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Automated Ribotyping Using Different Enzymes To Improve Discrimination of *Listeria monocytogenes* Isolates, with a Particular Focus on Serotype 4b Strains

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To develop improved automated subtyping approaches for *Listeria monocytogenes*, we characterized the discriminatory power of different restriction enzymes for ribotyping. When 15 different restriction enzymes were used for automated ribotyping of 16 selected *L. monocytogenes* isolates, the restriction enzymes *Eco*RI, *Pvu*II, and *Xho*I showed high discriminatory ability (Simpson's index of discrimination > 0.900) and produced complete and reproducible restriction cut patterns. These three enzymes were thus evaluated for their ability to differentiate among isolates representing the two major serotype 4b epidemic clones, those having ribotype reference pattern DUP-1038 (51 isolates) and those having pattern DUP-1042 (20 isolates). Among these isolates, *Pvu*II provided the highest discrimination for a single enzyme (nine different subtypes; index of discrimination = 0.518). A combination of *Pvu*II and *Xho*I showed the highest discriminatory ability (index of discrimination = 0.590) for these isolates. A group of 44 DUP-1038 isolates and a group of 12 DUP-1042 isolates were identical to each other even when the combined data for all three enzymes were used. We conclude that automated ribotyping using different enzymes allows improved discrimination of *L. monocytogenes* isolates, including epidemic serotype 4b strains. We furthermore confirm that most of the isolates representing the genotypes linked to the two major epidemic *L. monocytogenes* clonal groups form two genetically homogeneous groups.

Listeria monocytogenes is a pathogen that causes a severe human food-borne disease (26). Molecular subtyping provides a crucial tool for the detection of human listeriosis outbreaks and single-source clusters, which are often difficult to detect by classical epidemiological methods without molecular subtyping-based surveillance data. Clinical characteristics of human listeriosis complicating the detection and tracking of outbreaks include a long incubation period (1 to 90 days) in comparison to that of many other food-borne diseases. *L. monocytogenes* has also been shown to persist in food plants and thus can lead to prolonged contamination of food products, which may be distributed over a wide geographic range. As a consequence, this organism may cause widespread multistate and possibly multicountry outbreaks, with relatively few related cases in each geographic area. Rapid and standardized subtyping methods for *L. monocytogenes* are thus particularly important for effective detection of human listeriosis outbreaks.

Various methods can be used to distinguish *L. monocytogenes* subtypes. Traditional subtyping methods include serotyping (34) and phage typing (23, 24). Serotyping is of restricted value because most human clinical *L. monocytogenes* isolates belong to only 3 (1/2a, 1/2b, and 4b) of the 13 serotypes known for this species (11, 22, 32, 33). Worldwide, most sporadic human cases and most outbreaks have reportedly been caused by *L. monocytogenes* serotype 4b (11, 30), including one of the more recent outbreaks in the United States (9, 10). Most of these epidemic isolates can be grouped into two closely related homogeneous groups (so-called "epidemic clones") represented by two multilocus enzyme electrophoresis types and two ribotypes (14, 20, 27, 29, 37). These observations indicate that sensitive strain discrimination among serotype 4b strains is particularly crucial for surveillance and monitoring of human listeriosis cases.

The use of several subtyping methods, including multilocus enzyme electrophoresis, total DNA restriction endonuclease analysis, ribotyping, pulsed-field gel electrophoresis (PFGE), and random amplified polymorphic DNA analysis, for sensitive subtyping of *L. monocytogenes* has been explored by various groups (15). Many molecular typing methods offer the advantage of high discriminating ability and typeability and do not require specialized reagents such as typing sera and bacteriophages. PFGE using one or more enzymes is a commonly used and apparently highly discriminatory molecular subtyping method for *L. monocytogenes* (15). Major limitations of most molecular typing methods include a lack of standardization, a need for highly skilled technical staff, and significant hands-on time required for performance. Automation may allow laboratories to apply molecular typing more broadly. The RiboPrinter microbial characterization system (Qualicon Inc., Wilmington, Del.) is a completely automated subtyping system based on the principle of ribotyping (16). This system automates and standardizes all process steps required for ribotyping, from cell lysis to image analysis, and provides subtyping results within 8 h. While setup of an automated ribotyping laboratory requires considerable capital investment, for labo-

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ratories subtyping >1,500 isolates/year, costs on a per-isolate basis may be lower than for other, more labor-intensive subtyping methods (39).

Both manual and automated ribotyping methods have been widely used for subtyping of bacterial isolates (1, 2, 13, 14, 16, 19), detection and tracking of human and animal listeriosis cases and outbreaks (9, 36, 38), and tracking of *L. monocytogenes* contamination patterns in food processing plants (28). While the restriction enzymes *Eco*RI and *Pvu*II have been used most commonly for ribotyping (13, 14), other restriction enzymes have also been used (2, 19). Some previous studies indicated that single-enzyme ribotyping may be less discriminatory than some other subtyping methods, particularly for *L. monocytogenes* serotype 1/2b and 4b strains (4, 35).

To develop improved automated subtyping approaches for *L. monocytogenes*, we characterized the discriminatory power of different restriction enzymes for automated ribotyping. We also specifically explored the use of ribotyping with different restriction enzymes to improve discrimination of isolates representing the two epidemic *L. monocytogenes* serotype 4b clonal groups.

Automated ribotyping. Ribotyping was performed using the RiboPrinter microbial characterization system. Briefly, overnight bacterial cells were picked from brain heart infusion agar plates, suspended in sample buffer, inactivated by a heat kill step, and treated with lytic enzymes to release the DNA. The DNA was cut with a restriction enzyme, and the fragments were electrophoretically separated and simultaneously transferred to a membrane. A DNA probe for the *Escherichia coli rrnB* operon was then hybridized to the genomic DNA on the membrane. The genetic fingerprint was visualized and captured using a chemiluminescent detection system and a charge-coupled device camera. Analysis software automatically characterized and identified the digitalized image. Characterization consists of combining patterns within a specific similarity range to form a dynamic ribogroup that reflects the genetic relatedness of the isolates (6).

To allow the use of restriction enzymes requiring different time-temperature combinations for optimum performance (Table 1), the RiboPrinter system software has been modified to allow different digestion times (20 and 120 min) and temperatures (37 and 60°C). All restriction enzymes were obtained from New England Biolabs (Cambridge, Mass.).

Subtyping results with automated ribotyping using 15 restriction enzymes. In an initial screening, 15 restriction enzymes were tested for their ability to discriminate 16 *L. monocytogenes* isolates. These isolates had previously been shown to represent 16 distinct *Eco*RI ribotypes through the use of manual ribotyping (7), which allowed for longer gel run times and better band separation. These initial isolates represented the *Eco*RI ribotypes dd 0647, dd 0653, dd 3581, dd 1049, dd 1288, dd 7674, dd 7730, dd 7745, dd 0566, dd 1070, dd 6439, dd 6481, dd 1966, dd 6296, dd 7696, and dd 6323 (7).

Three of the enzymes tested (*Bso*BI, *Cla*I, and *Sty*I) consistently produced incomplete digests under the conditions used. Thus, patterns were not reproducible and these enzymes are not recommended for automated ribotyping of *L. monocytogenes* using the digestion conditions outlined in Table 1. With the remaining 12 restriction enzymes, we were able to differentiate between 1 and 15 different ribotypes (Table 1). The suitability of ribotyping for differentiation of strains was quantitated using Simpson's index of discrimination (SID) (18). The numerical value of this index indicates the discriminatory power of a given typing method by estimating the probability that two unrelated strains are differentiated by this method. As the numerical index approaches the maximum value of 1 (representing 100% discriminatory ability of a method), the probability increases that a given method will be able to discriminate between two unrelated strains. The five restriction enzymes with the highest discriminatory ability (SID $\geq$ 0.900) were *Pvu*II, *Eco*RI, *Bst*EII, *Ban*I, and *Xho*I (Fig. 1). *Pvu*II was the most discriminatory enzyme, yielding 15 different ribotypes. A combination of *Pvu*II ribotypes with ribotypes created by either *Xho*I, *Bst*EII, *Ase*I, *Ban*I, or *Bgl*III allowed discrimination of all 16 isolates. No other combination of two restriction enzymes allowed discrimination of all 16 isolates. While this is the first study reporting comparison of a large number of restriction enzymes for subtyping of *L. monocytogenes*, our results are in general agreement with other studies. For example, Gendel and Ulaszek (13) showed that *Pvu*II ribotyping discriminated more subtypes among a collection of 72 smoked salmon isolates than *Eco*RI ribotyping did. In earlier studies, *Eco*RI ribotyping was shown to be more discriminatory and suitable for *L. monocytogenes* subtyping than *Hin*dIII or *Hae*III ribotyping (2, 19).

TABLE 1. Digestion time, incubation temperature, and discriminatory ability of 15 restriction enzymes used for characterization of 16 *L. monocytogenes* isolates

Enzyme	Incubation temp (°C)	Digestion time (min)	No. of ribotypes among 16 isolates	SID
*Pvu*II	37	20	15	0.992
*Eco*RI	37	20	12	0.950
*Bst*EII	60	120	10	0.925
*Ban*I	37	120	10	0.917
*Xho*I	37	120	10	0.900
*Bgl*II	37	120	8	0.883
*Eag*I	37	120	9	0.883
*Ase*I	37	120	7	0.817
*Hin*cII	37	120	5	0.792
*Pst*I	37	120	6	0.617
*Nco*I	37	120	4	0.617
*Xba*I	37	120	1	0
*Bso*BI	60	120	ND[a]	ND
*Cla*I	37	120	ND	ND
*Sty*I	37	120	ND	ND

[a] ND, not determined. The enzymes *Bso*BI, *Cla*I, and *Sty*I showed incomplete digests with spurious and variable high-molecular-weight bands, which did not allow assignment of distinct ribotypes.

Discrimination of epidemic serotype 4b-associated genotypes using ribotyping with three selected restriction enzymes. Sensitive subtyping of *L. monocytogenes* serotypes 4b and 1/2b is of particular importance, as these two serotypes form a distinctive subset (lineage) that is responsible for the majority of human listeriosis cases (22, 33, 37). Within the lineage containing serotype 1/2b and 4b strains, two distinct clonal groups show particular prevalence among human listeriosis cases and outbreaks (29, 37). Isolates representing one clonal group have previously been characterized as ribotype reference pattern DUP-1038 and ribotype dd 0647 (37). This clonal group was linked to human listeriosis outbreaks in France (8), Nova Scotia, Canada (31), Switzerland (3), and Los Angeles,

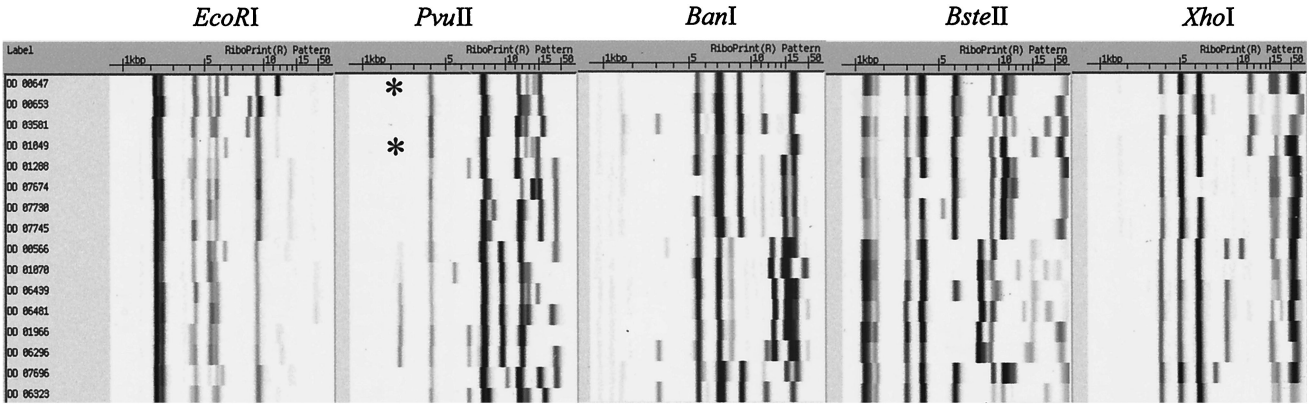

FIG. 1. Ribotypes obtained with enzymes *Eco*RI, *Pvu*II, *Bst*EII, *Ban*I, and *Xho*I for 16 genotypically distinctive *L. monocytogenes* isolates. The two isolates with indistinguishable *Pvu*II ribotypes are marked with asterisks.

Calif. (21). A second clonal group (ribotype reference pattern DUP-1042, ribotype dd 0653 [37]) has been linked to human listeriosis outbreaks in Boston (17), Massachusetts (12), and the United Kingdom (25). Reference patterns DUP-1042 and DUP-1038 each represent a group of closely related *Eco*RI ribotypes. These reference patterns were created by averaging individual closely related ribotype patterns obtained for multiple isolates. For example, DUP-1042 was previously shown to contain at least two closely related *Eco*RI ribotypes (dd 3581 and dd 0653) (7).

We used 71 *L. monocytogenes* isolates from humans, food, and other sources, representing the two major serotype 4b epidemic clones of *L. monocytogenes*, to further evaluate the discriminatory power of automated ribotyping using different restriction enzymes. Specifically, 51 and 20 of these isolates had previously been characterized as the *Eco*RI ribotype reference patterns DUP-1038 and DUP-1042, respectively. From the five restriction enzymes that allowed the most sensitive discrimination (SID $\geq$ 0.900) in our initial experiments on 16 diverse isolates, three enzymes (*Pvu*II, *Eco*RI, and *Xho*I) were used to determine their ability to differentiate among these isolates. Restriction enzymes *Ban*I and *Bst*EII were not included because they produced weak low-molecular-weight bands, which often required manual refinement for accurate analysis. Enzymes *Pvu*II, *Eco*RI, and *Xho*I produced more distinct patterns amenable to automated analysis and differentiation. Automated ribotyping with *Pvu*II provided the highest discrimination (SID = 0.518) (Table 2) among these groups of closely related isolates. Combined analysis of ribotype patterns obtained with two enzymes allowed a significant increase in discriminatory power, and a combination of *Pvu*II and *Xho*I data allowed for the highest discriminatory ability (Table 2). Forty-four of the 51 DUP-1038 as well as 12 of the 20 DUP-1042 isolates gave identical patterns with all three enzymes. Figure 2 shows the ribotype patterns obtained for the DUP-1038 and DUP-1042 isolates. DUP-1038 and DUP-1042 showed distinct ribotype patterns, and careful examination of *Eco*RI ribotype patterns allowed differentiation of four different ribotypes among DUP-1042 isolates and three different ribotypes among DUP-1038 isolates. The three DUP-1038 ribotypes differed by the presence or absence of weak bands in the 8- to 10-kb range, while the four DUP-1042 isolates differed by the presence or absence of weak bands in the 10- to 13-kb range. *Pvu*II ribotype patterns, on the other hand, generally showed much more distinct differences in banding patterns.

Although considered a highly discriminatory subtyping method for *L. monocytogenes* (15), even PFGE with three different restriction enzymes often appears not to discriminate within these clonal groups, including among isolates from temporally and/or geographically distinct outbreaks. For example, isolates from the 1983 listeriosis outbreak in Massachusetts and isolates from the 1987–1989 outbreak in England were indistinguishable by PFGE using the restriction enzymes *Asc*I, *Apa*I, and *Sma*I. Similarly, the same enzymes could not differentiate isolates from the outbreak in Los Angeles in 1985 and from the outbreak in Vaud, Switzerland, from 1983 to 1987 (5). Our results are thus consistent with previous results, which show the highly clonal nature of the serotype 4b clonal groups (5, 29). Sensitive subtyping of these isolates represents a significant challenge and may require novel, possibly genomics-based, approaches.

Conclusions. Our results show that hierarchical ribotyping using different enzymes allows improved discrimination of *L. monocytogenes* isolates, including strains in the epidemic serotype 4b clonal groups. While surveillance and epidemiological investigations of human listeriosis cases may profit considerably from subtyping using multiple enzymes and automated ribotyping, currently more labor-intensive and possibly less standardized additional typing methods may be necessary to further discriminate strains in these clonal groups.

TABLE 2. Subtype discrimination among epidemic clones DUP-1038 (n = 51) and DUP-1042 (n = 20) by ribotyping with different restriction enzymes

Enzyme or enzyme combination	Total no. of subtypes	SID	No. of subtypes	
			DUP-1038	DUP-1042
*Pvu*II	9	0.518	4	7
*Eco*RI	7	0.478	3	4
*Xho*I	3	0.444	2	3
*Pvu*II/*Eco*RI	13	0.538	4	9
*Pvu*II/*Xho*I	11	0.590	5	7
*Eco*RI/*Xho*I	11	0.551	4	7
*Pvu*II/*Eco*RI/*Xho*I	14	0.591	5	9

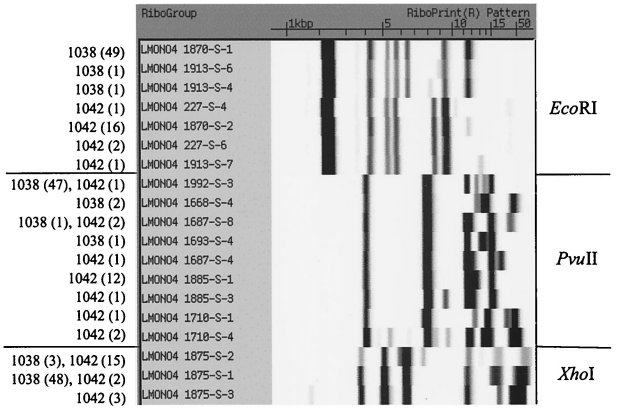

FIG. 2. Ribotype patterns obtained with enzymes *Eco*RI, *Pvu*II, and *Xho*I among all DUP-1038 and DUP-1042 isolates included in this study. The number of isolates within each ribotype (separated by DUP-1038 and DUP-1042 isolates) is indicated on the left.

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REFERENCES

1. **Allersberger, F., and S. J. Fritschel.** 1999. Use of automated ribotyping of Austrian *Listeria monocytogenes* isolates to support epidemiological typing. J. Microbiol. Methods **35:**237–244.
2. **Baloga, A. O., and S. K. Harlander.** 1991. Comparison of methods for discrimination between strains of *Listeria monocytogenes* from epidemiological surveys. Appl. Environ. Microbiol. **57:**2324–2331.
3. **Bille, J.** 1990. Anatomy of a foodborne listeriosis outbreak, p. 39–46. *In* Foodborne listeriosis: proceedings of a symposium on September 7, 1988, in Wiesbaden, FRG. Technomic Publishing Co., Inc., Hamburg, Germany.
4. **Bille, J., and J. Rocourt.** 1996. W.H.O. International Multicenter *Listeria monocytogenes* Subtyping Study: rationale and setup of the study. Int. J. Food Microbiol. **32:**251–262.
5. **Brosch, R., M. Brett, B. Catimel, J. B. Luchansky, B. Ojeniyi, and J. Rocourt.** 1996. Genomic fingerprinting of 80 strains from the WHO multicenter international typing study of *Listeria monocytogenes* via pulsed-field gel electrophoresis (PFGE). Int. J. Food Microbiol. **32:**343–355.
6. **Bruce, J.** 1996. Automated system rapidly identifies and characterizes microorganisms in food. Food Technol. **50:**77–81.
7. **Bruce, J. L., R. J. Hubner, E. M. Cole, C. I. McDowell, and J. A. Webster.** 1995. Sets of *Eco*RI fragments containing ribosomal RNA sequences are conserved among different strains of *Listeria monocytogenes*. Proc. Natl. Acad. Sci. USA **92:**5229–5233.
8. **Carbonelle, B., J. Cottin, F. Parvery, G. Chambreuil, S. Kouyoumdjian, M. Le Lirzin, G. Cordier, and F. Vincent.** 1978. Epidemie de listeriose dans l'ouest de la France (1975–1976). Rev. Epidemiol. Sante Publique **26:**451–467.
9. **Centers for Disease Control and Prevention.** 1998. Multistate outbreak of listeriosis—United States, 1998. Morb. Mortal. Wkly. Rep. **47:**1085–1086.
10. **Centers for Disease Control and Prevention.** 1999. Update: multistate outbreak of listeriosis—United States, 1988–1999. Morb. Mortal. Wkly. Rep. **47:**1117–1118.
11. **Farber, J. M., and P. I. Peterkin.** 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. **55:**476–511.
12. **Fleming, D. W., S. L. Cochi, K. L. MacDonald, J. Brondum, P. S. Hayes, B. D. Plikaytis, M. B. Holmes, A. Audurier, C. V. Broome, and A. L. Reingold.** 1985. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. N. Engl. J. Med. **312:**404–407.
13. **Gendel, S. M., and J. Ulaszek.** 2000. Ribotype analysis of strain distribution in *Listeria monocytogenes*. J. Food Prot. **63:**179–185.
14. **Graves, L. M., B. Swaminathan, M. W. Reeves, S. B. Hunter, R. E. Weaver, B. D. Plikaytis, and A. Schuchat.** 1994. Comparison of ribotyping and multilocus enzyme electrophoresis for subtyping of *Listeria monocytogenes* isolates. J. Clin. Microbiol. **32:**2936–2943.
15. **Graves, L. M., B. Swaminathan, and S. B. Hunter.** 1999. Subtyping *Listeria monocytogenes*, p. 279–297. *In* E. T. Ryser and E. H. Marth (ed.), *Listeria*, listeriosis, and food safety. Marcel Dekker, Inc., New York, N.Y.
16. **Grimont, F., and P. A. D. Grimont.** 1986. Ribosomal ribonucleic acid gene restriction patterns as potential taxonomic tools. Ann. Inst. Pasteur/Microbiol. (Paris) **137B:**165–175.
17. **Ho, J. L., K. N. Shands, G. Friedland, P. Eckind, and D. W. Fraser.** 1986. An outbreak of type 4b *Listeria monocytogenes* infection involving patients from eight Boston hospitals. Arch. Intern. Med. **146:**520–524.
18. **Hunter, P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
19. **Jacquet, C., J. Bille, and J. Rocourt.** 1992. Typing *Listeria monocytogenes* by restriction polymorphism of the ribosomal ribonucleic acid gene region. Zenbl. Bakteriol. **276:**356–365.
20. **Jacquet, C., B. Catimel, R. Brosch, C. Buchrieser, P. Dehaumont, V. Goulet, A. Lepoutre, P. Veit, and J. Rocourt.** 1995. Investigations related to the epidemic strain involved in the French listeriosis outbreak in 1992. Appl. Environ. Microbiol. **61:**2242–2246.
21. **Linnan, M. J., L. Mascola, X. D. Lou, V. Goulet, S. May, C. Salminen, D. W. Hird, M. L. Yonekora, P. Hayes, R. Weaver, A. Audurier, B. D. Plikaytis, S. L. Fannin, A. Kleks, and C. V. Broome.** 1988. Epidemic listeriosis associated with Mexican-style cheese. N. Engl. J. Med. **319:**823–828.
22. **McLauchlin, J.** 1990. Distribution of serovars of *Listeria monocytogenes* isolated from different categories of patients with listeriosis. Eur. J. Clin. Microbiol. Infect. Dis. **9:**210–213.
23. **McLauchlin, J., A. Audurier, and A. G. Taylor.** 1986. The evaluation of a phage typing system for *Listeria monocytogenes* for use in epidemiological studies. J. Med. Microbiol. **22:**357–365.
24. **McLauchlin, J., A. Audurier, and A. G. Taylor.** 1986. Aspects of the epidemiology of human *Listeria monocytogenes* infections in Britain 1967–1984; the use of serotyping and phage typing. J. Med. Microbiol. **22:**367–377.
25. **McLauchlin, J., S. M. Hall, S. K. Velani, and R. J. Gilbert.** 1991. Human listeriosis and pâté: a possible association. Br. Med. J. **303:**773–775.
26. **Mead, P., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin, and R. V. Tauxe.** 1999. Food-related illness and death in the United States. Emerg. Infect. Dis. **5:**607–625.
27. **Nørrung, B., and N. Skovgaard.** 1993. Application of multilocus enzyme electrophoresis in studies of the epidemiology of *Listeria monocytogenes* in Denmark. Appl. Environ. Microbiol. **59:**2817–2822.
28. **Norton, D. M., M. A. McCarney, K. L. Gall, J. M. Scarlett, K. J. Boor, and M. Wiedmann.** 2001. Molecular studies on the ecology of *Listeria monocytogenes* in the smoked fish processing industry. Appl. Environ. Microbiol. **67:**198–205.
29. **Piffaretti, J.-C., H. Kressebuch, M. Aeschenbacher, J. Bille, E. Bannerman, J. M. Musser, R. K. Selander, and J. Rocourt.** 1989. Genetic characterization of clones of the bacterium *Listeria monocytogenes* causing epidemic disease. Proc. Natl. Acad. Sci. USA **86:**3818–3822.
30. **Rocourt, J.** 1988. Identification and typing of listeria, p. 19–37. *In* Foodborne listeriosis: proceedings of a symposium on September 7, 1988, in Wiesbaden, FRG. Technomic Publishing Co., Inc., Hamburg, Germany.
31. **Schlech, W. F., III, P. M. Lavigne, 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. N. Engl. J. Med. **308:**203–206.
32. **Schuchat, A., B. Swaminathan, and C. V. Broome.** 1991. Epidemiology of human listeriosis. Clin. Microbiol. Rev. **4:**169–183.
33. **Schwartz, B., D. Hexter, C. V. Broome, A. W. Hightower, R. B. Hirschhorn, J. D. Porter, P. S. Hayes, W. F. Bibbs, B. Lorber, and D. G. Faris.** 1989. Investigation of an outbreak of listeriosis: new hypotheses for the etiology of epidemic *Listeria monocytogenes* infections. J. Infect. Dis. **159:**680–685.
34. **Seeliger, H. P. R., and K. Hohne.** 1979. Serotyping of *Listeria monocytogenes* and related species. Methods Microbiol. **13:**31–49.
35. **Swaminathan, B., S. B. Hunter, P. M. Desmarchelier, P. Gerner-Smidt, L. M. Graves, S. Harlander, R. Hubner, C. Jacquet, B. Pedersen, K. Reineccius, A. Ridley, N. A. Saunders, and J. A. Webster.** 1996. WHO-sponsored international collaborative study to evaluate methods for subtyping *Listeria monocytogenes*: restriction fragment length polymorphism (RFLP) analysis using ribotyping and Southern hybridization with two probes derived from *L. monocytogenes* chromosome. Int. J. Food Microbiol. **32:**263–278.
36. **Wiedmann, M., J. Czajka, N. Bsat, M. Bodis, M. C. Smith, T. J. Divers, and C. A. Batt.** 1994. Diagnosis and epidemiological association of *Listeria monocytogenes* strains in two outbreaks of listerial encephalitis in small ruminants. J. Clin. Microbiol. **32:**991–996.
37. **Wiedmann, M., J. L. Bruce, C. Keating, A. E. Johnson, P. L. McDonough, and C. A. Batt.** 1997. Ribotypes and virulence gene polymorphisms suggest three distinct *Listeria monocytogenes* lineages with differences in pathogenic potential. Infect. Immun. **65:**2707–2716.
38. **Wiedmann, M., S. Mobini, J. R. Cole, Jr., C. K. Watson, G. Jeffers, and K. J. Boor.** 1999. Molecular investigation of a listeriosis outbreak in goats caused by an unusual strain of *Listeria monocytogenes*. J. Am. Vet. Med. Assoc. **215:**369–371.
39. **Wiedmann, M., D. Weilmeier, S. S. Dineen, R. Ralyea, and K. J. Boor.** 2000. Molecular and phenotypic characterization of *Pseudomonas* spp. isolated from milk. Appl. Environ. Microbiol. **66:**2085–2095.