Survival of *Listeria monocytogenes* in Simulated Milk Cooling Systems

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**ABSTRACT**

Survival of *Listeria monocytogenes* under conditions that might be found in milk cooling systems was studied. Sterile solutions of 0.1 and 0.01% peptone, 0.1 and 0.01% nonfat dry milk (NFDM), 30% propylene glycol, and 30% propylene glycol with 0.01% NFDM were inoculated with 6000 *L. monocytogenes* Scott A/ml and were incubated at 4°C. The temperature was increased to 7°C when little growth was observed. At 7°C, populations approached 10^9 organisms/ml in NFDM and peptone. Growth was greater in the higher concentrations of each, and there was limited survival in the glycol media. Growth in minimal media, 0.01% peptone, 0.01% NFDM, 30% propylene glycol with 0.01% NFDM, and 1% tryptic soy broth (TSB), was studied. These media were inoculated with 3500 *L. monocytogenes* Jalisco cheese/ml. At 4°C, more growth was observed in the NFDM than in the peptone, no survival was seen in the glycol media, and the most growth was observed in the TSB. Growth in sterile 10, 20, and 30% propylene glycol solutions (with 0.1% NFDM) was studied by inoculation with 8800 *L. monocytogenes* Jalisco cheese/ml and incubation at 4°C. Growth in the 10% solution was observed. However, there was survival in the 20 and 30% solutions with no increase in numbers apparent over the time studied. Presence of *L. monocytogenes* in milk cooling systems may pose a hazard, especially in sweet water systems that might contain a small amount of milk.

*Listeria monocytogenes* has been classified as a rapidly growing mesophile with an unusually low minimal temperature for growth. Its growth rate at 10°C is typical of psychro­ trophs. Experiments and mathematical manipulations by Wilkins et al. (13) determined that the optimum growth temperature for listeria is 38°C. The range of temperatures over which listeria is observed to have growth is 3 to 45°C. Welshimer and Donker-Voet (12) explained the enhanced survival of listeria in natural environments, by the psychrotrophic nature of the organism. These psychrotrophic properties of listeria have been applied in isolation procedures. Hayes et al. (7) found that the best isolation procedure for *L. monocytogenes* from raw milk included a dilution of the milk, then a primary enrichment at 4°C for one month, followed by a secondary enrichment and selective plating.

*L. monocytogenes* is a very resilient organism. It has been shown to survive to the final product when added to pasteurized milk used to make nonfat dry milk (4) and cottage cheese (10). In the manufacture of these products, processing steps could allow for the proliferation of *L. monocytogenes* if it were present as a contaminant.

The incidence of *L. monocytogenes* in raw milk is approximately 4.2%, and is generally found at a concentration of less than one organism per ml (9). The interaction of the organism and pasteurized milk has also been studied. When *L. monocytogenes* was added to sterile skim milk, the peak of the log phase was reached in 10 d at 6°C. At this population, the milk did not have any overt spoilage characteristics and could have been mistaken for good milk, based on aesthetic characteristics alone (3). An outbreak of listeriosis in Massachusetts in 1983 was associated with consumption of whole and 2% pasteurized milk. To study the potential for post-pasteurization contamination, the dairy plant was thoroughly examined: no listeria was found. The raw milk coming into the plant was not clarified, and the potential exists that intracellular lipid-shielded *L. monocytogenes* might have been able to survive the pasteurization process (5).

Post-pasteurization contamination may come from many sources. One area that has not been examined extensively is the coolant system used in the HTST pasteurizer. Currently, there are no regulations governing pressure differentials between milk and coolant in the cooling part of the HTST unit. Ideally, a higher pressure should be maintained on the milk side than on the coolant side so that if there are any pinholes or cracks in the coolant plates, the direction of flow will be from milk to coolant. This is especially important since these small holes may be very hard to detect.

Studies in our laboratory and by Ginn et al. (6) demonstrated the incidence and survival of psychro­ trophs in both sweet water and glycol from dairies across the United States. Although *L. monocytogenes* was not found in any of the coolant samples examined (8), other psychrophils and pathogens including *Yersinia enterocolitica* and *Salmonella typhimurium* were found.

Zottola and Smith (14) studied the growth of *Salmonella*
in coolants. Survival of the organism in cold water was demonstrated for 8 d at 1°C. Concentrations of glycol solutions in excess of 12% were inhibitory to the growth of *Salmonella*, but the organism was not inactivated. Survival of *Salmonella* in a nutrient-deficient solution of 0.1% peptone was observed for 11 months at 7°C (2). It was the goal of this research to characterize the growth of *L. monocytogenes* in various media that may mimic those found in high-temperature-short-time (HTST) milk pasteurization coolant systems.

**MATERIALS AND METHODS**

To simplify this study, it was divided into three separate experiments. The initial study was done in the fall of 1986; growth in minimal media and in glycol were done in winter and spring of 1987, respectively. All cultures of *L. monocytogenes* used in this study were obtained from the Food and Drug Administration microbiological laboratory in Minneapolis, Minnesota and their identity was confirmed by gram and catalase reactions and wet mounts. Inocula were prepared by placing 1 loopful of the stock culture in 10 ml of tryptic soy broth (Difco) with 0.6% yeast extract (Difco) and incubating at 35°C for 18 h. Before inoculation into the broths, the 18-h-old culture was enumerated by the pour plate procedure (11) using Plate Count Agar.

**Initial study**

Solutions of 0.1 and 0.01% nonfat dry milk (NFDM), 0.1 and 0.01% peptone, 30% propylene glycol, and 30% propylene glycol with 0.01% NFDM were sterilized at 121°C for 15 min. These media were inoculated with 6000 *L. monocytogenes* Scott A/ml. Initially, the glycol was a 30% solution of propylene glycol and was obtained from the stock supply in our laboratory. These broths were placed in a circulating water bath at 4°C (Model 2067, Bath/Circulator, Forma Scientific, Division Mallinkrodt, Inc., Marietta, OH 45750). The bath had a capacity of 26 L and could range in temperature from -20 to 70°C. A single stage pump circulated the water in the bath at a rate of 10 L per minute. After 3 weeks, the temperature was increased to 7°C because initial numbers declined sharply. Populations were determined by the pour plate procedure (11) using Plate Count Agar every 2-3 d, then weekly after the populations stabilized (in approximately 2 weeks), for a period of 16 weeks at 4°C.

**Growth in minimal media**

A minimal medium was defined as that which contained less than the optimal concentration of nutrients. Sterile solutions of 0.01% peptone, 0.01% NFDM, 30% propylene glycol with 0.01% NFDM, and 1% tryptic soy broth (TSB, Difco) were inoculated with 3500 *L. monocytogenes* Jalisco cheese/ml. The inoculated media were placed in a circulating water bath at 4°C. Populations were determined weekly, as previously described, for a period of 10 weeks.

**Growth in glycol**

Sterile solutions of 0.1% NFDM, 10% propylene glycol with 0.1% NFDM, 20% propylene glycol with 0.1% NFDM, and 30% propylene glycol with 0.1% NFDM were inoculated with 8800 organisms of *L. monocytogenes* Jalisco cheese/ml. Before inoculation, the identification of this isolate was reconfirmed as *L. monocytogenes* by biochemical and serological tests. The inoculated broths were placed in a circulating water bath at 4°C. Populations were determined weekly, as previously described, for a period of 10 weeks.

**RESULTS**

**Initial study**

In all six broths used in this initial experiment, no growth was observed after 3 weeks, and initial populations declined. The temperature was then increased to 7°C. Figures 1, 2, and 3 demonstrate growth of listeria in these broths. After 5 weeks, there were greater than 10⁹ organisms per milliliter in both concentrations of peptone and NFDM. Growth was greater in NFDM than peptone, approaching 10⁹ organisms per milliliter and at the higher concentrations of both media. There was limited survival in 30% glycol with 0.01% NFDM, and no survival in the 30% glycol solution. Negative
controls were done with each experiment and consisted of an uninoculated flask containing sterile tryptic soy broth (Difco) incubated with the experimental media. No growth was observed in this negative control.

Growth in minimal media

Once again, it was demonstrated that *L. monocytogenes* was able to grow better in NFDM than peptone. Data from this experiment are presented in Fig. 4. In this experiment, listeria was not able to survive in a 30% solution of glycol. Little growth was observed in the peptone broth; instead, the population fluctuated around 10^2 organisms per milliliter. The 1.0% TSB medium served as a control. In TSB, *L. monocytogenes* was able to grow to a maximum of >10^9 organisms per milliliter, with a slow decline after 7 weeks.

DISCUSSION

The dilute solutions of nonfat dry milk and peptone may represent conditions found in a sweet water coolant system. In this study, sweet water was defined as refrigerated potable water. Glycol, as used by the dairy industry, is a propylene glycol solution, at approximately 30% concentration. In this series of experiments, nonfat dry milk was added to the coolants to simulate conditions of milk leaking into the coolant if small holes were present in the high-temperature short-time pasteurizer (HTST) plates. It has generally been assumed that no organisms will grow in these coolants, but results from our laboratory (8) indicate that there is a great deal of contamination.

In this study, survival of *L. monocytogenes* was seen in both concentrations of NFDM and peptone. More growth was demonstrated at the 0.1% level of each of these media. Previous results from our laboratory (14) have indicated that organisms are inhibited in concentrations of propylene glycol greater than 12%. This characteristic was also demonstrated with listeria, since only limited survival of the organism was seen in concentrations of glycol greater than 10%. In this study, dilution of the glycol while plating would com-
pensate for any potentially inhibitory effects. So, there
appears to be less of a threat of \textit{L. monocytogenes}
contamination in glycol systems, since most dairies use their
glycol at a concentration of approximately 30\% \cite{1}.

However, in a survey of dairy processing plants across
the United States \cite{8}, it was found that those dairies with
glycol cooling systems represented only about 12\% of plants
surveyed. In light of this information, it appears that there
should be more concern over the presence of \textit{L. monocytogenes}
in coolants. The effect on \textit{L. monocytogenes}
of different sanitizers which may be added to the coolants is
not known, and this may play a role in the inhibition of the
organism. To decrease the potential for contamination with
\textit{L. monocytogenes}, dairy plant personnel should frequently
examine the plates in the cooling section of the HTST for
small holes or cracks. Since these holes are often hard to
detect, the added protection of a higher pressure on the milk
side of flow should be maintained.

Because \textit{listeria} is such a ubiquitous organism, it could
come from many sources within the dairy processing plant.
If \textit{L. monocytogenes} were present in the coolant, these results
indicate that it is likely that the organism would survive with
the potential to contaminate the milk supply flowing through
the HTST pasteurizer.

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