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## Role of Potassium Tellurite and Brain Heart Infusion in Expression of the Hemolytic Phenotype of *Listeria* spp. on Agar Plates

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**The influence of potassium tellurite (PT) and brain heart infusion agar (Difco), two components of modified *Listeria* selective agar medium (LSAMm), on the hemolytic phenotype of *Listeria* spp. was studied. *L. monocytogenes* and *L. ivanovii* displayed bigger zones of hemolysis on brain heart infusion agar compared with on Columbia agar base. The addition of PT increased the sizes of zones of hemolysis displayed by *L. monocytogenes*. This effect seemed to be produced by the enhancement of the cytolytic effect of listeriolysin O. PT decreased the hemolysis produced by *L. ivanovii*, and this effect seemed to be due to an inhibition of the sphingomyelinase C produced by this species.**

The isolation of *Listeria* spp. from sources with low levels of contamination by these microorganisms, such as environmental or food samples, is often impaired by the naturally occurring background microflora. This makes it necessary to use highly selective media. These media have been progressively improved to enhance their selectivity and ability to detect small numbers of *Listeria* colonies, thus avoiding previous selective enrichment steps (2, 4). The goal of these improvements has been to provide a reliable, sensitive, and rapid isolation method for *Listeria monocytogenes* and other *Listeria* spp., and there are several selective plating media available to isolate and enumerate *Listeria* colonies (1-4).

The hemolytic phenotype is currently a fundamental criterion for the differentiation and identification of *Listeria* spp. (6), and it is also a marker of pathogenicity (16). Consequently, hemolytic activity can be used as an indicator of the presence of pathogenic listeriae in a plating medium. The only exception is *Listeria seeligeri*, which is weakly hemolytic but nonpathogenic. This does not substantially affect the validity of this indicator, as this species is isolated only sporadically. Therefore, it is important to easily determine the hemolytic character of *Listeria* isolates on plating media. In accordance with these observations, a further improvement introduced in selective *Listeria* isolation has been the detection of hemolytic colonies of *L. monocytogenes* directly on selective agar by using an erythrocyte top layer (RBCTL) technique (1). This RBCTL technique provides an easy method for the differentiation of hemolytic and nonhemolytic *Listeria* colonies isolated from the same sample, avoiding further subcultures to determine the hemolytic character either on blood agar or by the CAMP test, both of which are less sensitive or unreliable (1, 5, 6, 19). The performance of the RBCTL technique was higher when used with *Listeria* selective agar medium (modified) (LSAMm) than it was when used with other types of *Listeria* selective agar (1). In the work described here, we analyzed the reasons why such enhancement of hemolysis of *L. monocytogenes* is observed on LSAMm. We found that potassium tellurite (PT) and the basal medium are factors that influence this phenomenon.

The following *Listeria* strains were used: *Listeria monocytogenes* NCTC (National Culture Type Collection) 7973, NCTC 5105, NCTC 6348, and P-9 (animal clinical isolate); *Listeria innocua* SLCC (Special *Listeria* Culture Collection) 3379 and C-645 (from the collection of the Microbiology Department, University of Leicester, Leicester, United Kingdom); *Listeria ivanovii* ATCC (American Type Culture Collection) 19119 and C-659; *Listeria seeligeri* CIP (Collection of the Institut Pasteur, Paris, France) 100100 and NCTC 11806; and *Listeria welshimeri* CIP 8149 and SLCC 5334. Additionally, the following three mutant strains of *L. monocytogenes* were used: a Tn1545-induced nonhemolytic *L. monocytogenes* mutant strain (7) (a producer of phospholipases but not of listeriolysin O [LLO]) (LLO<sup>-</sup>) (obtained from P. Berche, Laboratoire de Microbiologie, Faculté de Médecine Necker-Enfants Malades, Paris, France), BUG 206 (15) (a producer of LLO and lecithinase but not of phosphatidylinositol-specific phospholipase C [PI-PLC]), and LUT-12 (a Tn917-lac lecithinase-negative mutant, a producer of LLO and PI-PLC). BUG 206 and LUT-12 were obtained from P. Cossart, Unité de Génie Microbiologique, Institut Pasteur, Paris, France.

The hemolytic activities of the *Listeria* strains were screened on brain heart infusion agar (BHIA) (Difco) and Columbia agar base (CAB) (Oxoid). Both media were prepared and sterilized according to the manufacturer's instructions. When these media were used to grow mutant strains, they were supplemented with the appropriate antibiotics. Hemolysis was determined by the top-layer technique proposed by Blanco et al. (1). After incubation at 37°C for 48 h, plates were chilled at 4°C for 2 h before being covered by 15 ml of an RBCTL for hemolysis detection. The composition, preparation, and storage of the top layer have been described previously (1, 5). After top-layer addition, plates were reincubated for 24 h at room temperature and screened for hemolysis.

The influence of BHIA and CAB on the hemolysis of streaked *Listeria* spp. was determined by streaking each strain on plates of both media (four strains per plate), which were incubated at 37°C for 48 h. After incubation, RBCTL was added and hemolysis screening was performed as described above. The zone of hemolysis was measured as the

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TABLE 1. Effect of PT on zone of hemolysis diameters of streaked strains of *L. monocytogenes* and *L. ivanovii*

Species and strain	Medium	Zone of hemolysis diam (mm) with PT concn (% [wt/vol]) of <sup>a</sup> :	
		0	0.02
<i>L. monocytogenes</i>			
NCTC 7973	BHIA	4.3 ± 1.2	7.0 ± 1.7
	CAB	3.5 ± 0.8	3.9 ± 0.6
NCTC 5101	BHIA	5.3 ± 0.8	8.0 ± 0.7
	CAB	4.4 ± 1.1	4.9 ± 1.5
P-9	BHIA	2.5 ± 0.9	6.5 ± 1.3
	CAB	1.6 ± 0.9	2.1 ± 0.7
NCTC 6384	BHIA	2.0 ± 0.5	5.7 ± 1.1
	CAB	1.6 ± 2.1	2.0 ± 1.7
<i>L. ivanovii</i>			
ATCC 19119	BHIA	9.1 ± 1.9	5.3 ± 2.0
	CAB	5.8 ± 1.7	3.0 ± 1.0
C-659	BHIA	8.0 ± 2.0	6.2 ± 1.8
	CAB	4.6 ± 2.0	2.4 ± 0.7

<sup>a</sup> Values are means ± standard deviations of nine determinations.

distance between the edge of the streak of growth and the limit of the zone of hemolysis.

The influence of PT on the hemolysis and growth of streaked strains of *Listeria* was determined as follows. In a first trial, 200 mg of PT per liter (0.02% [wt/vol]) (the concentration of PT used in the formulation of LSAMM) was added to BHIA and CAB (BHIA-PT and CAB-PT). In a second trial, BHIA was supplemented with PT to reach concentrations of 0.004, 0.008, 0.01, 0.02, 0.1, 0.5, and 1%, and the effect of each mixture on the growth and hemolysis of all *Listeria* strains was determined. Simultaneously, BHIA plates without PT were used as controls. BHIA and CAB were prepared and sterilized as described above. After they had cooled to about 50°C, the appropriate amount of filter-sterilized PT was added. BHIA and CAB plates in the first trial and BHIA plates in the second trial were cultured, incubated, and screened for hemolysis as mentioned above.

To determine the hemolytic activities of *Listeria* supernatants in the presence of PT, *Listeria* strains were grown in 1,000 ml of brain heart infusion broth (Difco) at 37°C for 24 h with shaking (1,000 rpm). After incubation, cells were harvested by centrifugation (12,000 × g for 30 min), and the

supernatant fluid was concentrated to 65 ml by ultrafiltration through 10-kDa-exclusion-limit membranes (Minitan system, Millipore Corp.) and filtered through a sterile filter (pore size, 0.22 μm). BHIA and CAB were supplemented with bacteriological agar (Difco) to a final concentration of 2%. Media were boiled, distributed in aliquots of 25 ml in glass tubes, and sterilized (121°C for 15 min). After being cooled to about 50°C, PT was added to final concentrations of 0.004, 0.008, 0.01, and 0.02%. The tubes were mixed vigorously and dispensed immediately into petri dishes. When the mixture had solidified, wells (7 mm diameter) were made in the medium by using a drill bit. The agar plugs were removed with a sterile needle. After drying (37°C for 24 h), 200 μl of the supernatant fluid was placed in wells as follows. Wells were filled with 100 μl of supernatant fluid, and plates were stored for 12 h at room temperature to allow supernatant absorption. Wells were filled again with another 100 μl of supernatant fluid, and plates were stored for another 12 h at room temperature. The nonabsorbed supernatant was removed, and RBCTL was added. Further incubation of plates to screen the hemolytic activities of the supernatants was done as described above. The hemolytic activity was measured as the diameter of the zones of hemolysis.

Selective sequestration of thiol-activated *L. ivanovii* cytolysin (ivanolysin O) was performed by the method described by Vazquez-Boland et al. (20, 21). To the concentrated and filtered supernatant of *L. ivanovii*, 200 μl of a 10-mg/ml cholesterol (Panreac, Barcelona, Spain) solution in absolute ethanol was added (final concentration of cholesterol was 35 μg/ml). After incubation (30 min at 37°C) with shaking, the resultant suspension of cholesterol and thiol-activated cytolysin complexes was pelleted by centrifugation (40,000 × g for 45 min at 4°C). The supernatant obtained, which lacked the thiol-activated cytolysin activity, was tested for hemolytic activity.

All strains of *L. monocytogenes* and *L. ivanovii* displayed bigger and clearer zones of hemolysis on BHIA than they did on CAB (Table 1). The zones of hemolysis displayed by *L. monocytogenes* (an average of 36 determinations [9 determinations per strain]) and *L. ivanovii* (an average of 18 determinations [9 determinations per strain]) were about 1 and 3.5 mm larger, respectively, on BHIA than they were on CAB. *L. seeligeri* displayed very small hemolytic zones (always smaller than 1 mm) on both BHIA and CAB.

TABLE 2. Effect of PT on zone of hemolysis diameters of supernatants of *L. monocytogenes* and *L. ivanovii* strains

Species and strain	Medium	Zone of hemolysis diam (mm) with PT concn (% [wt/vol]) of <sup>a</sup> :				
		0	0.004	0.008	0.01	0.02
<i>L. monocytogenes</i>						
NCTC 7973	BHIA	14.6 ± 1.0	17.0 ± 0.7	15.5 ± 0.8	15.5 ± 0.6	15.2 ± 1.2
	CAB	14.5 ± 1.0	16.7 ± 1.2	15.8 ± 0.7	15.5 ± 0.5	15.2 ± 1.3
NCTC 5101	BHIA	17.8 ± 2.9	20.5 ± 2.6	19.5 ± 2.3	19.5 ± 0.6	15.2 ± 1.3
	CAB	17.3 ± 2.0	20.3 ± 2.1	19.8 ± 1.7	18.7 ± 0.5	18.5 ± 1.6
P-9	BHIA	10.0 ± 1.9	17.0 ± 1.6	16.0 ± 2.4	16.2 ± 2.1	14.3 ± 2.0
	CAB	11.5 ± 2.8	15.2 ± 2.6	14.2 ± 2.1	14.1 ± 1.8	14.0 ± 1.5
<i>L. ivanovii</i>						
ATCC 19119	BHIA	20.0 ± 2.6	19.0 ± 2.3	15.3 ± 2.5	17.6 ± 1.3	17.4 ± 2.5
	CAB	22.1 ± 1.8	20.3 ± 2.7	20.0 ± 2.1	19.7 ± 0.9	19.6 ± 1.8
C-659	BHIA	16.5 ± 1.6	14.6 ± 2.3	14.0 ± 1.7	12.7 ± 0.7	12.2 ± 1.5
	CAB	15.4 ± 2.0	12.3 ± 1.9	12.5 ± 1.8	12.1 ± 2.4	11.5 ± 1.9

<sup>a</sup> Values are means ± standard deviations of six determinations.

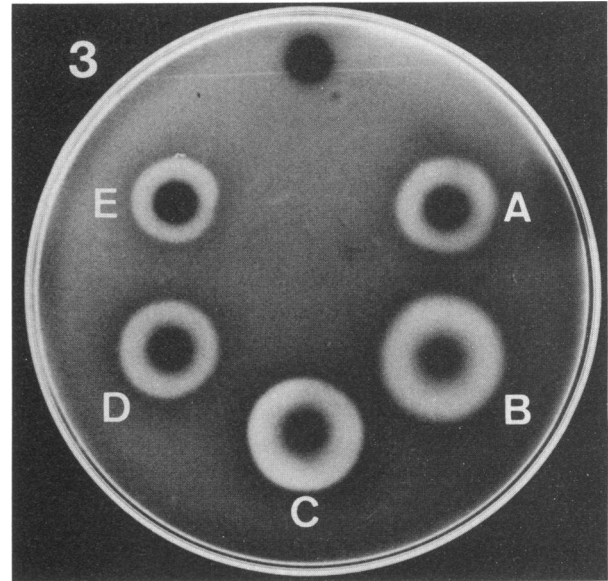
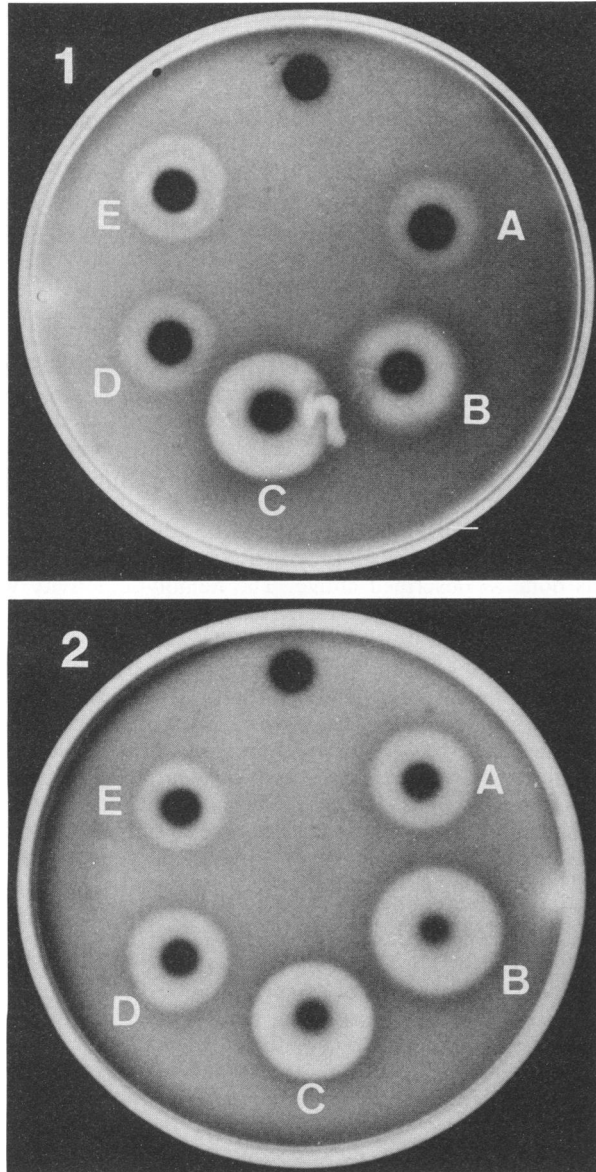


FIG. 1. Hemolysis displayed by supernatants of *Listeria* spp. (A, *L. monocytogenes* NCTC 7973; B, *L. monocytogenes* NCTC 5105; C, *L. ivanovii* C-659; D, *L. monocytogenes* P-9; E, *L. ivanovii* ATCC 19119) on BHIA without PT (panel 1) and on BHIA supplemented with 0.004 (panel 2) and 0.02% (panel 3) PT.

zones of hemolysis displayed by the streaked strains and supernatants of *L. ivanovii* decreased in parallel with the increase in the concentration of PT (Table 2). Thus, streaked strains of *L. ivanovii* displayed hemolytic zones similar to or even smaller than those produced by *L. monocytogenes* strains on BHIA supplemented with 0.02% PT (Table 1). The zones of complete hemolysis of *L. ivanovii* (Fig. 1, panels 2 and 3 [E]) were bigger in the presence of PT than they were in the absence of PT (Fig. 1, panel 1 [E]); nevertheless, it was impossible to recognize the external ring of incomplete hemolysis. Overall, in the presence of PT, the streaked strains of *L. monocytogenes* and *L. ivanovii* always had bigger zones of hemolysis on BHIA than on CAB (Table 1), whereas the hemolysis displayed by the supernatants of these species was similar on BHIA and CAB (Table 2). *L. seeligeri* did not show any detectable enhancement in its hemolysis with any of the PT concentrations used. *L. innocua* and *L. welshimeri* strains were always nonhemolytic, as expected.

Both streaked strains and supernatants of the LLO<sup>-</sup> *L. monocytogenes* mutant strain were always nonhemolytic on BHIA and CAB with or without PT. On the other hand, streaked cells and supernatants of *L. monocytogenes* mutant strains affected either in PI-PLC or in lecithinase production displayed clear zones of hemolysis which were enhanced in the presence of PT.

The cholesterol-treated supernatants of *L. ivanovii* did not display the inner halo of complete hemolysis. Without PT, the external ring of incomplete hemolysis on BHIA and CAB was of a size similar to that of the nontreated supernatants. Nevertheless, the zone of incomplete hemolysis was strongly inhibited in both media when PT was added.

PT was first recommended, at concentrations between 0.5 and 1%, for its use in media for isolating *L. monocytogenes* more than 40 years ago (4, 10). Although further studies reported that PT inhibited the growth of some strains of *L.*

All *Listeria* strains grew perfectly at PT concentrations up to 0.02%, while concentrations of PT equal to or higher than 0.1% decreased or completely inhibited their growth (data not shown). The hemolysis of both streaked strains and supernatants of *L. monocytogenes* was enhanced in the range of concentrations from 0.004 to 0.02% PT, compared with the same medium without PT (Table 2 and Fig. 1, panels 1 and 2 [A, B, and D]). The increase in hemolysis was comparatively higher for weakly hemolytic strains (NCTC 6384 and P-9) than for moderate or strongly hemolytic strains (NCTC 7973 and NCTC 5105); this fact was more evident with the streaked strains. The greatest increase in the zone of hemolysis was detected with 0.004% PT on both BHIA and CAB.

*L. ivanovii* ATCC 19119 usually displayed a characteristic bizonal hemolytic area on BHIA without PT (Fig. 1, panel 1 [E]). *L. ivanovii* C-659 did not display a bizonal hemolysis in most cases (Fig. 1, panel 1 [C]). In the presence of PT, the

*monocytogenes* (12), it is still incorporated in different isolation media for *Listeria* spp. associated with other selective agents (1, 2, 11). Since PT is reduced by *Listeria* spp., it is also used as a differential agent (3). In this report, we provide evidence that PT enhances the hemolytic phenotype of *L. monocytogenes*, which was reflected in a slight increase in the width of the zone of hemolysis and, especially, in the greater clarity of these zones. Hemolysis of *L. monocytogenes* is currently related to the secretion of a 60-kDa, thiol-activated cytolysin, termed LLO; however, two additional putative cytolytic factors have to be considered, a 34-kDa PI-PLC and a lecithinase (9, 13, 15, 20). The PT could act on some of these membrane-damaging proteins. The results obtained with the phospholipase-negative (but LLO-producing) mutants of *L. monocytogenes*, which displayed increases in hemolysis in the presence of PT, and those obtained with the LLO<sup>-</sup> *L. monocytogenes* mutant strain (a producer of phospholipases but not of LLO), which did not show any zone of hemolysis in the presence of PT, would suggest that the effect of PT is exerted on LLO activity. The fact that supernatants of *L. monocytogenes* showed increases in their hemolytic activities on PT-containing plates rules out a possible stimulatory effect of this compound in the production and/or secretion of the hemolysin. Whether PT acts directly on the toxin activity, affects its diffusion, or sensitizes the erythrocyte membranes remains to be determined.

*L. ivanovii* displays a characteristic strong hemolysis, which is often bizonal, in sheep blood agar (17, 21). A thiol-activated cytolysin of 61 kDa, termed ivanolysin O, is the cytolytic factor responsible for the inner zone of complete hemolysis, and a 27-kDa hemolytic sphingomyelinase C is the responsible for the external halo of incomplete hemolysis (21). In accordance with our results, the PT could interact with one or both cytolysins. Without PT, *L. ivanovii* ATCC 19119 displayed a typical bizonal hemolytic area on BHIA and CAB, reflecting the presence of both cytolytic factors. The addition of PT produced an increase in size of the zones of complete hemolysis, denoting an increase in the cytolytic effect of the ivanolysin O. As previously demonstrated, selective sequestration of ivanolysin O by cholesterol leads to a culture supernatant in which sphingomyelinase C would be the only cytolytic factor (21). As expected, the cholesterol-treated supernatants of *L. ivanovii* did not display zones of complete hemolysis, and the zones of incomplete hemolysis did not change. On the other hand, these supernatants displayed almost no hemolytic activity in the presence of PT, which strongly suggests an inhibition of the sphingomyelinase C by PT. However, the mechanism of this inhibition also remains to be determined.

*L. seeligeri* did not show any detectable increase in its hemolytic effect in the presence of PT under our experimental conditions, which could be explained by the low levels of thiol-activated hemolysin production by this species (8).

The similar hemolytic activities displayed by the supernatants of *L. monocytogenes* and *L. ivanovii* in diffusion assays in both types of media indicate that the cytolysin activity was not affected by the basal medium composition. On the other hand, the results obtained with the streaked strains of these species, which always yielded larger zones of hemolysis on BHIA than they did on CAB, suggest a higher production of cytolysins in the former medium. This is most likely due to the fact that BHIA supports the growth of *Listeria* spp. much better than does CAB (data not shown).

The stronger hemolysis displayed by *L. monocytogenes* on BHIA and the enhancement of its hemolytic effect in the

presence of PT could explain the better recognition and differentiation of colonies of *L. monocytogenes* grown on LSAMm (formulated with BHIA as the basal medium and 200 mg of PT per liter) compared with other selective plating media, which, although they have similar compositions, use different basal media and do not include PT in their formulations (5). The addition of PT to *Listeria* selective plating media for direct isolation and enumeration of hemolytic *Listeria* spp. could replace the use of other hemolysis-enhancing substances (e.g., culture supernatants of *Staphylococcus aureus* or *Rhodococcus equi*) (6, 18), the use of which is more difficult, less easily standardized, and more time consuming. According to our results, the performance of the RBCTL technique could be improved by decreasing the concentration of PT in LSAMm to 40 mg/liter (0.004%), as the hemolysis of *L. monocytogenes* and *L. ivanovii* is slightly higher at this concentration and the selectivity of LSAMm is not affected (unpublished results).

In summary, the combination of BHIA with PT provides a reliable manner for differentiating and enumerating hemolytic *Listeria* spp. in *Listeria* selective plating media.

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#### REFERENCES

- Blanco, M., J. F. Fernández-Garayzábal, L. Domínguez, V. Briones, J. A. Vázquez-Boland, J. A. García, and G. Suárez. 1989. A technique for the direct identification of haemolytic-pathogenic listeria on selective plating media. *Lett. Appl. Microbiol.* 9:125-128.
- Buchanan, R. L. 1990. Advances in cultural methods for the detection of *Listeria monocytogenes*, p. 85-95. In A. J. Miller, J. L. Smith, and G. A. Somkuti (ed.), *Foodborne listeriosis*. Elsevier, Amsterdam.
- Buchanan, R. L., H. G. Stahl, and D. L. Archer. 1987. Improved plating media for simplified, quantitative detection of *Listeria monocytogenes* in foods. *Food Microbiol.* 4:269-275.
- 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.
- Domínguez, L., J. F. Fernández-Garayzábal, M. Blanco, V. Briones, J. A. Vázquez-Boland, J. L. Blanco, and G. Suárez. 1990. Overlay technique for direct detection and identification of haemolytic *Listeria* on selective plating media: comparison of five media. *Z. Lebensm. Unters. Forsch.* 191:16-19.
- Domínguez, L., J. A. Vázquez-Boland, J. F. Fernández-Garayzábal, P. Echalecu Tranchant, E. Gómez-Lucia, E. F. Rodríguez Ferri, and G. Suárez Fernández. 1986. Microplate technique to determine hemolytic activity for routine typing of *Listeria* strains. *J. Clin. Microbiol.* 24:99-103.
- Gaillard, J. L., P. Berche, and P. Sansonetti. 1986. Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of *Listeria monocytogenes*. *Infect. Immun.* 52:50-55.
- Geoffroy, C., J. L. Gaillard, J. E. Alouf, and P. Berche. 1989. Production of thiol-dependent haemolysins by *Listeria monocytogenes* and related species. *J. Gen. Microbiol.* 135:481-487.
- Geoffroy, C., J. Raveneau, J. L. Beretti, A. Lecroisey, J. A. Vázquez-Boland, J. E. Alouf, and P. Berche. 1991. Purification and characterization of an extracellular 29-kilodalton phospholipase C from *Listeria monocytogenes*. *Infect. Immun.* 59:2382-2388.
- Gray, M. L., H. J. Stafseth, and F. Thorp, Jr. 1950. The use of

- potassium tellurite, sodium azide, and acetic acid in a selective medium for the isolation of *Listeria monocytogenes*. *J. Bacteriol.* **59**:443–444.
11. Khan, M. A., A. Seaman, and M. Woodbine. 1973. Differential media in the isolation of *Listeria monocytogenes*. *Zentralbl. Bakteriol. Mikrobiol. Hyg. Abt. 1 Orig. A* **224**:362–375.
  12. Leighton, I. 1979. Use of selective agents for the isolation of *Listeria monocytogenes*. *Med. Lab. Sci.* **36**:283–288.
  13. Leimeister-Wächter, M., E. Domann, and T. Chakraborty. 1991. Detection of a gene encoding a phosphatidylinositol-specific phospholipase C that is coordinately expressed with listeriolysin in *Listeria monocytogenes*. *Mol. Microbiol.* **5**:361–366.
  14. Linder, R. 1984. Alteration of mammalian membranes by the cooperative and antagonistic actions of bacterial proteins. *Biochim. Biophys. Acta* **779**:423–475.
  15. Mengaud, J., C. Braun-Breton, and P. Cossart. 1991. Identification of phosphatidylinositol-specific phospholipase C activity in *Listeria monocytogenes*: a novel type of virulence factor? *Mol. Microbiol.* **5**:367–372.
  16. Seeliger, H. P. R., and D. Jones. 1986. Genus *Listeria* Pirie 1940, p. 1235–1245. In P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 2. Williams & Wilkins, Baltimore.
  17. Skalka, B., J. Smola, and K. Elischerová. 1982. Routine test for in vitro differentiation of pathogenic and apathogenic *Listeria* strains. *J. Clin. Microbiol.* **15**:503–507.
  18. Skalka, B., and J. Smola. 1983. Selective diagnostic medium for pathogenic *Listeria* spp. *J. Clin. Microbiol.* **18**:1432–1433.
  19. Vázquez-Boland, J. A., L. Domínguez, J. F. Fernández-Garayzábal, E. F. Rodríguez-Ferri, V. Briones, M. Blanco, and G. Suárez. 1990. Revision of the validity of CAMP-test for *Listeria* identification. Proposal of an alternative method for the determination of haemolytic activity by *Listeria* strains. *Acta Microbiol. Hung.* **37**:201–206.
  20. Vázquez-Boland, J. A., L. Domínguez, E. F. Rodríguez-Ferri, J. F. Fernández-Garayzábal, and G. Suárez. 1989. Preliminary evidence that different domains are involved in cytolytic activity and receptor (cholesterol) binding in listeriolysin O, the *Listeria monocytogenes* thiol-activated toxin. *FEMS Microbiol. Lett.* **65**:95–100.
  21. Vázquez-Boland, J. A., L. Domínguez, E. F. Rodríguez-Ferri, and G. Suárez. 1989. Purification and characterization of two *Listeria ivanovii* cytolytins, a sphingomyelinase C and a thiol-activated toxin (ivanolysin O). *Infect. Immun.* **57**:3928–3935.