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Improved *Listeria monocytogenes* Selective Agar

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By increasing the LiCl concentration to 5 g/liter and adding 20 mg of moxalactam per liter to modified McBride agar base, it was possible to inhibit the growth of many bacteria which interfered with the recovery of *Listeria monocytogenes* from beef.

Recent outbreaks of *Listeria monocytogenes* infections caused by the consumption of contaminated cole slaw (14), pasteurized milk (7), and cheese made with pasteurized milk (4) have raised concern about the effects on public health of this pathogen in our food supply. Fenlon (6) recovered *L. monocytogenes* from silage and wild birds. *L. monocytogenes* causes diseases in cattle and sheep (8) and was present in 12% of raw milk tested (9). However, our initial efforts using previously described methods (13, 16), to recover low numbers of *L. monocytogenes* inoculated into beef were unsuccessful owing to the profuse growth of numerous other bacterial colonies on McBride (12) and modified McBride (McB) (15) agar bases and on Difco Columbia CNA agar. It was observed that *L. monocytogenes* grew on Baird-Parker agar (1), which selects for *Staphylococcus aureus* but controls the growth of *Streptococcus* sp. Larsson et al. (11) reported that 175 clinical strains of *L. monocytogenes* were resistant to 128 μg or more per ml of moxalactam. Moxalactam has a very broad spectrum of activity against many gram-positive and gram-negative bacteria including *Staphylococcus*, *Proteus*, and *Pseudomonas* spp. (2, 17). An improved selective agar for *L. monocytogenes*, (LiCl-phenylethanol-moxalactam; LPM agar) was developed by combining the best features of Baird-Parker agar (1), McB agar (15), and moxalactam.

LPM agar consists of phenylethanol agar (Difco Laboratories) with 10 g of glycine anhydride (15) per liter and 5 g of LiCl per liter (1). Glycine inhibited *L. monocytogenes* in this agar. After the agar was autoclaved and cooled to 46°C, 20 mg of filter-sterilized moxalactam (Eli Lilly & Co.) per liter in 0.1 M potassium phosphate buffer (pH 6.0) was added. Thin agar plates with 10 to 12 ml/10-cm plate were prepared; these could be refrigerated for 2 weeks.

Possible *L. monocytogenes* colonies from LPM agar were selected with 45° oblique transillumination (10) after 16 to 20 h of incubation at 30°C. Oblique lighting was achieved with an inverted microscope (25×) or with a concave mirror to reflect a concentrated light source (AO 653H; American Optical Co.) and a thin Plexiglas stage of a dissecting microscope (15×). Even pure cultures of *L. monocytogenes* showed variations of size and blue to white iridescence on the same 20-h plate under oblique lighting. Older colonies tended to lose the distinctive coloration. For best results, the oblique lighting was readjusted each day with 20-h *L. monocytogenes* colonies as a reference.

Suspected colonies were screened for characteristic umbrella motility on Difco motility test medium (3) and for narrow β-hemolytic zones on a thin blood layer of CNA agar (a 10-ml base layer covered by a 5-ml top layer that contained 4% horse blood). Streaked surface colonies of *L. monocytogenes* produced clear hemolytic zones on layered CNA agar after 1 to 2 days of incubation at 35°C; it was not necessary to stab the colonies into the agar to observe hemolysis. Umbrella motility appeared after 2 days of incubation at 25°C.

First, LPM agar was evaluated by comparing the growth of various bacteria on it and on McB agar (15). Inocula were grown overnight in brain heart infusion broth and streaked with a loop on blood or nutrient, LPM, and McB agars. LPM agar inhibited the growth of most of the bacterial strains tested other than *L. monocytogenes* (Table 1). It should be noted that the growth after 1 day had no practical significance for *L. monocytogenes* selection, since the colonies were chosen after 16 to 20 h of incubation. Then LPM agar was evaluated for recovery of *L. monocytogenes* (seven strains listed in Table 1) at 20 to 40 CFU/25 g of beef. Inoculated beef was cultured in 225 ml of enrichment broth containing acriflavine, nalidixic acid, and esculin (5) for 24 to 48 h at 30°C and then streaked on CNA, McB, and LPM agars. *L. monocytogenes* was recovered from LPM agar but not from CNA or McB agars. To ensure successful recovery from beef, it was necessary to select and examine a large number of blue to white colonies owing to variations of color and size of *Listeria* colonies. Many *Listeria* isolates were nonhemolytic. Numerous enterococci and occasional *Pseudomonas* sp. were also recovered from LPM agar. These contaminants were suppressed or grew poorly on LPM agar but were difficult to avoid during colony selection, since they grew well on the nonselective motility agar. Thus it was necessary to restreak and purify the primary isolates on layered horse blood CNA agar before biochemical identification. Our results demonstrate that LPM agar controls the growth of many bacteria and allows the formation of *L. monocytogenes* colonies. Its use should facilitate the recovery of this bacterium from mixed cultures.

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TABLE 1. Growth of streaked bacterial cultures on LPM and McB agars without blood at 30°C

Strain	Source[a]	Colonies/days[b] on:	
		LPM	McB
Listeria monocytogenes V7	Milk (CDC via J. Hunt, FDA)	+/1	+/1
Listeria monocytogenes V37CE	Milk (CDC via J. Hunt, FDA)	+/1	+/1
Listeria monocytogenes Scott A	Human (CDC via J. Hunt, FDA)	+/1	+/1
Listeria monocytogenes Murray B	Human (CDC via J. Hunt, FDA)	+/1	+/1
Listeria monocytogenes RM I	Bovine (R. Miller, FSIS)	+/1	+/1
Listeria monocytogenes RM II	Bovine (R. Miller, FSIS)	+/1	+/1
Listeria monocytogenes ATCC 7644	Human (ATCC)	+/1	+/1
Corynebacterium equi	Swine (C. Tate, FSIS)	0/3	t/1, ++/2
Corynebacterium pseudotuberculosis	Swine (C. Tate, FSIS)	0/3	t/1-2
Corynebacterium pyogenes	Bovine (C. Tate, FSIS)	t/3	t/1, ++/2
Corynebacterium renale	Bovine (C. Tate, FSIS)	t/3	t/1, ++/2
Corynebacterium sp. (yellow)	Bovine (C. Tate, FSIS)	t/3	0/1-2
Bacillus subtilis ATCC 6633	ATCC	±/1	++/1
Bacillus subtilis		++/1	++/1
Bacillus cereus 1		0/4	++/1
Bacillus cereus 4		0/1, ±/2	++/1
Bacillus cereus 1s		0/4	++/1
Enterobactor cloacae ATCC 23355	Difco set	0/4	t/1
Escherichia coli ATCC 25922	Difco set	0/4	t/1, ++/4
Klebsiella pneumoniae ATCC 13883	Difco set	0/4	0/1, ++/4
Klebsiella sp. LT 5A	J. Leslie, FDA	0/4	±/1
Klebsiella sp. LT 808	J. Leslie, FDA	0/4	±/1
Micrococcus sp.		0/4	±/1
Proteus vulgaris ATCC 13315	Difco set	0/1, ±/2	t/1
Pseudomonas aeroginosa ATCC 27853	Difco set	0/4	++/1
Pseudomonas sp.		0/4	t/1
Salmonella typhimurium ATCC 14028	Difco set	0/4	±/1, +/4
Serratia marcescens ATCC 8100	Difco set	0/4	0/4
Serratia sp.		0/4	0/1, t/4
Staphylococcus aureus ATCC 25923	Difco set	0/4	+/1
Staphylococcus aureus 98 T	J. Leslie, FDA	0/4	++/1
Staphylococcus aureus LT 802	J. Leslie, FDA	0/4	t/1, ++/4
Staphylococcus epidermidis ATCC 12228	Difco set	0/4	t/1
Streptococcus agalactiae NIRD	USDA-ARS	0/3	t/1
Streptococcus faecalis subsp. *liquefaciens* ATCC 27959	USDA-ARS	0/2, t/3	+/1
Streptococcus faecalis LD-2	USDA-ARS	0/4	+/1
Streptococcus faecalis		0/1, ±/4	++/1
Streptococcus pyogenes ATCC 19615	Difco set	0/4	t/1

[a] CDC, Centers for Disease Control, Atlanta, Ga., FDA, Food and Drug Administration, Cincinnati, Ohio; FSIS, Food Safety and Inspection Service, Beltsville, Md.; ATCC, American Type Culture Collection, Rockville, Md.; USDA-ARS, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Md.

[b] Symbols: 0, no visible growth; t, ± 0.1-mm colonies; ±, few 1- to 2-mm (resistant) colonies; +, 1-mm colonies (typical for *L. monocytogenes*); ++, >1-mm colonies.

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