GROWTH AND SURVIVAL OF *YERSINIA ENTEROCOLITICA, LISTERIA MONOCYTOGENES* AND *STAPHYLOCOCCUS AUREUS* IN BRINE AND PROPYLENE GLYCOL CHILLER CONDITIONS

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Background: Recycled solutions are 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 secondly, it aides 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 solution, 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. Listeriosis and yersiniosis outbreaks have heightened awareness of risks from these organisms, which can grow below temperatures that are normally inhibitory to other pathogenic bacteria. While recycled cooling solutions may serve as a reservoir for psychrotrophs or halophiles, there are no reports of challenge studies in cooling solutions using pathogens. In addition, there are few quantitative data available about the food safety risks of salt-tolerant or psychrotrophic pathogens in these harsh environments.

Objective: 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 or propylene glycol concentration levels, to simulate conditions in a recycled solution chiller.

Methods: *L. monocytogenes* (Scott A) and *S. aureus* (196 E) were inoculated separately in BHI broth (Difco) and incubated aerobically at 37°C for 18-24 hours prior to the start of each experiment. *Y. enterocolitica* (serotype 0:3, strain GER) was incubated in BHI broth at 12°C for 48 hours. Sodium chloride or propylene glycol (PG) solutions were first prepared by combining in volumetric flasks with distilled water to yield 1 liter. BHI (37g/L) was hydrated using the various solutions. Final NaCl concentrations, including the 0.5% from BHI, were 0.5, 5, 9, 15, and 20%, while PG concentrations were 1, 8, 13, 27, and 60%. Solutions were dispensed into Erlenmeyer flasks, then autoclaved at 121°C for 20 minutes. 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 either -12°,-7°,-2°,5°,12° or 28°C. 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 $\log_{10}$ cfu/ml. Following inoculation, an initial aliquot was withdrawn and transferred to sterile culture tubes for enumeration. Samples were removed periodically 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 until the population of each flask fell below the threshold of detection (1.31 $\log_{10}$ cfu/ml). Bacteriostatic conditions were sampled for up to 30 days. Withdrawn samples were maintained at 5°C and were diluted using pH 7.2, 0.1% peptone buffer (Difco). Each dilution was plated in duplicate using a spiral plater (Spiral Systems, Cincinnati, OH) onto BHIA + 1% pyruvate (BHIA+P). Petri dishes were enumerated using a bacterial 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.

Results *Y. enterocolitica*. Exponential growth was observed at 28°C and 12°C, when the NaCl level was 0.5%. Growth rate slowed as the temperature was decreased and as NaCl concentration was increased. Growth occurred at -2°C and 5°C at 0.5% and 5% NaCl, respectively. No growth was observed below -2°C. Bacteriostatic or killing conditions prevailed in 9% NaCl or greater. Growth was observed at 5°C using 8% PG. Bacteriostatic or killing occurred at -2°C or lower or when PG concentration was greater than 13%. *L. monocytogenes*. Growth occurred at 28°, 12°, and 5°C in 9%, 9%, and 5% NaCl, respectively. Static conditions were observed at -2°C, in 9% NaCl, and at all NaCl levels at -7° and -12°C. It is noteworthy that bacteriostasis was observed at -12°C in 20% NaCl.. The organism appeared to be more susceptible to the lethal effect of NaCl than to cold temperature, since bacteriocidal conditions occurred predominately in 15-20% NaCl, at 28°, 12°, 5°, and -2°C. At all NaCl levels, lowering temperature appeared to offer some survival protection. Growth was observed in 8% PG at 12°C. Bacteriostatic or killing was observed at 13% PG or greater or at 5°C or less. *S. aureus*. Growth was observed in 5% NaCl at 12°C and at 9% NaCl at 28°C. Static conditions or death were observed in ≥15% NaCl, at all temperatures. Bacteriocidal conditions prevailed at 5°C or lower at all NaCl concentrations. When PG was used as the humectant, growth occurred only at 28°C at 1 or 8% PG. All other conditions were bacteriostatic or bacteriocidal. General conditions that were found to ensure that growth cannot occur in these solutions are presented in Tables 1 and 2.

Discussion The guiding principle that enables such controls to be effective is the "Hurdle Theory," described by Leistner and Gorris. It is based on the observation that combinations of sublethal factors can synergistically function to inhibit growth or kill bacteria. "Hurdles" include: temperature, reduced water activity, extremes of oxidation-reduction potential, extremes of pH, and the presence of antimicrobial agents. Cooling solutions inhibit bacteria by cold temperature, low water activity, and direct solute effects.

A key finding of this study was the determination that there was no temperature and solute concentration combination that ensured

death of all three pathogenic bacteria, since *L. monocytogenes* survived the harshest temperature/humectant 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 then to contaminate the food plant environment or the food itself. A worse-case scenario was employed in the present study by incubating the organisms in a nutrient rich medium, a situation unlikely to occur in a food plant. Nonetheless, the nonfastidious nutrient requirement of many bacteria, particularly Gram-negative species, suggests that even low levels of nutrients would permit growth, if other conditions were permissive. Smith et al. (1994) 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 chilling systems. It should also be noted that the potential for pathogen destruction by chlorination was not studied in the research described here, and may serve as an additional safeguard.

Conclusions: This study demonstrates that bacteriostatic, but not bacteriocidal, conditions can be maintained in recycled cooling solutions. In order to prevent pathogen growth it is necessary to maintain strict control over temperature and humectant concentrations.

Pertinent literature

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Table 1. Growth (+) and Non-growth (-) Conditions of Three Bacterial Pathogens in Brine Solutions

NaCl, %	Temperature, °C					
	28	12	5	-2	-7	-12
0.5	+	+	+	+	-	-
5	+	+	-	-	-	-
9	+	+	-	-	-	-
15	-	-	-	-	-	-
20	-	-	-	-	-	-

Table 2. Growth (+) and Non-growth (-) Conditions of Three Bacterial Pathogens in Propylene Glycol (PG) Solutions.

PG%	Temperature, °C					
	28	12	5	-2	-7	-12
1	+	+	+	+	-	-
8	+	+	+	-	-	-
13	-	-	-	-	-	-
27	-	-	-	-	-	-
60	-	-	-	-	-	-