[b] ± 0.17	6.62[c] ± 0.14
22	9.76[a] ± 0.14	9.63[a] ± 0.09	9.21[a] ± 0.98	9.14[a] ± 0.14	8.81[a] ± 0.14	7.16[b] ± 0.46	6.50[b] ± 0.25
24	9.70[a] ± 0.15	9.67[a] ± 0.16	9.79[a] ± 0.14	9.02[a] ± 0.10	8.56[a] ± 0.09	7.44[b] ± 0.29	6.62[b] ± 0.05

[1]Inoculum levels were at ratios of 1:1, 2:1 and 3:1 log CFU of *C. piscicola: L. monocytogenes* (CFU/g). Ratio example = 1:1 was 2 log CFU/ml of both microorganisms; 2:1 was 4 log CFU/ml of *C. piscicola* and 2 log CFU/m of *L. monocytogenes*.
[2a, b, c] Means sharing the same lowercase superscript letter within the same row for growth of *C. piscicola* or *L. monocytogenes* are not significantly different (P > 0.05)

the preservation of cold-smoked salmon, production of bacteriocin was not reliable, and thus may not consistently ensure the safety of the food product.

Optimal production of bacteriocin occurs between a pH of 6–7. Differences in pH were not expected between the fresh and pasteurized crab meat since they originated from the same processor. Khouiti et al. *(15)* saw maximum bacteriocin productivity in their supernatant occurring at pH 7, concluding that a controlled pH increased the volumetric activity of the bacteriocin *(9–11, 16)*. In this study, the pH of neither fresh nor pasteurized crab meat was altered, but consistently measured higher than 7. In fresh crab meat, this may indicate that there was indigenous spoilage bacteria present and that food matrix deterioration occurred prior to testing day 0.

Financially weighing the benefits of a food product with a short shelf life is necessary when considering the use of bacteriocins. In many instances, imported crab meat is removed from its container and repacked for domestic sales *(13)*. It is not uncommon, or illegal as long as the cups do not state "Fresh Crab Meat," for pasteurized crab meat to be repacked into cups for refrigerated sales. During the re-packing process, the crab meat may become contaminated with *L. monocytogenes*. Re-packed refrigerated previously pasteurized crab meat can have a shelf life of approximately 25 days. The addition of *C. piscicola* to re-packed crab meat can be a secondary hurdle to inhibit and/or control the growth of *L. monocytogenes* during extended refrigerated storage. Future work is needed in this area, both for fresh and pasteurized crab meat. Adjusting the pH of the crab meat may also help stimulate bacteriocin activity and the addition of glucose may result in better control of *L. monocytogenes*.

ACKNOWLEDGMENT

This research was funded through the Virginia Sea Grant "Develop, Evaluate and Characterize Different Package Types on the Quality, Shelf-Life and Market Acceptability of Pasteurized and Fresh Crab Meat."

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