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Isolation of *Listeria monocytogenes* from Food Products on Four Selective Plating Media

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ABSTRACT

The suitability of four selective media for isolation of *Listeria monocytogenes* strains has been evaluated. Samples of cheese, processed meat, fresh vegetables, and raw milk were inoculated with low numbers of *L. monocytogenes* cells. Inoculated samples were enriched at 30°C and plated on selective media for isolation. Modified McBride Agar was found to be substantially inferior in comparison with other media. Acriflavine-Ceftazidime Agar was relatively more efficient but was not satisfactory for all classes of foods and all strains of *L. monocytogenes*. With *L. monocytogenes* serotype 4b and strain 286, Lithium Chloride-Phenylethanol-Moxalactam Agar (LPM) and Oxford Agar (OA) were approximately equally effective for isolation from all classes of foods. However, LPM performed poorly with *L. monocytogenes* serotype 3a. OA medium was consistently superior and gave higher recovery with all strains studied. Incubation of enrichment broth culture for just one day was not sufficient and 2 d of incubation was necessary to achieve a satisfactory level of performance.

Listeria monocytogenes has recently become of much concern to the food processing industry, public health professionals, and regulators. This organism has long been known to cause human infections (*20*) and has now been recognized as an important foodborne pathogen (*7,13,19*).

A modification of McBride agar (*18*) called modified McBride agar (MMA) has extensively been used as a selective plating medium for *Listeria* isolation (*6,10,16,17*). However, there have been several reports suggesting the inferior quality of MMA for isolation of listeriae from food products (*1,5,11,14,15*). Attempts to formulate a procedure using selective plating media that are suitable for isolation of *Listeria* from a wide range of food products have met with failure (*3,4,8,9*). In a collaborative study using four selective media (G.A. Prentice, P. Neaves, and A. D. Hitchins; unpublished data), Oxford modification of *Listeria* Selective Medium (Oxford Agar) (Oxoid Canada Ltd., Toronto, Ontario) was found to be the best selective medium for isolation of *L. monocytogenes* from cheese samples.

The objective of this study was to evaluate the suitability of Oxford Agar (OA) for isolation of *L. monocytogenes* from various categories of food products. Three other media which have been found convenient for isolation work in this laboratory were used for comparison.

MATERIALS AND METHODS

Microorganisms

L. monocytogenes 4b (81-861) and *L. monocytogenes* 3a (7834) were obtained from Dr. J. Farber, Health Protection Branch, Ottawa, Ontario. A strain isolated from raw milk in this laboratory and designated *L. monocytogenes* 286 was also used. The organisms were maintained on Trypticase Soy Agar (Difco Laboratories, Detroit, MI) slants containing 0.6% yeast extract (TSA-YE) and subcultured every month. For preparing inoculum to spike the samples, the organisms were cultured in Trypticase Soy Broth (Difco) containing 0.6% yeast extract (TSB-YE) at 35°C for 20 to 24 h. To assess the number of cells inoculated, the culture was diluted and dilutions were plated on TSA-YE plates.

Media

In addition to OA, three other selective media were used in this study. These were, Lithium-Chloride-Phenylethanol-Moxalactam Agar (LPM) medium (*14*), Acriflavine-Ceftazidime Agar (AC) (*2*), and Modified McBride Agar (MMA) consisting of McBride Listeria Agar (Difco) containing 200 mg cycloheximide (Sigma Chemical Co., St. Louis, MO) per L.

Samples

Fresh vegetable and cheese samples were purchased from a local grocery store. Processed, sliced ham and wiener packages were obtained from a meat processing plant. Raw milk samples were submitted to the laboratory by dairy inspectors or dairy plant personnel.

Enrichment and isolation

Twenty-five gram portions of the food products were weighed in Stomacher bags. Each bag received 225 ml FDA enrichment broth (EB) (*16*). The bags containing solid samples were stomached for 2 min. Diluted *L. monocytogenes* culture was added to the food suspension to obtain the desired number of *Listeria* cells per 25 g sample. The number of 25 g portions taken from the same batch or package of the product for a recovery experiment was called "number of replicates." An uninoculated control for each product was run in parallel to determine if it was naturally contaminated with *Listeria*. Naturally contaminated

samples were excluded from this study. The inoculated samples and appropriate controls were incubated at 30°C for 2 d. A loopful of enrichment culture was streaked on a plate of each of the four selective media after 1 and 2 d. The plates were incubated at 35°C and observed after 24 and 48 h with 45° incident-transmitted light (*12*). Although an oblique lighting arrangement was not necessary for examination of OA and AC plates, they could be examined for *Listeria* colonies with this set up. Typical *Listeria* colonies were bluish to bluish grey on MMA, bluish or grey on LPM, yellowish green on AC, and dark brown with dark halos on OA. On AC and LPM, *Listeria* colonies exhibited ground glass like structure. Known *Listeria* cultures were streaked on the selective agar plates to study typical characteristics.

Confirmation of Listeria *isolates*

Five typical colonies (all, if less than five developed on a plate) were picked from selective media plates and streaked on TSA-YE. The streaked plates were incubated at 35°C for 24 h and examined with oblique transmitted light. *Listeria* colonies were light bluish or bluish grey with ground glass structure. Further confirmation of *L. monocytogenes* colonies was carried out by examining for morphology, motility, catalase and gram stain reaction, and ß-hemolysis by procedures described elsewhere (*16*).

RESULTS AND DISCUSSION

Presence of a large number of *Listeria* cells in the enrichment culture would conceivably result in abundant growth on all selective media plates. Profuse growth may not allow determination of efficiency of the media. Therefore, in recovery experiments attempts were made to inoculate a low number of *L. monocytogenes* cells. The results of the recovery of *L. monocytogenes* strain 286 in three types of cheese samples at two inoculum levels are shown in Table 1. In this experiment recovery was studied after 2 d incubation. At lower levels of inoculum recovery was less frequent. OA media was found to be superior to the other media with LPM a close second. AC medium was clearly inferior to OA and LPM, and it allowed more non-*Listeria* colonies to develop. MMA was found to be very inferior in comparison to the other three media. As observed by Lee and McClain in their study with beef (*14*), we found that numerous competing non-*Listeria* colonies developed on MMA. Some of these colonies resembled *Listeria* colonies in appearance, but turned out to be *Streptococcus* spp. Occasionally, lactobacilli also produced typical bluish colonies.

In preliminary trials meat products supported good growth of *Listeria*. Inoculation of one or two organisms per g of meat sample resulted in a profuse growth at 1 d enrichment. Selective media plates streaked with this EB culture were crowded and comparison of efficacy was not possible. Therefore, a very low level of inoculum was used. Table 2 shows the data on recovery of *L. monocytogenes* serotype 4b from processed meat products. On MMA recovery was possible only from 30% of the inoculated samples, while the organisms could be isolated from 90% of the samples on the other three media. The recovery on selective media was low after 1 d enrichment, but the growth was profuse after 2 d. It is apparent that a minimum of 2 d was required to fully enrich the culture for isolation of *Listeria* under these conditions of low initial contamination.

MMA again proved to be less desirable for recovery of *L. monocytogenes* 4b from vegetable products (Table 3). The organism was recovered on MMA from less than half (45.8%) of the samples after 1 d enrichment, while OA and LPM recovered from 87.5% samples. Recovery on MMA improved to 95.8% after 2 d enrichment. However, it should be emphasized that although MMA showed high recovery rate, the isolations were successful from all the replicate samples on the other three media, including AC, after 2 d enrichment. This was likely due to very little of the competing microflora in the vegetables developing in EB as observed from the growth pattern on the selective media plates. Very few cells of *L. monocytogenes* were isolated after 1 d enrichment from carrots on all four media

TABLE 1. *Recovery of* L. monocytogenes *strain 286 from inoculated cheese samples on four selective plating media after 2d enrichment.*

Cheese sample	No. of cells inoculated per 25 g	No. of replicates	No. of replicates positive on[a]			
			OA	AC	LPM	MMA
Blue	25	6	2(8)[b]	1(15)	2(11)	1(1)
	100	6	5	4	5	1(1)
Brie	25	6	4	3	3	1(12)
	100	6	6	6	6	2
Swiss	25	6	3(11)	0	2(5)	0
	100	6	6	3	6	2(2)

[a]Colonies developing on streaked plates were too numerous to count unless specified in parentheses.
[b]Counts in parentheses are average number of colonies per plate.

TABLE 2. *Recovery of* L. monocytogenes *serotype 4b from inoculated meat products on four selective plating media.*

Sample	No. of cells inoculated per 25 g	No. of replicates	Days EB culture incubated	No. of replicates positive on			
				OA	AC	LPM	MMA
Ham	3	6	1	5(49)[a]	5(43)	5(34)	1(60)
			2	5[b]	5	5	1
Wieners	3	4	1	4(42)	4(42)	4(28)	2(30)
			2	4	4	4	2(62)

[a]Counts in parentheses are average number of colonies per plate.
[b]Counts at 2d incubation of EB were too numerous to count unless specified in parentheses.

TABLE 3. *Recovery of* L. monocytogenes *serotype 4b from inoculated*[a] *vegetable samples on four selective plating media.*

Sample	No. of replicates	Days EB culture incubated	No. of replicates positive on			
			OA	AC	LPM	MMA
Lettuce	4	1	4(47)[b]	4(18)	4(54)	2(9)
		2	4[c]	4	4	4
Broccoli	4	1	4(12)	3(10)	3(14)	2(17)
		2	4	4	4	3
Cabbage	4	1	4(13)	4(8)	4(9)	2(2)
		2	4	4	4	4
Carrots	4	1	2(5)	2(3)	3(3)	1(1)
		2	4	4	4	4
Salad mix	4	1	4(25)	4(21)	4(29)	2(11)
		2	4	4	4	4
Bean Sprouts	4	1	3(65)	3(17)	3(37)	2(5)
		2	4	4	4	4

[a]All samples received 12 cells per 25 g.
[b]Counts in parentheses are average number of colonies per plate.
[c]Counts at 2d incubation of EB were too numerous to count.

suggesting a possible inhibitory effect of carrots on the growth of listeriae.

Performance of the selective media with inoculated milk samples is shown in Table 4. Again MMA and AC media were found to be inferior for isolation. OA and LPM were equally effective and incubation of EB for 2 d was essential to recover *L. monocytogenes* 4b from all the samples. Why AC and MMA were not as effective at higher inoculum level as at low levels is not clear from this study.

In preliminary experiments *L. monocytogenes* serotype 3a could not be recovered when milk samples were inoculated in small numbers that were used to recover serotype 4b. Therefore, this serotype was inoculated in larger numbers (Table 5). At inoculum levels of 99 and 198 per 25 g sample, MMA and AC were completely ineffective in recovering the organisms. Recovery on LPM was obtained only from one replicate with higher inocu-

TABLE 4. *Recovery of* L. monocytogenes *serotype 4b from inoculated raw milk samples on four selective plating media.*

Sample No.	No. of cells inoculated per 25 g	No. of replicates	Days EB culture incubated	No. of replicates positive on			
				OA	AC	LPM	MMA
1	17	5	1	2(3)[a]	0	2(2)	1(3)
			2	5[b]	4	5	3
2	34	5	1	4(4)	1(5)	4(3)	1(1)
			2	5	2	5	1

[a]Counts in parentheses are average number of colonies per plate.
[b]Counts at 2d incubation of EB were too numerous to count.

TABLE 5. *Recovery of* L. monocytogenes *serotype 3a from inoculated raw milk samples on four selective plating media.*

Sample	No. of cells inoculated per 25 g	No. of replicates	Days EB culture incubated	No. of replicates positive on			
				OA	AC	LPM	MMA
1	99	5	1	2(2)[a]	0	0	0
			2	5(10)	0	0	0
2	198	5	1	3(2)	0	1(2)	0
			2	4(44)	0	1(2)	0

[a]Counts in parentheses are average number of colonies per plate.

TABLE 6. *Recovery of* L. monocytogenes *serotype 3a from inoculated wiener samples on four selective plating media.*

Sample	No. of cells inoculated per 25 g	No. of replicates	Days EB culture incubated	No. of replicates positive on			
				OA	AC	LPM	MMA
1	70	6	1	6(60)[a]	0	3(57)	0
			2	6[b]	1(1)	0	0
2	140	6	1	6(85)	0	3(80)	0
			2	6[b]	0	1(3)	0

[a]Counts in parentheses are average number of colonies per plate.
[b]Number of colonies developed on streaked plates were too numerous to count.

lum. OA was the only medium which was effective in recovering the organism from 90% of the samples after 2 d incubation of EB. However, even after 2 d incubation of EB, the *Listeria* population did not achieve good growth as seen from the number of *Listeria* colonies developing on OA (Table 5).

The experiments with *L. monocytogenes* 3a were extended to wiener samples (Table 6). MMA and AC again did poorly. LPM, which proved very effective for isolation of other strains, was not as useful as OA. OA recovered the inoculated organisms from all the replicate samples. These observations with *L. monocytogenes* 3a strain suggest that with present methodology certain strains of *Listeria* may go undetected in food products, especially if they are present in small numbers.

In conclusion, OA medium has been found to be very effective for isolation of *L. monocytogenes* strains. In comparison, MMA was found to be much less satisfactory. These observations, along with previous reports (*1,5,15*), suggest that MMA is not a selective medium of choice for *Listeria* isolation. AC medium also did not perform with distinction and performed poorly with cheese samples and with *L. monocytogenes* 3a. LPM, while performing well with *L. monocytogenes* strain 4b and strain 286 in all categories of food products, was not equally effective with strain 3a. In contrast, OA has been shown to be a superior isolation medium for all classes of foods and all three *L. monocytogenes* strains used in this study. In most instances *Listeria* colonies on OA were recognizable within 24 h incubation. The colonies could be picked at this time for further identification, reducing the total time required for confirmatory work. Further studies using other strains of *Listeria* organisms are required to ascertain if OA medium could be used as a sole selective *Listeria* isolation medium. This study has also shown that 1 d incubation of FDA EB was not enough for *Listeria* isolation. Incubation at 35°C for 2 d was essential to achieve substantial population density of inoculated *L. monocytogenes* for a satisfactory level of isolation.

REFERENCES

1. Bailey, J. S., D. L. Fletcher, and N. A. Cox. 1989. Recovery and serotype distribution of *Listeria monocytogenes* from broiler chickens in the southeastern United States. J. Food Prot. 52:148-150.
2. Bannerman, E. S., and J. Bille. 1988. A new selective medium for isolating *Listeria* spp. from heavily contaminated material. Appl. Environ. Microbiol. 54:165-167.
3. Cassiday, P. K., and R. E. Brackett. 1989. Methods and Media to isolate and enumerate *Listeria monocytogenes*: A review. J. Food Prot. 52:207-214.
4. Cassiday, P. K., R. E. Brackett, and L. R. Beuchat. 1989. Evaluation of three newly developed direct plating media to enumerate *Listeria monocytogenes* in foods. Appl. Environ. Microbiol. 55:1645-1648.
5. Datta, A. R., B. A. Wentz, D. Shook, and M. W. Trucksess. 1988. Synthetic oligodeoxyribonucleotide probes for detection of *Listeria monocytogenes*. Appl. Environ. Microbiol. 54:2933-2937.
6. Doyle, M. P., and J. L. Schoeni. 1986. Selective enrichment procedure for isolation of *Listeria monocytogenes* from fecal and biologic specimens. Appl. Environ. Microbiol. 51:1127-1129.
7. Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Pilkaytis, M. B. Homes, A. Audurier, C. V. Broome, and A. L. Reginald. 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. New Engl. J. Med. 312:404-407.
8. Golden, D. A., L. R. Beuchat, and R. E. Brackett. 1988. Direct plating technique for enumeration of *Listeria monocytogenes* in foods. J. Assoc. Off. Anal. Chem. 71:647-650.
9. Golden, D. A., L. R. Beuchat, and R. E. Brackett. 1988. Evaluation of selective direct plating media for their suitability to recover uninjured, heat-injured, and freeze-injured *Listeria monocytogenes* from foods. Appl. Environ. Microbiol. 54:1451-1456.
10. 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.
11. Heisick, J. E., F. M. Harrell, E. H. Peterson, S. McLaughlin, D. E. Wagner, I. V. Wesley, and J. Bryner. 1989. Comparison of four procedures to detect *Listeria* spp. in foods. J. Food Prot. 52:154-157.
12. Henry, B. S. 1933. Dissociation in the genus *Brucella*. J. Infect. Dis. 52:374-402.
13. James, S. M., S. L. Fannin, B. A. Agee. B. Hall, E. Parker, J. Vogt, G. Run, J. Williams, L. Lieb, C. Salminen, T. Prendergast, S. B. Werner, and J. Chin. 1985. Listeriosis outbreak associated with Mexican-style cheese-California. Morbid. Mortal. Weekly Rep. 34:357-359.
14. Lee, W. H., and D. McClain 1986. Improved *Listeria monocytogenes* selective agar. Appl. Environ. Microbiol. 52:1215-1217.
15. Loessner, M. J., R. H. Bell, J. M. Jay, L. A. Shelef. 1988. Comparison of seven plating media for enumeration of *Listeria* spp. Appl. Environ. Microbiol. 54:3003-3007.
16. Lovett, J. 1987. *Listeria* isolation. pp. 29.01-29.09. *In* FDA Bacteriological Analytical Manual, supplement to 6th edn. Association of Official Analytical Chemists, Arlington, VA.
17. Lovett, J., D. W. Frances, and J. M. Hunt. 1987. *Listeria monocytogenes* in raw milk: detection, incidence, and pathogenicity. J. Food Prot. 50:188-192.
18. McBride, M. E., and K. F. Girard. 1960. A selective method for the isolation of *Listeria monocytogenes* from mixed bacterial populations. J. Lab. Clin. Med. 55:153-157.
19. Schlech, W. F., P. M. Levigne, R. A. Bortolussi, A. C. Allen, E. V. Haldane, A. J. Wort, A. W. Hightower, S. E. Johnson, S. H. King, E. S. Nicholls, and C. V. Broome. 1983. Epidemic Listeriosis - evidence for transmission by food. New Engl. J. Med. 308:203-206.
20. Seeliger, H. P. R. 1961. Listeriosis. Hafner Publishing Co., Inc., New York.