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Behavior of *Listeria monocytogenes* when Incubated Together with *Pseudomonas* Species in Tryptose Broth at 7 and 13°C

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ABSTRACT

Tryptose broth (TB) was inoculated with *Listeria monocytogenes* (strain Scott A or California), *Pseudomonas aeruginosa*, *Pseudomonas flourescens*, or a combination of *L. monocytogenes* plus *Pseudomonas* species, and incubated at 7 or 13°C for 8 weeks. McBride Listeria Agar was used to determine numbers of *L. monocytogenes* and *Pseudomonas* Isolation Agar to enumerate *Pseudomonas* species at 0, 7, 14, 28, 42, or 56 d. At 13°C, presence of *P. fluorescens* had a slight negative effect on growth of *L. monocytogenes* strain Scott A, and was somewhat detrimental to its survival during the extended incubation. Growth of *L. monocytogenes* strain California was retarded by presence of *P. fluorescens* although the maximum population achieved by the pathogen was greater in the presence rather than absence of the pseudomonad; the pseudomonad did have a negative effect on survival of the pathogen. At the same temperature, *P. aeruginosa* had a negative effect on survival of *L. monocytogenes* strain California, but had essentially no effect on the other strain of the pathogen. Neither strain of *L. monocytogenes* affected growth of *P. fluorescens* nor *P. aeruginosa*. At 13°C the pH of TB generally decreased when *L. monocytogenes* grew by itself but increased when either pseudomonad grew by itself or together with the pathogen. At 7°C, growth of both pseudomonads was minimal. Presence of non-growing cells of *P. fluorescens* retarded somewhat growth of both *L. monocytogenes* strains early during the incubation. *P. aeruginosa* had no detectable effect on either strain of *L. monocytogenes*. The pH of TB decreased when *L. monocytogenes* grew by itself or together with either pseudomonad, and remained unchanged in TB inoculated with either pseudomonad.

Listeria monocytogenes is a pathogenic bacterium that was responsible for several food-related outbreaks of disease or has contaminated foods which have been recalled from the marketplace *(1,2,3,4)*. The pathogen is psychrotrophic (i.e. it can grow at or below 7°C); accordingly, refrigerated storage does not completely control growth of this bacterium. The ability of bacteria to grow at refrigeration temperatures was demonstrated by Foster in 1887 *(10)*, and such bacteria are ubiquitous in various natural habitats *(15,16,17,20,24)*.

The psychrotrophic nature of *L. monocytogenes* has been firmly documented *(9,13,14,21,25)*, although the temperature range for its optimum growth is 30-37°C *(12)*. The bacterium not only survives, but grows at temperatures as low as 3°C in tryptose phosphate broth *(14)*, 4°C in milk *(9,11,12)*, and 0°C in sterile meat after 16-20 d of storage *(18)*. *L. monocytogenes* also can withstand freezing temperatures, as is shown by its recent discovery in bulk ice cream and ice cream novelties *(5,6)*.

Cold storage selects for psychrotrophic bacteria that are able to grow, and carry out metabolic activities at or below 7°C. Hence, with the increased use of refrigerated storage of food, growth of these bacteria has become of great importance in the food industry over the last 30 years *(8)*. In general, psychrotrophic microorganisms are important because they can cause spoilage in foods. Furthermore, some foods such as milk and meat products can be contaminated by *L. monocytogenes*. Consequently, *L. monocytogenes* and other psychrotrophic bacteria such as *Pseudomonas* are likely to be present together in some raw foods or through post-heating contamination of products such as pasteurized milk. Hence, it is important to know if either has an affect on growth of the other. Consequently, this study was done to investigate the behavior of *L. monocytogenes* and of *Pseudomonas* spp. when incubated alone and in the presence of each other at 7 or 13°C for up to 8 wk.

MATERIALS AND METHODS

Cultures

Two strains of *L. monocytogenes*, Scott A (clinical isolate, serotype 4b, 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) were inoculated separately into Tryptose Broth (TB) (Difco) and incubated at 35°C for 24 h *(7)*. The number of cells/ml of the cultures was adjusted by dilution; a sufficient volume of the diluted culture was dispersed in 25 ml of TB to yield desired initial levels of *L. monocytogenes* in test substrates.

Pseudomonas aeruginosa ATCC 10145 and *P. fluorescens* (McCoy strain) were obtained from the culture collection of the Food Microbiology Laboratory in the Department of Food Science, University of Wisconsin-Madison. *Pseudomonas* species were each inoculated separately into TB and incubated at 35°C for 24 h. The number of cells/ml of the cultures was adjusted by dilution; a sufficient volume of the diluted culture was dispersed in 25 ml of TB to yield desired initial levels of *Pseudomonas* species in test substrates. The following pure or mixed cultures of the test

bacteria, in 25 ml of TB, were prepared: *(a) L. monocytogenes* strain Scott A, *(b) L. monocytogenes* strain California, *(c) P. fluorescens, (d) P. aeruginosa, (e) L. monocytogenes* strain Scott A + *P. fluorescens, (f) L. monocytogenes* strain Scott A + *P. aeruginosa, (g) L. monocytogenes* strain California + *P. fluorescens,* and *(h) L. monocytogenes* strain California + *P. aeruginosa.* These cultures were held at 7 or 13°C. All cultures were sampled after 0, 7, 14, 28, 42, and 56 d to determine populations of the bacteria.

Enumeration of L. monocytogenes *and* Pseudomonas *species*

One-milliliter portions from each well-mixed culture were appropriately diluted in sterile 0.5% peptone (Difco) buffer, followed by duplicate surface plating of 0.1 ml of specific dilutions on McBride Listeria Agar (MLA) *(19). Pseudomonas* Isolation Agar (Difco) was used to enumerate *Pseudomonas* spp. Both agar media were incubated at 35°C for 48 h. After plate counts were obtained, averages were calculated, and results are given as the $\log_{10}$ of such values.

RESULTS AND DISCUSSION

Behavior of L. monocytogenes *in the presence of* P. fluorescens

The population achieved by *L. monocytogenes* strain Scott A after 7 d at 13°C was quite similar with or without the presence of *P. fluorescens* (Table 1). However, the fold-increase in mixed cultures was less than in pure cultures. The decrease in population of viable cells began sooner and was greater in magnitude during the extended incubation period when *P. fluorescens* was present rather than absent. In contrast to this, a somewhat higher population of *L. monocytogenes* strain California developed in the presence rather than in the absence of *P. fluorescens* but the fold-increase was less (Table 1). Aslo, the population of this strain of the pathogen was more stable than that of the other during the prolonged incubation regardless of whether or not *P. fluorescens* was present. Growth of *P. fluorescens* at 13°C did not appear to be affected appreciably by the presence of either strain of *L. monocytogenes.* Reducing the incubation temperature to 7°C prohibited growth of our strain of *P. fluorescens* (Table 2). There was a reduction in the population of viable cells of *P. fluorescens* during the prolonged incubation period, but this decrease did not seem to be related to the absence or presence of either strain of *L. monocytogenes* in the culture (Table 2).

Growth of both strains of *L. monocytogenes* at 7°C was retarded somewhat during the first 7 d of incubation when *P. fluorescens* was present in the culture, even though the pseudomonad was not actively growing. This effect was not evident at 14 d and beyond when populations of both strains of *L. monocytogenes* growing by themselves were similar to populations developed by the strains when *P. fluorescens* was present (Table 2).

At 7°C, both strains of *L. monocytogenes* reduced the pH of the TB, strain Scott A did more so than strain California, regardless of whether or not *P. fluorescens* was present in the culture (Table 3). Incubation at 13°C was accompanied by a similar decrease in pH when the strains of *L. monocytogenes* grew by themselves. However, at this temperature *P. fluorescens* grew (Table 1) and the pH of cultures of the pseudomonad by itself and together with *L. monocytogenes* increased by more than one pH unit during the incubation period (Table 3). Absence of growth by the pseudomonad (Table 2) caused the pH results observed at 7°C.

Behavior of L. monocytogenes *in the presence of* P. aeruginosa

Presence of *P. aeruginosa* had little or no effect on the population of *L. monocytogenes* strain Scott A during 56 d at 13°C (Table 4). In contrast, beginning at 7 d of incubation, the pseudomonad had a detrimental effect on survival of *L. monocytogenes* strain California. Presence of either strain of *L. monocytogenes* had little or no effect, when compared to the control, on the population of *P. aeruginosa* that developed and then persisted during the incubation period (Table 4).

P. aeruginosa failed to grow appreciably at 7°C, but a major portion of the initial population in all cultures survived through at least 42 d of incubation (Table 5). The viable but non-growing cells of the pseudomonad had little or no effect on growth and survival of either strain of *L. monocytogenes.* Changes in pH of TB cultures at 7 and 13°C (Table 6) were similar to those noted with *P. fluorescens* (Table 3).

Earlier work by Cousin and Marth *(8)* showed that preculturing pasteurized milk with psychrotrophic bacteria served to enhance acid production (and growth) by lactic acid bacteria in such milk. This was attributed to increased availability of nitrogen to the lactic acid bacteria through

TABLE 1. *Behavior of* Listeria monocytogenes *and* Pseudomonas fluorescens *alone and in the presence of each other in tryptose broth held at 13 °C.*

Incubation time (d)	Log10 cfu/ml (fold increase) on McBride Listeria Agar[a]				Pseudomonas Agar[a]		
	L_1[b]	L_1 + P[c]	L_2[d]	L_2 + P	P	L_1 + P	L_2 + P
0	4.9	5.3	4.0	5.1	5.1	4.9	4.9
7	9.2 (17,000)	9.2 (7,200)	8.9 (66,000)	9.1 (9,800)	8.8 (6,100)	8.3 (2,300)	7.9 (800)
14	9.3 (22,000)	8.9 (4,000)	8.7 (45,000)	9.4 (19,000)	9.5 (30,000)	9.1 (14,000)	9.2 (16,000)
28	8.9 (10,000)	8.2 (730)	8.8 (60,000)	9.5 (28,000)	10.2 (150,000)	9.8 (63,000)	9.6 (40,000)
42	8.3 (2,400)	7.9 (450)	8.7 (54,000)	9.1 (12,000)	9.7 (41,000)	9.3 (21,000)	9.4 (27,000)
56	8.1 (1,400)	7.3 (98)	8.6 (34,000)	8.4 (2,100)	8.4 (2,200)	8.2 (1,900)	8.7 (5,500)

[a]Average of four trials.
[b]L_1 = *L. monocytogenes* strain Scott A.
[c]P = *P. fluorescens* strain McCoy.
[d]L_2 = *L. monocytogenes* strain California.

TABLE 2. *Behavior of* Listeria monocytogenes *and* Pseudomonas fluorescens *alone and in the presence of each other in tryptose broth held at 7°C.*

Incubation time (d)	Log$_{10}$ cfu/ml (fold increase) on McBride Listeria Agar[a]				Pseudomonas Agar[a]		
	L_1[b]	L_1 + P[c]	L_2[d]	L_2 + P	P	L_1 + P	L_2 + P
0	4.5	4.9	3.6	5.1	4.9	5.0	4.9
7	8.4 (7,800)	7.5 (340)	8.4 (62,000)	6.8 (48)	5.0 (1.1)	5.0 (0)	5.0 (1.1)
14	8.9 (21,000)	8.9 (10,000)	8.3 (53,000)	8.4 (1,900)	4.8 (-0.65)	4.9 (-0.83)	5.0 (1.1)
28	8.8 (18,000)	8.8 (7,300)	8.7 (140,000)	8.5 (3,200)	4.8 (-0.65)	4.7 (-0.50)	4.7 (-0.57)
42	8.7 (16,000)	8.5 (3,600)	8.7 (140,000)	8.4 (2,300)	4.7 (-0.54)	4.7 (-0.50)	4.7 (-0.57)
56	8.4 (6,400)	8.2 (2,000)	8.3 (56,000)	8.3 (1,700)	4.4 (-0.29)	4.4 (-0.24)	4.4 (-0.32)

[a]Average of four trials.
[b]L_1 = *L. monocytogenes* strain Scott A.
[c]P = *P. fluorescens* strain McCoy.
[d]L_2 = *L. monocytogenes* strain California.

TABLE 3. *Changes in pH caused by* L. monocytogenes, Pseudomonas fluorescens *or* Listeira monocytogenes *plus* Pseudomonas fluorescens *incubated in tryptose broth at 7 or 13°C.*[a]

Incubation time (d)	pH at 7°C					pH at 13°C				
	L_1[b]	L_1 + P[c]	L_2[d]	L_2 + P	P	L_1	L_1 + P	L_2	L_2 + P	P
0	7.13	7.13	7.12	7.14	7.14	7.14	7.15	7.16	7.16	7.16
7	5.92	6.82	6.10	6.65	7.11	5.43	6.16	5.60	6.58	7.40
14	5.31	5.42	6.07	6.06	7.05	5.48	6.99	5.83	7.12	7.61
28	5.30	5.48	5.84	5.82	5.98	5.54	7.52	5.99	7.47	7.69
42	5.40	5.41	6.08	5.60	7.16	5.71	8.09	6.18	8.08	8.04
56	5.60	5.65	6.80	6.30	7.26	5.63	8.34	6.34	8.30	8.13

[a]Average of four trials.
[b]L_1 = *L. monocytogenes* strain Scott A.
[c]P = *P. fluorescens* strain McCoy.
[d]L_2 = *L. monocytogenes* strain California.

TABLE 4. *Behavior of* Listeria monocytogenes *and* Pseudomonas aeruginosa *alone and in the presence of each other in tryptose broth held at 13°C.*

Incubation time (d)	Log$_{10}$ cfu/ml (fold increase) on McBride Listeria Agar[a]				Pseudomonas Agar[a]		
	L_1[b]	L_1 + P[c]	L_2[d]	L_2 + P	P	L_1 + P	L_2 + P
0	4.6	4.6	3.7	3.8	5.0	5.0	4.9
7	9.2 (41,000)	9.1 (33,000)	8.8 (107,000)	8.8 (99,000)	8.0 (1,200)	7.3 (200)	6.6 (56)
14	9.2 (41,000)	9.1 (33,000)	8.7 (85,000)	8.3 (33,000)	9.6 (43,000)	9.2 (14,000)	9.3 (34,000)
28	8.8 (15,000)	8.0 (2,300)	8.9 (135,000)	7.5 (4,800)	9.6 (43,000)	9.9 (81,000)	9.8 (76,000)
42	7.9 (2,300)	7.9 (1,900)	8.8 (114,000)	7.1 (1,900)	9.3 (23,000)	9.9 (81,000)	9.8 (76,000)
56	7.0 (240)	7.8 (1,400)	8.6 (78,000)	6.8 (1,000)	8.6 (4,700)	8.6 (4,000)	9.0 (14,000)

[a]Average of four trials.
[b]L_1 = *L. monocytogenes* strain Scott A.
[c]P = *P. aeruginosa.*
[d]L_2 = *L. monocytogenes* strain California.

TABLE 5. *Behavior of* Listeria monocytogenes *and* Pseudomonas aeruginosa *alone and in the presence of each other in tryptose broth held at 7°C.*

Incubation time (d)	Log$_{10}$ cfu/ml (fold increase) on McBride Listeria Agar[a]				Pseudomonas Agar[a]		
	L_1[b]	L_1 + P[c]	L_2[d]	L_2 + P	P	L_1 + P	L_2 + P
0	4.7	4.6	3.7	3.8	4.7	4.7	4.8
7	6.0 (23)	6.2 (38)	5.5 (65)	5.6 (67)	5.9 (3.5)	4.6 (-0.74)	4.8 (0)
14	9.0 (21,000)	8.8 (18,000)	8.3 (41,000)	8.2 (2,800)	4.5 (-0.93)	4.2 (-0.32)	43. (-0.27)
28	9.1 (28,000)	8.9 (21,000)	8.7 (94,000)	8.7 (92,000)	3.8 (-0.13)	3.5 (-0.1)	4.2 (-0.2)
42	9.4 (47,000)	9.3 (48,000)	8.8 (130,000)	9.0 (170,000)	3.5 (-0.07)	3.2 (-0.03)	4.0 (-0.2)
56	9.0 (24,000)	8.9 (23,000)	7.5 (62,000)	8.8 (110,000)	3.0 (-0.02)	2.9 (-0.02)	3.4 (-0.03)

[a]Average of four trials.
[b]L_1 = *L. monocytogenes* strain Scott A.
[c]P = *P. aeruginosa.*
[d]L_2 = *L. monocytogenes* strain California.

TABLE 6. *Changes in pH caused by* L. monocytogenes, Pseudomonas aeruginosa *or* Listeria monocytogenes *plus* Pseudomonas aeruginosa *incubated in tryptose broth at 7 or 13°C.*[a]

Incubation time (d)	pH at 7°C					pH at 13°C				
	L_1[b]	L_1 + P[c]	L_2[d]	L_2 + P	P	L_1	L_1 + P	L_2	L_2 + P	P
0	7.27	7.26	7.22	7.28	7.25	7.11	7.13	7.11	7.13	7.10
7	7.01	7.01	7.03	7.02	7.02	5.30	6.06	6.09	6.28	7.28
14	6.02	6.10	6.34	6.35	7.07	5.27	6.98	5.60	7.49	7.49
28	5.81	5.41	6.00	5.84	7.10	5.50	7.47	6.22	7.40	7.65
42	5.36	5.37	5.80	5.87	7.02	5.52	7.62	6.14	7.95	7.98
56	5.44	5.41	5.95	6.26	7.25	5.30	8.16	7.14	8.16	8.12

[a]Average of four trials.
[b]L_1 = *L. monocytogenes* strain Scott A.
[c]P = *P. aeruginosa.*
[d]L_2 = *L. monocytogenes* strain California.

partial degradation of milk proteins by the psychrotrophs. In this work, growth of the pseudomonad did not seem to improve the substrate for listeriae, probably because nitrogen-bearing substances did not need to be hydrolyzed to make them more readily available to the pathogen.

Schaack and Marth *(22)* incubated *L. monocytogenes* together with lactic acid bacteria and found that growth of the pathogen was markedly restricted. This restriction, at least in part, resulted from an appreciable lowering of the pH of the medium. Such a change did not occur in the present experiments.

The limited effect of psychrotrophic bacteria on *L. monocytogenes* probably resulted from some metabolites produced by those bacteria. Speck *(23)* indicated that variations in lactic acid production in milk by lactic cultures resulted from inhibitory substances produced by growth of antagonistic bacteria such as psychrotrophs.

In conclusion, growth and/or survival of *L. monocytogenes* at low temperatures was affected by presence of the psychrotrophic *Pseudomonas* spp. in the medium. In general, somewhat more activity was noted with *P. fluorescens* than with *P. aeruginosa* present in mixed cultures. The limited effects we noted were not related to incubation temperature, except for the absence of effects when pseudomonads failed to grow at 7°C. Results of this work have indicated, that *L. monocytogenes* is an effective competitor when in the presence of growing or non-growing cells of *Pseudomonas* spp. Finally, it should be mentioned that we used two selective agar media in this work and thus the actual number of both species of bacteria may have been underestimated. However, this is unlikely to have had an effect on the conclusions drawn from these experiments.

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REFERENCES

1. Anonymous. 1985. Listeriosis outbreak associated with Mexican-style cheese. California. Morbid. Mortal. Weekly Rep. 34:357-359.
2. Anonymous. 1985. Listeriosis transmitted by contaminated Jalisco brand cheese. Calif. Morbid. No. 46. November 22, 1985.
3. Anonymous. 1986. FDA finds *Listeria* in Brie cheese from French certified plant. Food Chem. News 27(50):35.
4. Anonymous. 1986. Brie cheese recalls extended by General Foods, U.S. importer. Food Chem. News 27(52):25.
5. Anonymous. 1986. Class I recall made of ice cream bars because of *Listeria.* Food Chem. News 28(19):31.
6. Anonymous. 1986. More ice cream recalled because of *Listeria.* Food Chem. News 28(35):25.
7. Bearns, R. E., and K. F. Girard, 1958. The effect of pasteurization on *L. monocytogenes.* Can J. Microbiol. 4:55-61.
8. Cousin, M. A., and E. H. Marth. 1977. Lactic acid production by *Streptococcus lactis* and *Streptococcus cremoris* in milk precultured with psychrotrophic bacteria. J. Food Prot. 40:406-410.
9. Donnelly, C. W., and E. H. Briggs. 1966. Psychrotrophic growth and thermal inactivation of *Listeria monocytogenes* as a function of milk composition. J. Food Prot. 49:994-998.
10. Foster, J. 1887. Über einige Eigenschaften leuchtender Bakterien. Zbl. Bakteriol. Parasitenk. 2:337-340.
11. Gitter, M., R. Bradely, and P. H. Blampied. 1980. *Listeria monocytogenes* infection in bovine mastitis. Vet. Rec. 107:390-393.
12. Gray, M. L., and A. H. Killinger. 1966. *Listeria monocytogenes* and listeric infections. Bacteriol. Rev. 30:309-371.
13. Hayes, P. S., J. C. Feeley, L. M. Graves, G. W. Ajello, and D. W. Fleming. 1986. Isolation of *Listeria monocytogenes* from raw milk. Appl. Environ. Microbiol. 51:438-440.
14. Hof, H., H. P. R. Seeliger, A. Schrettenbrunner, and S. Chatzipanagiotou. 1986. The role of *Listeria monocytogenes* and other *Listeria* spp. in foodborne infections. pp. 220-223. *In* Proc. 2nd World Congress, Foodborne Infections and Intoxications, Berlin, W. Germany.
15. Ingraham, J. L., and G. F. Bailey. 1959. Comparative effect of temperature on the metabolism of mesophilic and psychrophilic bacteria. J. Bacteriol. 77:609-613.
16. Kampelmacher, E. H., and L. M. van Noole Jansen. 1972. Further studies on the isolation of *L. monocytogenes* in clinically healthy individuals. Zbl. Bakteriol. Parasitenk. Infektionsk. Hyg., Abt. I. Orig. A 221:70-77.
17. Kampelmacher, E. H., E. D., Maas, and L. M. van Noorle Jansen. 1976. Occurrence of *Listeria monocytogenes* in feces of pregnant women with and without direct animal contact. Zbl. Bakteriol. Parasitenk. Infektionsk. Hyg., Abt. I. Orig. A 234:238-242.
18. Khan, M. A., C. V. Palmas, A. Seaman, and M. Woodbine. 1972. Survival versus growth of facultative psychrotroph. Acta Microbiol. Acad. Sci. Hung. 19:357-362.
19. McBride, M. E., and K. F. Girard. 1960. A selective method for the isolation of *Listeria monocytogenes* from mixed populations. J. Lab. Clin. Med. 55:153-157.
20. Morita, R. Y. 1975. Psychrophilic bacteria. Bacteriol. Rev. 39:146-167.
21. Rosenow, E. M., and E. H. Marth. 1987. Growth of *Listeria monocytogenes* in skim, whole, and chocolate milk, and in whipping cream during incubation at 4, 8, 13, 21, and 35°C. J. Food Prot. 50:452-459.
22. Schaack, M. M., and E. H. Marth. 1988. Behavior of *Listeria monocytogenes* in skim milk during fermentation with mesophilic lactic starter cultures. J. Food Prot. 51:600-606.
23. Speck, M. L. 1962. Starter culture growth and action in milk. J. Dairy Sci. 45:1281-1286.
24. Weiss, J., and H. P. R. Seeliger. 1975. Incidence of *Listeria monocytogenes* in nature. Appl. Microbiol. 30:29-32.
25. Wilkins, P. O., R. Bourgeois, and R. G. E. Murray. 1972. Psychrotrophic properties of *Listeria monocytogenes.* Can. J. Microbiol. 18:543-551.