Journal of Food Protection, Vol. 52, No. 12, Pages 852-855 (December 1989)

Growth of *Listeria monocytogenes* in the Presence of *Pseudomonas fluorescens* at 7 or 13°C in Skim Milk

SEHAM A. FARRAG and ELMER H. MARTH[1]*

Department of Food Science and The Food Research Institute, University of Wisconsin-Madison, Madison, Wisconsin 53706

(Received for publication April 10, 1989)

ABSTRACT

Autoclaved samples of skim milk were inoculated with *Listeria monocytogenes* (strain Scott A, California or V7), *Pseudomonas fluorescens* (strain P26 or B52), or a combination of *L. monocytogenes* plus *P. fluorescens*, and incubated at 7 or 13°C for 8 weeks. McBride Listeria Agar was used to determine populations of *L. monocytogenes* (at 0, 7, 14, 28, 42, or 56 d), and *Pseudomonas* isolation agar to enumerate *P. fluorescens*. Growth of *L. monocytogenes* was somewhat enhanced after 7 d of incubation at 7 but not at 13°C in the presence of pseudomonads. However, after 14 d and until the end of the incubation period (56 d), slight inactivation of *L. monocytogenes* in the presence of *P. fluorescens* was observed. *L. monocytogenes* did not affect growth or survival of *P. fluorescens*; also, no marked changes in pH of the milk were caused either by *L. monocytogenes* alone or by *L. monocytogenes* plus *P. fluorescens*.

Listeria monocytogenes is a gram-positive, nonspore-forming, psychrotrophic, rod-shaped bacterium which is widely distributed in the environment. This organism quite likely has been present in our environment and food supply for years. Reports of bacteria resembling *Listeria* appear in the scientific literature beginning as early as 1896, but a complete description of the organism we now recognize as *L. monocytogenes* was not published until 1926 *(6)*.

L. monocytogenes can cause illness in a variety of animals and in humans, particularly individuals in certain high risk groups *(9)*. High risk individuals include immunocompromised hosts such as patients undergoing chemotherapy for cancer treatment or individuals receiving corticosteroids to prevent organ transplant rejection, pregnant females, and infants. Normal healthy humans who have no underlying illness appear to be relatively resistant to acquiring a listeric infection.

The ability of *L. monocytogenes* to grow at low temperatures is a characteristic which is not common among animal pathogens. This peculiarity has been exploited in procedures to isolate this organism from clinical specimens *(4,6)*. Psychrotrophic properties of *L. monocytogenes* also affect its pathology and epidemiology; growth at low temperatures increases the amount of its agent which affects monocyte production *(1)* and enhances its survival in natural environments *(19)*. This psychrotrophic nature has been firmly documented *(4,7,8,10,14,18,20)*. *L. monocytogenes* not only survives but grows at temperatures as low as 3°C in tryptose phosphate broth *(8)*, 4°C in milk *(4,14)*, and at 0°C in sterile meat after 16-20 d *(10)*. Therefore, cold storage, as is provided for most dairy products, can not be depended on to prevent growth of the bacterium if a product becomes contaminated with *L. monocytogenes*.

Psychrotrophic spoilage organisms present in milk and milk products usually come from soil, water, and vegetation and are primarily in the genus *Pseudomonas (2,17)*. Often raw milk destined for processing is held refrigerated for an extended time (3 to 4 d) before pasteurization. During this time, psychrotrophic bacteria grow and cause significant chemical changes in the milk. Another concern is the frequent appearance of psychrotrophic bacteria in pasteurized dairy products as post-processing contaminants. *L. monocytogenes* could occur in raw milk as a component of the normal psychrotrophic microflora or in pasteurized milk most likely as a post-processing contaminant.

Pseudomonads can enhance growth of nonpathogenic *(3,11)* and pathogenic *(5,15)* bacteria in dairy products. Since psychrotrophs, and especially pseudomonads, are in milk as natural contaminants, and since milk also can be contaminated with *L. monocytogenes*, it is important to know how the fate of each bacterium is affected by the other one. Hence, this study was done to learn about interactions between both organisms in skim milk during extended storage at 7 or 13°C.

MATERIALS AND METHODS

Preparation of cultures and samples

Three strains of *L. monocytogenes* were used in these experiments. Included were Scott A (clinical isolate, serotype 4b), V7 (milk isolate, serotype 1) both provided by R. M. Twedt, Food and Drug Administration, Cincinnati, OH, and California (serotype 4b isolate from Mexican-style cheese) obtained from Silliker Laboratories, Inc., Carson, CA. Stock cultures were maintained through bimonthly transfer on Tryptose Agar (TA) (Difco Laboratories, Detroit, MI) and storage at 4°C. To prepare

[1]*Present address: Department of Food Science, University of Wisconsin, 1605 Linden Drive, Madison, WI 53706*

for an experiment, inocula from stock cultures were transferred to Tryptose Broth (TB) (Difco), and incubated for 24 h at 35°C under normal atmospheric conditions. Subsequent transfers of these cultures to new TB were then made followed by incubation as just described. Inocula (0.1 ml) from these second TB cultures were then added to 500 ml screw-capped Erlenmeyer flasks, each containing 200 ml of autoclaved (15 min, 121°C) skim milk. Following incubation for 24 h at 35°C, another 0.1 ml transfer was made into two more flasks of sterile skim milk followed by incubation for 48 h at 35°C. One of these cultures was diluted to yield between 1 x 10^5 and 3 x 10^5 *L. monocytogenes*/ml as starting inoculum for test substrates.

Preparation of Pseudomonas *cultures*

Pseudomonas fluorescens strains P26 and B52 were obtained from Robin McKellar of the Food Research Institute, Ottawa, Canada. Both were initially isolated from milk and can grow at refrigeration temperatures. Stock cultures were maintained through bimonthly transfers on Plate Count Agar (PCA) (Difco) and storage at 4°C. Activation of cultures and inoculation of samples were done as with *Listeria* cultures, but the incubation temperature was 25°C. The following pure or mixed cultures of these microorganisms in 200 ml of sterile skim milk were prepared: *(a) L. monocytogenes* strain Scott A, *(b) L. monocytogenes* strain California, *(c) L. monocytogenes* strain V7, *(d) P. fluorescens (e) L. monocytogenes* strain Scott A + *P. fluorescens, (f) L. monocytogenes* strain California + *P. fluorescens,* and *(g) L. monocytogenes* strain V7 + *P. fluorescens.* Cultures were held at 7 or 13°C for 56 d.

Enumeration of L. monocytogenes *and* P. fluorescens

Flasks were agitated just before sampling, and 1 ml portions from each well-mixed culture were appropriately diluted in sterile 0.5% peptone (Difco) solution. One-tenth milliliter of specific dilutions was streaked onto McBride Listeria Agar (MLA) *(13)* and similarly onto *Pseudomonas* Isolation Agar (Difco).

RESULTS AND DISCUSSION

Growth of *L. monocytogenes* strains Scott A, California, or V7 was somewhat enhanced in the presence of *P. fluorescens* P26 after 7 d of incubation at 7°C (Fig. 1). These results are similar to those observed by Marshall and Schmidt *(12)* when they studied growth of *L. monocytogenes* at 10°C in milk pre-incubated with selected pseudomonads. In contrast, by 14 d, the populations of all strains of *L. monocytogenes* decreased in the presence of *P. fluorescens* P26, when compared to populations of *L. monocytogenes* grown by itself. Also, *P. fluorescens* P26, with or without *L. monocytogenes* present, grew well in skim milk at 7°C throughout the entire incubation period (Fig. 2).

At 13°C, growth of *L. monocytogenes* decreased slightly in the presence of *P. fluorescens* P26 after 7d compared to growth of the pathogen by itself. By 14 d, and for the remainder of the incubation period, the inhibitory effect of *P. fluorescens* P26 on *L. monocytogenes* was moderate; this is true for all strains of *L. monocytogenes* used in these experiments (Fig. 3).

Results in Fig. 4 indicate there was no marked change in growth or survival of *P. fluorescens* P26 whether or not

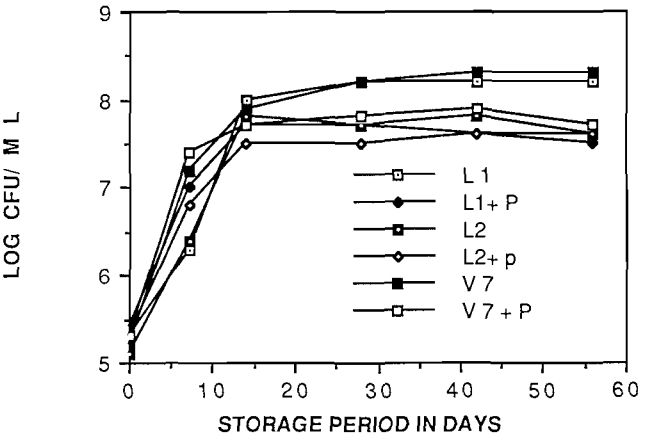

Figure 1. *Behavior of* L. monocytogenes *alone and in the presence of* P. fluorescens *P26 in skim milk held at 7°C for 56 d.* L_1 = L. monocytogenes *strain Scott A,* L_2 = L. monocytogenes *strain California, V7* = L. monocytogenes *strain V7 and P* = P. fluorescens.

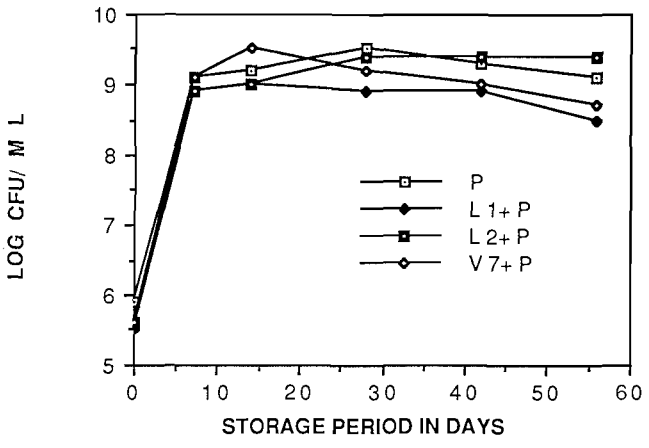

Figure 2. *Behavior of* P. fluorescens *P26 in the presence of* L. monocytogenes *in skim milk held at 7°C for 56 d. See Fig. 1 for explanation of symbols.*

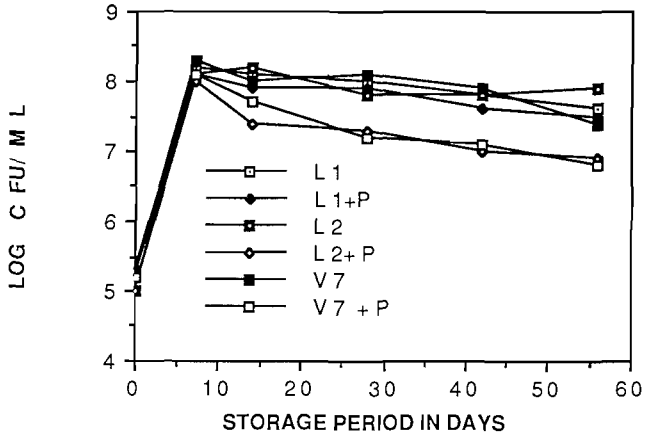

Figure 3. *Behavior of* L. monocytogenes *alone and in the presence of* P. fluorescens *P26 in skim milk held at 13°C for 56 d. See Fig. 1 for explanation of symbols.*

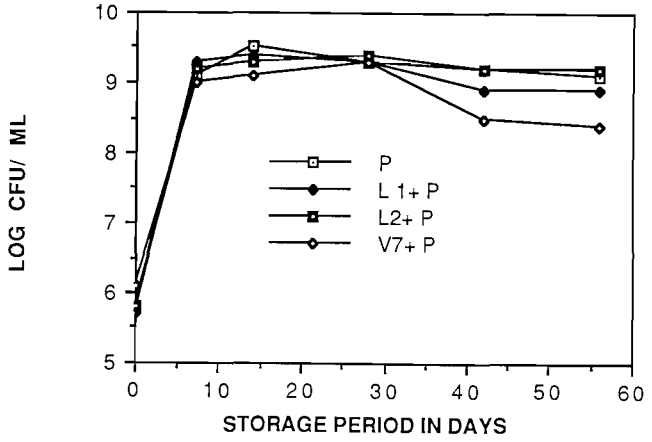

Figure 4. *Behavior of* P. fluorescens *P26 in the presence of* L. monocytogenes *in skim milk held at 13°C for 56 d. See Fig. 1 for explanation of symbols.*

L. monocytogenes was present. These results are in agreement with those of Marshall and Schmidt *(12)*.

Behavior of *L. monocytogenes* alone or in the presence of *P. fluorescens* B52 in skim milk held at 7°C also was examined (Fig. 5). Results indicate growth of *L. monocytogenes* strain Scott A or California was enhanced modestly in the presence of *P. fluorescens* B52 after 7 d of incubation. By the end of the incubation period there was a slight decrease in numbers of all strains of *L. monocytogenes* in mixed cultures compared to pure cultures. In contrast, strain V7 was inhibited somewhat at 7°C after 7 d of incubation in the presence of *P. fluorescens* B52. The extent of inhibition was greater at the end of the incubation period in mixed rather than pure cultures.

Proteolytic activity of *Pseudomonas* spp. in milk is generally thought to be responsible for providing substances responsible for enhanced growth or activity of other organisms. Furthermore, proteolytic enzymes of pseudomonads are heat stable and can survive pasteurization *(3)*. Speck *(16)* indicated that variation in lactic acid production in milk by lactic cultures may result from inhibitory substances produced by growth of antagonistic bacteria such as psychrotrophs. Initial numbers of *P. fluorescens* B52 were approximately the same for all treatments and control; this also was true after 56 d of incubation, regardless of whether or not *L. monocytogenes* was present (Fig. 6).

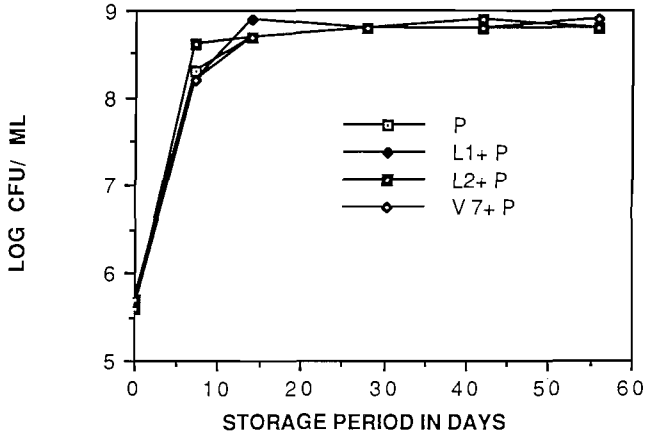

Figure 6. *Behavior of* P. fluorescens *B52 in the presence of* L. monocytogenes *in skim milk held at 7°C for 56 d. See Fig. 1 for explanation of symbols.*

At 13°C, populations of all *L. monocytogenes* strains in the presence of *P. fluorescens* B52 were somewhat less than when the pseudomonad was absent (Fig. 7). However, populations of all *L. monocytogenes* strains began to decrease more in the presence of *P. fluorescens* B52 than in its absence and continued to do so for the remainder of the incubation period (Fig. 7).

Growth or survival of *P. fluorescens* B52 was not affected appreciably by the presence of *L. monocytogenes* (Fig. 8). Generally, growth of all *L. monocytogenes* strains

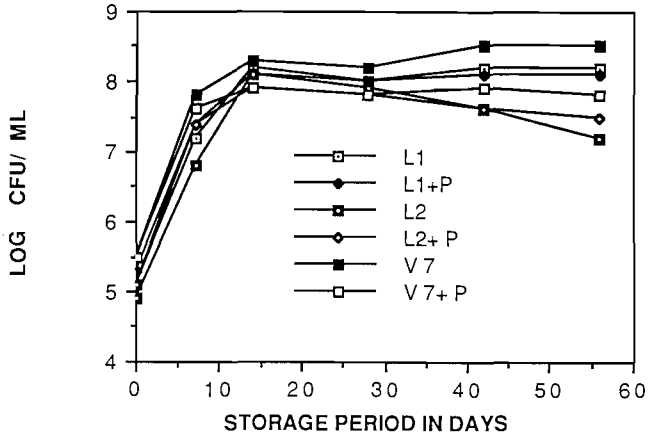

Figure 5. *Behavior of* L. monocytogenes *alone and in the presence of* P. fluorescens *B52 in skim milk held at 7°C for 56 d. See Fig. 1 for explanation of symbols.*

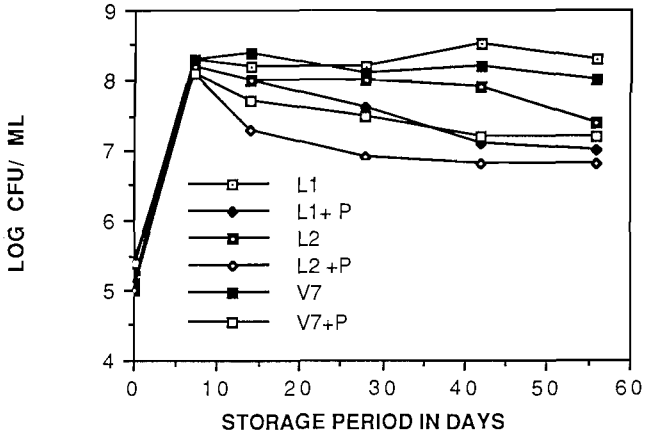

Figure 7. *Behavior of* L. monocytogenes *in the presence of* P. fluorescens *B52 in skim milk held at 13°C for 56 d. See Fig. 1 for explanation of symbols.*

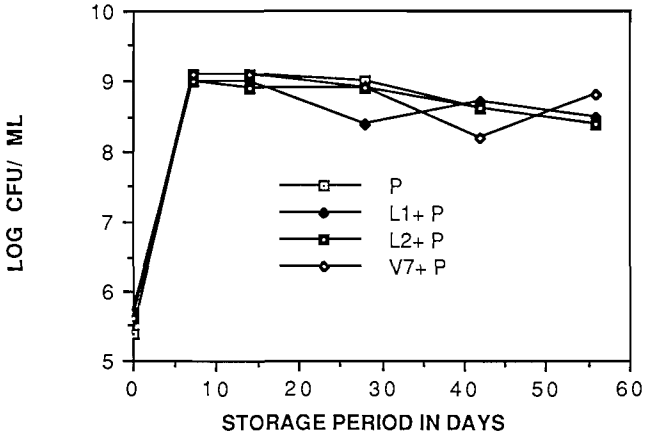

Figure 8. *Behavior of* P. fluorescens *B52 in the presence of* L. monocytogenes *in skim milk held at 13°C for 56 d. See Fig. 1 for explanation of symbols.*

in skim milk at 13°C was better than at 7°C; these results are in agreement with those Rosenow and Marth *(14)* obtained when they studied growth of *L. monocytogenes* in whole and skim milk at different temperatures.

Measurement of pH at the beginning and end of experiments revealed no marked changes during the incubation period either for pure or mixed cultures (data not shown). In no instance did the pH drop by more than 0.4 unit by the end of incubation at 7 or 13°C.

Results of this study suggest that *L. monocytogenes* can grow in the presence of other common psychrotrophic bacteria in milk. Also, growth and survival of the *Pseudomonas* species used in this study was not affected by the presence of *L. monocytogenes* in milk. Moreover, our results indicate that the limited inhibitory effect of pseudomonads was most evident at 13 rather than at 7°C. However, *L. monocytogenes* proved to be an effective competitor in the presence of *P. fluorescens*.

ACKNOWLEDGMENTS

Research supported by the College of Agricultural Life Sciences, University of Wisconsin-Madison and by the Wisconsin Milk Marketing Board, Madison, WI.

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