

Growth, Injury, and Survival Potential of *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Staphylococcus aureus* in Brine Chiller Conditions[†]

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ABSTRACT

A model brine system was used to evaluate growth, injury, and survival potential of *Yersinia enterocolitica*, *Listeria monocytogenes*, and *Staphylococcus aureus*. Each strain was incubated for up to 30 days at -12 to 28°C in brain heart infusion broth containing 0.5 to 20% NaCl. Samples were enumerated on a dual agar plating system to assess growth and injury. *Y. enterocolitica* grew at -2°C in 0.5% brine and at 5°C in 5% NaCl. *L. monocytogenes* grew at 5°C in 5% NaCl and at 12°C in 9% NaCl. *S. aureus* grew at 12°C in 5% NaCl. Significant injury was observed for two of the pathogens, but not for *L. monocytogenes*. Bacteriostatic or lethal conditions were maintained for the three organisms at -2°C and 9% NaCl. While lethal NaCl and temperature combinations were defined for *Y. enterocolitica* and *S. aureus*, *L. monocytogenes* survived for 30 days at -12°C in 20% NaCl. This study provides safety criteria and recommendations for use in the operation of recycle brine systems for cooling processed foods.

Key words: Chiller brine, pathogen survival, psychrotrophic bacteria, halotolerant bacteria

Recycled brine is used frequently to cool thermally processed or fresh food products. Rapid heat removal minimizes product exposure to temperatures that permit bacterial growth. This has two microbiological benefits. First, it assures adequate shelf life by preventing growth of spoilage organisms, and second, it aids microbiological safety by preventing growth of pathogenic organisms that may survive thermal processing or are present on fresh foods. Since heat and nutrients from product permeate the brine, the cooling solution may allow growth or survival of harmful bacteria. Thus, it is also important to maintain the microbial safety of the cooling solutions, particularly when recycled.

Psychrotrophic pathogenic bacteria have become an important public health concern for the food industry (12).

Listeriosis (13) and yersiniosis outbreaks (8) have heightened awareness of risks from these organisms, which can grow at temperature below those that are normally inhibitory to other pathogenic bacteria. Recycled cold brine solutions may serve as a reservoir for psychrotrophs, particularly *L. monocytogenes* (5). This organism is somewhat halotolerant, being able to grow at a water activity of 0.92 with salt as the humectant (3, 11). *Staphylococcus aureus*, as well, may survive in these harsh environments and is capable of growth at a water activity below 0.90 (1).

The USDA's Food Safety and Inspection Service (FSIS) issued MPI Bulletin 83-16 (15), which defines requirements for and limits to brine solution recycling for cooling meat and poultry products. The solution maintenance provisions for reuse are shown in Table 1. They range from recycling for one production shift, using no temperature or sodium chloride controls, to recycling for up to four weeks, with a requirement for maintaining 20% NaCl and a maximum temperature of -12.2°C. Chlorine may be added as well. These controls need to be coupled with a rigorous sanitation program and quality assurance system.

While the guidelines for brine solution reuse have served FSIS, the industry, and consumers well, they were empirically derived from in-plant experiences, using total aerobic plate counts and coliform data. There are no reports of challenge studies using pathogens. In addition, there are few quantitative data available about the food safety risks of

TABLE 1. Maintenance requirements and permitted duration for reuse of cooling brine solutions as defined in MPI Bulletin 83-16 (15)

Reuse duration	Solution maintenance conditions ^a	
	Minimum salt concentration (%)	Maximum temperature
One production shift	None	Undefined
Up to 24	5	40°F (4.4°C)
Up to 1 week	9	28°F (-2.2°C)
Up to 4 weeks	20	10°F (-12.2°C)

salt-tolerant or psychrotrophic pathogens in these harsh environments. Thus, the objective of this study was to evaluate growth, injury, and survival potential of *Y. enterocolitica*, *L. monocytogenes*, and *S. aureus* at low temperature and high sodium chloride concentration levels, to simulate conditions in a recycle brine chiller.

MATERIALS AND METHODS

Bacterial strains

Cultures of *L. monocytogenes* Scott A and *S. aureus* 196 E were from the frozen (-70°C) permanent collection at the Eastern Regional Research Center, and the subcultures were maintained on brain heart infusion agar (BHIA) plates (Difco, Detroit, MI). Individual colonies of each strain were inoculated separately in 9.9 ml of BHI broth (Difco) and incubated aerobically at 37°C for 18 to 24 hours prior to the start of each experiment. *Y. enterocolitica* serotype 0:3, strain GER, was from the ERRC culture collection, and was maintained in a 50:50 mixture of BHI and glycerol at -80°C . Culture tubes of *Y. enterocolitica* were kept on dry ice during the inoculation of BHI tubes to prevent thawing and possible plasmid loss. A scraped and thawed aliquot of approximately 10 μl of *Y. enterocolitica* was inoculated into 9.9 ml of BHI broth using a sterile loop. *Y. enterocolitica* cultures were inoculated then incubated at 12°C for 48 hours.

Media and conditions

Sodium chloride (Mallinckrodt Specialty Chemicals Co., Paris, KY) solutions were first prepared by combining in volumetric flasks 0 to 195 g of NaCl with distilled water to yield 1 liter. BHI (37 g/liter) was hydrated using the various salt solutions. Final NaCl concentrations, including the 0.5% from BHI, were 0.5, 5, 9, 15, and 20%. BHI/NaCl solutions were dispensed (100 ml) into 250-ml Erlenmeyer flasks, then autoclaved at 121°C for 20 minutes. The pH values for the solutions varied between 7.37 for 0.5% NaCl and 7.51 for 20% NaCl.

Incubation, inoculation, and sampling

Sterile flasks were tempered to experimental temperatures prior to inoculation. Flasks inoculated with stationary phase cells were incubated and monitored for up to 30 days at -12 , -7 , -2 , 5, 12, or 28°C . Flasks at 28, 12, and 5°C were incubated in an incubator shaker (model G26, New Brunswick Scientific Co. Inc., New Brunswick, NJ) and shaken at 150 rpm. Flasks incubated at -2 and -7°C were maintained on orbital shakers at 150 rpm (Model 5730 Lab Line Instruments, Melrose Park, IL) inside an environmental room (Forma Scientific, Marietta, OH) that was equilibrated to the desired temperature. Flasks were incubated at -12°C in a shaker bath (model 6250 Eberbach Corp., Ann Arbor, MI) located inside a -7°C environmental room. Based on the results of preliminary experiments, it was determined that the flasks could be maintained a -12°C by circulating a 70:30 (wt/wt) ethylene glycol-water mixture through the shaker bath at -20°C using a Polyscience 950 refrigerated circulator (Niles, IL). To minimize temperature rise, the flasks were shaken at 50 rpm.

Triplicate temperature-equilibrated flasks of each BHI solution were inoculated with 100 μl of each bacterial culture to yield approximately 6 log CFU/ml. Following inoculation, an initial aliquot was withdrawn and transferred to sterile culture tubes for enumeration. Samples were removed periodically using a sterile glass serological pipet (Thomas Scientific, Swedesboro, NJ) to determine population density. From those samples where growth occurred, portions were continually withdrawn until stationary

phase was determined to occur. For inhibitory conditions, flasks were sampled for 30 days or until the population within a flask fell below the threshold of detection (1.03 log CFU/ml). Flasks exhibiting bacteriostatic conditions were sampled for up to 30 days.

Bacteriological and water activity analyses

Withdrawn samples were maintained at 5°C prior to plating for no more than 30 minutes to ensure that no growth occurred. If required, samples were diluted using 0.1% peptone buffer (pH 7.2) (Difco). Samples were plated in duplicate using a spiral plater (Spiral Systems, Cincinnati, OH) onto a dual plating system consisting of BHIA medium plus 1% pyruvate (BHIA+P; Sigma Chemical Co., St. Louis, MO) and BHIA medium plus NaCl (BHIA+S). The dual plating system was employed to determine the degree of cellular injury occurring at each of the temperature and brine conditions. For *L. monocytogenes* and *S. Aureus* BHIA plus 5% NaCl was employed, while BHIA plus 2% NaCl was used for *Y. enterocolitica*. The reduced level of NaCl was used for *Y. enterocolitica* after preliminary trials demonstrated that unstressed cells of this pathogen would not grow on BHIA plus 5% NaCl. Plates were incubated at 37°C . Samples plated on BHIA+P were counted after 18 to 24 hours of incubation. Samples plated on BHIA+S often required incubations of up to 48 hours.

Water activity (a_w) was estimated on uninoculated BHI-NaCl solutions using a water activity meter (model CX 2 instrument, Aqua Lab, Pullman, WA) equilibrated to the corresponding experimental temperature. Water activity determinations could not be made for BHI-NaCl solutions incubated at temperatures below -2°C , based on the manufacturer's recommendation.

Data collection and analysis

Colonies from microbiological analyses were enumerated using a bacteria colony counter (model 500A, Spiral Systems, Cincinnati, OH) equipped with a CASBA software system (Spiral Biotech, Bethesda, MD). CASBA PRN files were exported into Lotus 4.01 for Windows (Lotus, Cambridge, MA) for further analysis. Growth curves were generated from the experimental data using the Gompertz equation (18). The four Gompertz parameters were subsequently used to calculate lag phase duration (LPD), generation time (GT), and exponential growth rate (EGR). For survival curves, D values (time in hours for a 10-fold population reduction) and shoulders (TL) were determined using the equation developed by Whiting (17), which was modified from the logistic equation of Kamau et al. (6). D_1 and D_2 values were calculated when inflection points were observed on the survival curves. The data were analyzed further using the SAS (SAS Institute, Cary, NC) General Linear Models Procedure. Regression analysis was performed by calculating second-order response surfaces for the responses of bacterial counts for each organism to NaCl concentration, time, and temperature for each plating media. To analyze for the degree of injury that occurred, an analysis of covariance was used for each strain to test for homogeneity of regression coefficients between the two types of media.

Industrial cooling brine solutions

Brine solutions (provided by A. Oser, Hatfield Meats, Inc., Hatfield, PA) from seven chillers were tested over three to four days. Solutions were from shower-type brine chillers which were maintained at temperatures below -2°C , prior to introduction of product. Brine recycle durations were 1 and ≤ 3 days, depending upon use for cooling exposed or protected products, respectively (see Table 7). Brine samples were aseptically collected and tested by company personnel. pH values were estimated using a temperature-compensating Orion pH meter (model 610, ATI Orion, Boston,

MA) equipped with a combination electrode. Brix readings for salinity were estimated using refractometry.

RESULTS

Water activity measurements

Water activities of the model brine solutions ranged from 0.99 (0.5% NaCl) to 0.85 to 0.86 (20% NaCl) and were generally uniform at a given NaCl concentration, regardless of temperature (Table 2). Measurements could not be obtained at -7° or below.

TABLE 2. Water activities of brain heart infusion brine solutions

NaCl, %	Temperature, $^{\circ}$ C					
	28	12	5	-2	-7	-12
0.5	0.99	0.99	0.99	0.99	ND ^a	ND
5	0.96	0.96	0.96	0.96	ND	ND
9	0.94	0.95	0.93	0.94	ND	ND
15	0.90	0.90	0.90	0.90	ND	ND
20	0.85	0.85	0.86	0.86	ND	ND

^a ND = not determined.

Growth, death, or survival

Y. enterocolitica. Exponential growth occurred within approximately 1 h and 24 h at 28° C and 12° C, respectively, when the NaCl level was 0.5% ($a_w = 0.99$) (Table 3). Growth rates slowed and generation times increased as temperature decreased and as NaCl concentration increased. This general relationship was observed for all three organisms studied. After a lag phase, growth resumed at -2° C and 0.5% NaCl and at 5° C and 5% NaCl ($a_w \geq 0.96$). No growth was observed below -2° C. Bacteriostatic or bactericidal conditions prevailed at 9% NaCl or greater ($a_w = 0.93$ to 0.95) regardless of temperature. At -2° C and 9% NaCl or greater there was a population decline. At -7° C or lower bacteriostasis prevailed, suggesting a protective condition. Killing due to cold temperature and/or NaCl was linear, except at -2° C and 9%, where an inflection (D_2) was observed, and at 28° C and 20% NaCl, where a 4-h shoulder (TL) was observed. The analysis of variance indicated significant treatment effects ($P < 0.01$) on the bacterial response due to NaCl concentration, time, temperature, and their two-way interactions, except time by temperature (Table 4).

L. monocytogenes. Exponential growth occurred within approximately 14 h at 28° C and 9% NaCl (Table 5). At 12° C

TABLE 3. Growth or inhibition kinetics of *Yersinia enterocolitica* in brine solutions at various temperatures

Temp., $^{\circ}$ C ^a	% NaCl ^a	Gompertz derived values ^b			Logistic decline values ^b		
		LPD	GT	EGR	TL	D_1	D_2
28	0.5	1.2 (0.5) ^c	0.7 (0.0)	0.43 (0.0)			
28	5	12.4 (1.1)	1.4 (0.1)	0.22 (0.0)			
28	9				0.0 (0.0)	40.2 (4.4)	0.0 (0.0)
28	15				0.0 (0.0)	17.6 (5.1)	0.0 (0.0)
28	20				4.1 (3.6)	11.2 (0.3)	0.0 (0.0)
12	0.5	8.76 (2.5)	3.5 (0.3)	0.1 (0.0)			
12	5	23.4 (0.5)	6.9 (1.4)	0.1 (0.0)			
12	9	<i>d</i>					
12	15	<i>d</i>					
12	20	<i>d</i>					
5	0.5	43.6 (15.7)	6.5 (2.2)	0.1 (0.0)			
5	5	308.5 (13.7)	24.7 (9.4)	0.0 (0.0)			
5	9	<i>d</i>					
5	15				0.0 (0.0)	171.9 (5.7)	0.0 (0.0)
5	20				0.0 (0.0)	233.8 (22.9)	0.0 (0.0)
-2	0.5	81.4 (25.2)	27.3 (5.7)	0.0 (0.0)			
-2	5	<i>d</i>					
-2	9				0.0 (0.0)	59.2 (1.6)	410.5 (52.9)
-2	15				0.0 (0.0)	144.7 (46.1)	0.0 (0.0)
-2	20				0.0 (0.0)	110.6 (2.7)	0.0 (0.0)
-7	5	<i>d</i>					
-7	9	<i>d</i>					
-7	15	<i>d</i>					
-7	20	<i>d</i>					
-12	20	<i>d</i>					

^a ANOVA indicated a significant treatment effect ($P < 0.01$) for temperature, time, NaCl concentration, and some two-way interactions (see Table 4).

^b LPD, lag phase duration (h); GT, generation time (h); EGR, exponential growth rate (CFU/h); TL, lag time (h); D_1 , time (h) for a 1-log decline.

^c Mean of three trials (standard deviation).

^d Bacteriostatic conditions were observed.

TABLE 4. Statistical significance,^a based on analysis of variance, of the effects of growth temperature, NaCl concentration, incubation time, type of medium (injury), and major interactions on the growth, survival, and injury potential of three pathogenic bacteria

Factor ^b	<i>Yersinia enterocolitica</i>	<i>Listeria monocytogenes</i>	<i>Staphylococcus aureus</i>
NaCl concentration	0.0001	0.9450	0.0001
Time of incubation	0.0001	0.0001	0.0001
Temperature	0.0001	0.0001	0.0001
NaCl × time	0.0001	0.0735	0.0008
NaCl × temperature	0.0001	0.0863	0.0001
Time × temperature	0.0449	0.0028	0.0771
Type of medium (injury)	0.0072	0.3827	0.0093
Overall model	0.0001	0.0001	0.0001

^a Numbers in columns are *P* values, where *P* < 0.01 is considered a significant treatment effect.

^b See Materials and Methods section for variable ranges for factors and for types of media used to determine injury potential.

growth occurred at 9% NaCl after a lag phase of 84.5 h. At 5°C exponential growth occurred at 0.5% and 5% NaCl. Lethal or static conditions were uniformly observed at greater than 9% NaCl. Static conditions were observed at

–2°C for 0.5 to 9% NaCl and for all NaCl levels at –7° and –12°C. It is noteworthy that bacteriostasis was observed at –12°C and 20% NaCl throughout the testing period. Where lethality occurred, the organism appeared to be more susceptible to the lethal effect of NaCl than to cold temperature. A shoulder was observed (TL) at 5°C where lethal conditions were maintained. Tailing (*D*₂) was observed at –2°C at 15 and 20% NaCl. Lowering the temperature appeared to enhance survival. The ANOVA showed significant treatment effects (*P* < 0.01) due to time and temperature and their two-way interaction (Table 4).

S. aureus. Exponential growth occurred at 28°C and 0.5 and 5% NaCl (Table 6). Growth rate slowed at 12°C, indicated by longer generation times and reduced exponential growth rates. Bacteriostasis or death was observed at 9% NaCl or above at all temperatures, although variable results were observed at 28°C and 9% NaCl. While two of six flasks tested had population growths of 1 log within 3 days, the remaining four flasks showed about a 1 log decline in the same time. Bactericidal or bacteriostatic conditions prevailed at temperatures of 5°C or lower at all NaCl concentrations. The ANOVA showed significant treatment effects (*P* < 0.01) due to NaCl concentration, time, temperature, and their two-way interactions, except for the time by temperature interaction (Table 4).

TABLE 5. Growth or inhibition kinetics of *Listeria monocytogenes* in brine solutions at various temperatures

Temp., °C ^a	% NaCl	Gompertz derived values ^b			Logistic decline values ^b		
		LPD	GT	EGR	TL	<i>D</i> ₁	<i>D</i> ₂
28	0.5	2.2 (0.3) ^c	0.6 (0.1)	0.5 (0.1)			
28	5	5.2 (0.1)	0.6 (0.0)	0.5 (0.0)			
28	9	14.5 (0.8)	1.9 (0.3)	0.2 (0.0)			
28	15				0.0 (0.0)	42.5 (11.9)	0.0 (0.0)
28	20				0.0 (0.0)	39.3 (8.2)	0.0 (0.0)
12	0.5	31.5 (0.7)	2.4 (0.2)	0.1 (0.0)			
12	5	29.0 (4.4)	4.7 (0.8)	0.1 (0.0)			
12	9	84.5 (14.6)	11.9 (7.0)	0.1 (0.1)			
12	15	<i>d</i>					
12	20	<i>d</i>					
5	0.5	148.9 (0.7)	12.5 (0.5)	0.0 (0.0)			
5	5	168.9 (4.1)	18.1 (1.0)	0.0 (0.0)			
5	9				312.6 (193.4)	163.7 (83.1)	0.0 (0.0)
5	15				394.2 (82.8)	124.4 (52.8)	0.0 (0.0)
5	20				378.3 (40.0)	139.0 (26.6)	0.0 (0.0)
–2	0.5	<i>d</i>					
–2	5	<i>d</i>					
–2	9	<i>d</i>					
–2	15				0.0 (0.0)	76.2 (10.6)	604.0 (11.1)
–2	20				0.0 (0.0)	90.2 (2.1)	655.1 (49.0)
–7	5	<i>d</i>					
–7	9	<i>d</i>					
–7	15	<i>d</i>					
–7	20	<i>d</i>					
–12	20	<i>d</i>					

^a ANOVA indicated a significant treatment effect (*P* < 0.01) for temperature, time and their two-way interactions (see Table 4).

^b LPD, lag phase duration (h); GT, generation time (h); EGR, exponential growth rate (CFU/h); TL, lag time (h); *D*, time (h) for a 1-log decline.

^c Mean of three trials (standard deviation).

^d Bacteriostatic conditions were observed.

TABLE 6. Growth or inhibition kinetics of *Staphylococcus aureus* in brine solutions at various temperatures

Temp., °C ^a	% NaCl ^a	Gompertz derived values ^b			Logistic decline values ^b		
		LPD	GT	EGR	TL	D ₁	D ₂
28	0.5	2.0 (0.3) ^c	0.5 (0.0)	0.7 (0.1)			
28	5	3.3 (0.2)	0.8 (0.1)	0.4 (0.1)			
28	9				0.0 (0.0)	42.5 (11.9)	0.0 (0.0)
28	15				0.0 (0.0)	55.6 (5.1)	0.0 (0.0)
28	20				0.0 (0.0)	131.5 (33.4)	0.0 (0.0)
12	0.5	49.6 (13.2)	10.5 (0.5)	0.0 (0.0)			
12	5	64.5 (13.3)	71.6 (53.0)	0.0 (0.0)			
12	9	<i>d</i>					
12	15	<i>d</i>					
12	20	<i>d</i>					
5	0.5				0.0 (0.0)	240.9 (8.7)	0.0 (0.0)
5	5				0.0 (0.0)	349.1 (30.8)	0.0 (0.0)
5	9				0.0 (0.0)	290.4 (16.6)	0.0 (0.0)
5	15				0.0 (0.0)	375.2 (51.5)	0.0 (0.0)
5	20				0.0 (0.0)	343.0 (6.53)	0.0 (0.0)
-2	0.5				0.0 (0.0)	166.7 (5.2)	0.0 (0.0)
-2	5				0.0 (0.0)	188.4 (24.0)	0.0 (0.0)
-2	9				0.0 (0.0)	188.4 (7.1)	0.0 (0.0)
-2	15				0.0 (0.0)	185.9 (3.2)	0.0 (0.0)
-2	20				0.0 (0.0)	228.0 (11.2)	0.0 (0.0)
-7	5	<i>d</i>					
-7	9	<i>d</i>					
-7	15	<i>d</i>					
-7	20	<i>d</i>					
-12	20	<i>d</i>					

^a ANOVA indicated a significant treatment effect ($P < 0.01$) for NaCl concentration, time, temperature, and some two-way interactions.

^b LPD, lag phase duration (h); GT, generation time (h); EGR, exponential growth rate (CFU/h); TL, lag time (h); D, time (h) for a 1-log decline.

^c Mean of three trials (standard deviation).

^d Bacteriostatic conditions were observed.

Injury

Y. enterocolitica exhibited a high degree of injury ($P < 0.01$) when plated onto a dual plating system (Table 4). Treatment combinations with NaCl levels of 5% or more and temperatures of 12°C and below yielded at least a 1-log difference between growth responses on the two types of media. However, generally, there was greater than a 2-log growth difference between media when cells were incubated in combinations with 9% NaCl or greater. There was no

evidence of *L. monocytogenes* injury throughout the experimental range ($P > 0.05$). The analysis of covariance indicated a highly significant degree of injury ($P < 0.01$) for *S. aureus*. This was most evident at -2°C or lower, where nearly all brine/temperature combinations produced differences between 1 and 2 log between the two types of media. There was no injury at any treatment combination at 5°C or higher.

TABLE 7. Characteristics of seven industrial recycled cooling brines

Products cooled	pH value ^a	% NaCl ^b	Target temperature, °C	Recycle duration, days
Exposed hams	6.52 (0.25)	12.5 (0.5)	<-2	1
Exposed hams	6.59 (0.36)	11.8 (0.3)	<-2	1
Potted hams	6.81 (0.38)	12.9 (0.4)	<-2	≤3
Potted hams	6.90 (0.22)	14.0 (0.9)	<-2	≤3
Mixed products	6.88 (0.37)	13.0 (1.4)	<-2	≤3
Mixed products	6.68 (0.42)	14.2 (1.0)	<-2	≤3
Mixed products	6.92 (0.37)	13.2 (0.0)	<-2	≤3

^a Mean (standard deviation) of four determinations over four days.

^b Mean (standard deviation) of two or three Brix determinations over three days.

Industrial cooling brine solutions

Brine strengths, pH values, and other characteristics of NaCl solutions from seven industrial cooling chillers are shown in Table 7. The overall mean pH value was 6.75 (SD = 0.37) and the overall mean brine strength was 13.02% NaCl (SD = 1.12%). The systems were initially maintained at -2°C or lower. The pH values in the model system were higher than those of the industrial brines. Brine strength, temperature, and recycle duration for the industrial brines were consistent with parameters used in the model system.

DISCUSSION

The "hurdle" or "barrier" concept described by Leistner and Gorris (9) is the guiding principle that enables controls on bacterial growth in recycled brine solutions to be

effective. It is based on the observation that combinations of sublethal factors can synergistically function to inhibit growth or kill bacteria. "Barriers" include high and low temperature, reduced water activity, extremes of oxidation-reduction potential, extremes of pH, and the presence of antimicrobial agents. Brine cooling solutions inhibit bacteria by means of cold temperature and high brine concentrations (low water activity).

The main objective of this study was to establish if specific vegetative pathogens would grow, survive, or die in conditions that simulated a brine chiller. The key observation indicated that growth of these psychrotrophic and halophilic pathogens could be controlled by maintaining -2°C and 9% NaCl. At these conditions bacteriostasis could be maintained. An important finding of this study, however, was the determination that there was no temperature and brine concentration combination that ensured death of all three pathogenic bacteria, since *L. monocytogenes* survived the harshest temperature and brine combination. This is an important consideration, because it demonstrates the risk if a viable pathogen is transferred to the product, either directly or via casings. It would be possible than to contaminate the food plant environment or the food itself.

This potential was demonstrated by Walls et al. (16), who used collagen sausage casings as a model system to show the attachment of *Salmonella typhimurium* in 0.15 M NaCl (ca. 1% NaCl) to type I collagen. Their study indicated that attachment occurred within 1 min of incubation, and the fraction of cells that attached increased with increasing time, increasing incubation temperature between 4 and 37°C , and increasing inoculum levels. Their data suggest, therefore, that product in collagen casings may bind pathogenic organisms and carry them to other sites. This potential underscores the importance of controlling bacterial loads in recycled brine by maintaining at least bacteriostatic levels of salt and temperature.

In the present study we incubated the organisms in a nutrient-rich medium, a situation unlikely to occur in food plant. Nonetheless, the nonfastidious nutrient requirements of many bacteria, particularly gram-negative species, suggest that even low levels of nutrients would permit growth if other conditions were permissive. For example, a wide range of pathogens including *E. coli* O157:H7, *A. hydrophila*, *S. aureus*, and *C. jejuni* have been observed to survive or grow in drinking water and other types of water, including seawater (4, 7, 10, 19). Smith et al. (14) demonstrated in a polar marine environment (-1.8°C) that restricting nutrients, rather than temperature, limited enteric bacterial activity, including *Y. enterocolitica*. They found that large nutrient inputs to low-temperature marine environments may allow for the long-term persistence of enteric bacteria in a nonculturable but viable state. This point emphasizes the essential need to restrict product leakage into brine chilling systems. It would also be prudent for processors to recycle solutions for shorter durations if exposed products may leach nutrients into the brine. The potential for pathogen destruction by chlorination was not studied in the research described here. However, chlorination is permitted for this application (15) and may offer a significant added degree of safety assurance.

Results from the present study also show that growth of these pathogens will not occur at the temperature, NaCl, and recycling time provisions specified in MPI Bulletin 83-16 (15). While *Y. enterocolitica* grew by less than 1 log within 4 weeks at 5°C and 5% NaCl, the bulletin specifies that the recycled brine must be discarded after 24 h at these conditions. Thus, the time-temperature-NaCl conditions prescribed by MPI 83-16 appear to be adequate to prevent growth and maintain bacteriostatic conditions in cooling brine solutions.

Moderate differences were observed between the pH values of the model system (range 7.47 to 7.51) and industrial brines (range 6.12 to 7.34). The differences are probably caused by the latter containing acidic residues where smoke was applied. While a pH variable was not incorporated into the model system, the higher pH of the model system should be more permissive for growth than the pH of the industrial brines. Thus, the model system would overstate the pathogen growth potential.

While growth occurs in some strains of *S. aureus* at water activity levels of 0.83, most strains are less osmoresistant, and require otherwise ideal growth conditions to grow at their minimum a_w (2). Thus, the observation that this pathogen did not grow in 15% NaCl ($a_w = 0.90$) at 28°C is to be expected. Holding the organism at cold temperatures and high NaCl concentrations has limited lethal effect on strain 196E, which is consistent with the observations obtained by others. Hudson (5) reported on the potential of low temperature and high sodium chloride concentration to destroy *Listeria monocytogenes*. He found that during 33 days in 6% brine the organism grew logarithmically at 10°C ; it grew less rapidly under refrigeration, and the population density remained unchanged at -18°C . With 26% NaCl, the numbers declined by 4 log cycles at 10°C , by 2 log cycles under refrigeration, and remained unchanged at -18°C . Those observations support those of the present study.

The behavior of bacteria in extreme environments, such as those presented in brine chillers, is bound to exhibit greater variability than at permissive growth conditions. Factors such as microenvironments, which may offer protection, and genetic variation may affect growth potential, survival, and injury or repair capability. While the conditions studied in this research were designed to mimic the industrial situation, differences undoubtedly will exist. Thus, the extrapolation from model systems to industrial conditions needs to be conservative. Yet, the guidelines from USDA MPI Bulletin 83-16 and the industrial data obtained here indicate that current practices should prevent growth of the three bacterial pathogens studied. This study suggests that bacteriostatic, but not bactericidal, conditions can be maintained in recycled cooling brine. In order to control pathogen growth, it is necessary to maintain strict control over temperature and brine conditions.

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