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Characterization of *Listeria monocytogenes* Isolated from Poultry Products and from the Poultry-Processing Environment by Random Amplification of Polymorphic DNA and Multilocus Enzyme Electrophoresis

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A total of 289 *Listeria monocytogenes* strains isolated from a poultry-processing environment and poultry products over a 6-month period were characterized by random amplification of polymorphic DNA, (RAPD) to pinpoint sources of contamination within the plant and gain some measure of the persistence of individual genotypes within this environment. Eighteen RAPD profiles (A through R) were identified within this group, with 64% (184 of 289) of all strains displaying a single RAPD profile, RAPD type A. This genotype was more prevalent in the raw-poultry-processing environment, where, although its origin within this environment appeared to be the incoming birds, it was also widespread on food contact surfaces, floors, and drains. This was the only genotype which persisted throughout the entire 6-month period, and it and RAPD type B were the only two genotypes found in both the raw- and cooked-poultry-processing environments. *L. monocytogenes* strains isolated from cooked poultry products and the cooked-poultry-processing environment up to 1 year later (17 strains) contained only RAPD types A and B, highlighting the potential which exists for persistent strains to cross-contaminate foods processed in that environment. The other genotypes (C through R) occurred more sporadically, suggesting varied sources of contamination. These were confined to either the raw- or the cooked-poultry-processing environment and were relatively short-lived. Further characterization of a selection of RAPD type A strains, together with strains of RAPD types B through R, was carried out by multilocus enzyme electrophoresis. Strains of RAPD type A contained two electrophoretic types, one of which was serotype 1/2a and the other was 1/2c. Each electrophoretic type appeared to be equally persistent and widespread, and their isolation from the same samples suggests that they may coexist within this environment. Multilocus enzyme electrophoresis provided no further differentiation within RAPD types B through R, demonstrating the high level of discrimination already achieved by RAPD. The latter method proved to be a valuable typing tool in this study and offers the potential for food processors to gain valuable information on sources of contamination and the persistence of strains within the processing environment.

Investigations of several large outbreaks of listeriosis (6, 11, 18, 29) and a number of sporadic cases (27) have demonstrated that contaminated food was responsible for the transmission of the causative organism, *Listeria monocytogenes*. Although some of these outbreaks may be considered zoonotic (6, 29), since the source of infection was domestic animals, listeriosis rarely seems to occur as a direct zoonosis (10), and there is increasing evidence that contamination of food products is more likely to originate from environmental sources (14), including the food-processing environment. The unusual growth and survival properties of *L. monocytogenes* (5, 25, 26) and its ability to adhere to various food contact surfaces (19) each contribute to the complexity of eliminating the organism from this environment. Therefore, to reduce or eliminate the potential for cross-contamination of foods from the processing environment, attention must be focused on detection of critical control points by using the hazard analysis critical control point concept (23).

Recent studies have demonstrated that for poultry processing, the processing environment is a major source of *Listeria* contamination of raw and cooked poultry products (7, 8, 13, 16, 31). In each of these studies, the incidence of *Listeria* spp. and *L. monocytogenes* within the product increased from the beginning to the end of processing, indicating the occurrence of cross-contamination from the environment. Various studies have shown the incidence of *L. monocytogenes* in cooked and ready-to-eat poultry products at 12 to 27% at the point of retail sale (9, 15, 28), in some cases at levels up to 700 CFU g^{-1} (1), thus emphasizing the need for better control measures. Although microbiological investigations of this nature are useful in monitoring the incidence of *Listeria* spp. at the various stages of processing, they fall short of detecting specific sources of contamination or identifying endemic strains within this environment. To enable more precise identification of sites of cross-contamination, or critical control points, and to give some measure of the persistence of individual strains within the poultry-processing environment, epidemiological tracking of strains over time is required. Achievement of this goal is enhanced by the availability of molecular typing techniques which allow rapid and sensitive differentiation between *L. monocytogenes* strains. Such methods include random amplification of polymorphic DNA (RAPD) (17, 20) and multilocus enzyme electrophoresis (MEE) (2, 3).

In the work reported here, *L. monocytogenes* strains isolated from raw poultry products at the various stages of processing and from various sites within a poultry-processing environment

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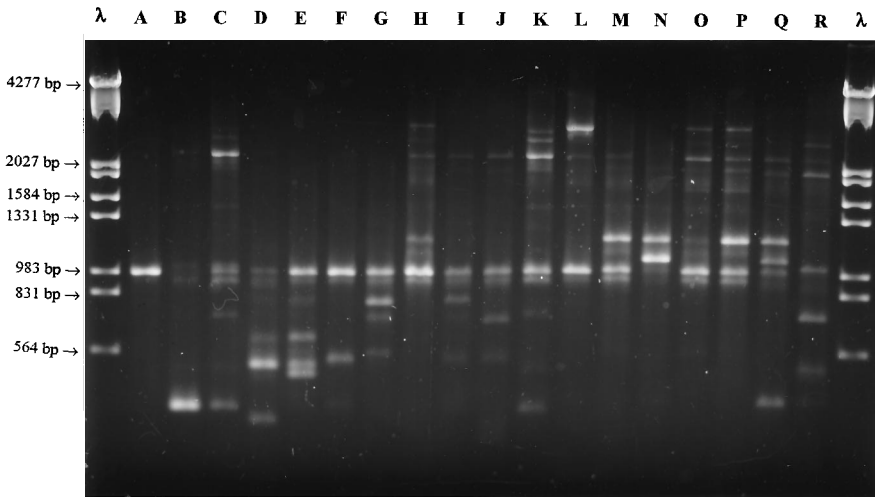

FIG. 1. RAPD profile types obtained with primer OPM-01 for 289 *L. monocytogenes* strains isolated from poultry products and a poultry-processing environment over a 6-month period. Lanes: λ, DNA molecular size marker (*Eco*RI-*Hin*dIII digest of λ DNA); A through R, RAPD profiles of types A through R, respectively (sample sources are identified in Table 1).

over a 6-month period (16) were further characterized by RAPD. The primer used in this investigation demonstrated maximum discrimination within *L. monocytogenes* in a previous study (17). However, to ensure that discrimination within this group of strains had been maximized, strains which gave identical RAPD profiles were further characterized by MEE. The aim of this study was to gain more information on potential sources of contamination for poultry products and to achieve some measure of the persistence of individual genotypes within this processing environment over the period of the study.

MATERIALS AND METHODS

Bacterial strains. A total of 289 *L. monocytogenes* strains which originated from 81 samples taken over a 6-month period (March through August 1992) from a single poultry-processing plant within Northern Ireland were investigated (16). These strains were isolated by a modification of the U.S. Department of Agriculture-Food Safety and Inspection Service procedure, as described by McClain and Lee (21). Up to five typical colonies were removed from each plate and transferred to nonselective media for purification. A single well-isolated *Listeria*-like colony on nonselective agar, tryptone soy agar (Oxoid) with an additional 0.6% yeast extract (TSAYE), demonstrating blue coloration by the Henry illumination technique (33) was transferred to a vial of beads in a cryopreservative fluid (Protect, Bury, England) and stored at −80°C for further identification and typing. In addition, a further 6 strains subsequently isolated from cooked poultry products (April through August 1993) and 11 strains isolated from the cooked-poultry-processing environment (March through August 1993) were examined.

RAPD analysis. All 306 bacterial strains were cultured from beads stored at −80°C in cryopreservative fluid. Cells were streaked onto TSAYE and incubated at 30°C overnight. To ensure culture purity, a single colony from TSAYE was then inoculated into 10 ml of brain heart infusion broth (Oxoid) and incubated at 30°C overnight, and the culture broth was used for RAPD analysis with the 10-mer primer OPM-01 (5′-GTT GGT GGC T-3′) as described previously (17). This primer was selected from 40 such primers in an earlier study, since it showed maximum discrimination within *L. monocytogenes* from a diverse range of sources. Thirty strains, selected at random, were analyzed a second time to assess the reproducibility of RAPD profiles throughout the course of the investigation.

MEE analysis. A total of 31 strains representative of those which gave an identical RAPD profile type (RAPD type A), together with all other strains (105 strains) which produced 17 distinct types (RAPD types B through R), were further characterized by MEE as described previously (12), except that the cell cytoplasmic contents were released by sonication on ice (model XL2020 Cup Horn Sonicator, 20-kHz energy; Heat Systems Inc., Farmingdale, N.Y.) for 3 min of 10-s bursts, each separated by 30 s. The electrophoretic mobilities of the enzymes 6-phosphogluconate dehydrogenase (EC 1.1.1.44), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), NAD-dependent glyceraldehyde-3-phosphate (EC 1.2.1.12), alanine dehydrogenase (EC 1.4.1.1), catalase (EC 1.11.1.6), phosphoglucomutase (EC 2.7.5.1), α-naphthyl propionyl esterases 1 and 2 (EC 3.1.1.1), acid phosphatase (EC 3.1.3.2), fumarase (EC 4.2.1.2), and mannose phosphate isomerase (EC 5.3.1.8) were investigated.

Electrophoretic types (ETs), genetic relationships between ETs, genetic diversity at each enzyme locus (h_i), and the mean genetic diversity over all loci (H) were determined as described previously (12). Strains of RAPD type A were then serotyped at the Centers for Disease Control and Prevention by using antisera purified from immunized rabbits and the methods of Seeliger and Höhne (30).

RESULTS

Characterization of *L. monocytogenes* by RAPD. For the group of 289 strains isolated from raw poultry products and a poultry-processing environment, 18 different RAPD profiles, arbitrarily designated A through R, were obtained. An example of each profile type is shown in Fig. 1. Control assays which contained no cell lysate yielded no detectable amplified product. Duplicate samples produced identical RAPD profiles.

The distribution of RAPD types within this group of strains in relation to their date of isolation is shown in Table 1. Of the 289 *L. monocytogenes* strains examined, 184 (64%) displayed a single RAPD profile, RAPD type A. This profile, which consisted of a single PCR product of around 1,000 bp, accounted for 83% (77 of 93) of all strains isolated from raw poultry products and 76% (68 of 90) and 37% (39 of 106) of those from the raw- and cooked-poultry-processing environments, respectively. This was the only genotype which was consistently isolated on each sampling occasion throughout the 6-month period. The percentage of strains from each source which displayed this profile on a monthly basis is also shown in Table 1.

The initial point at which RAPD type A appeared was on raw whole birds coming into the poultry-processing plant. Although the incidence of *L. monocytogenes* contamination at this stage was minimal, all strains detected on the incoming birds were of RAPD type A. The prevalence of type A was found to continue within raw poultry products which had been further processed and on contact surfaces of processing equipment within the raw-poultry-processing environment. Floor drains were also found to consistently harbour this RAPD type over the entire 6-month period. Within the cooked-poultry-processing environment, however, the percentage of *L. monocytogenes* strains of RAPD type A was reduced from that in the raw-poultry-processing environment, and on one sampling oc-

TABLE 1. Distribution of *L. monocytogenes* RAPD types (as shown in Fig. 1) in relation to the source and date of isolation of the strain(s)

Date of isolation	Source of strain(s)	No. of strains	RAPD type (%)
27 March 1992	Raw poultry products	25	A (100)
	Raw-poultry-processing environment	15	A (100)
	Cooked-poultry-processing environment	8	A (89)
		1	C (11)
24 April 1992	Raw poultry products	15	A (88)
		1	F (6)
		1	G (6)
	Raw-poultry-processing environment	9	A (75)
		1	B (8)
		1	F (8)
		1	G (8)
	Cooked-poultry-processing environment	11	A (48)
		10	B (43)
		1	D (4)
		1	E (4)
22 May 1992	Raw poultry products	24	A (100)
	Raw-poultry-processing environment	9	A (64)
		5	B (26)
	Cooked-poultry-processing environment	7	B (70)
		3	H (30)
26 June 1992	Raw poultry products	7	A (44)
		5	B (31)
		2	I (13)
		2	J (13)
	Raw-poultry-processing environment	11	A (78)
		2	I (14)
		1	J (7)
	Cooked-poultry-processing environment	1	A (14)
		6	B (86)
24 July 1992	Raw poultry products	4	A (80)
		1	B (20)
	Raw-poultry-processing environment	5	A (45)
		1	Q (9)
		5	R (45)
	Cooked-poultry-processing environment	2	A (10)
		6	K (30)
		12	L (60)
21 August 1992	Raw poultry products	2	A (33)
		4	O (67)
	Raw-poultry-processing environment	19	A (79)
		3	M (14)
		2	N (8)
	Cooked-poultry-processing environment	17	A (46)
		7	B (19)
		2	H (5)
		11	P (30)

TABLE 2. ET designation, RAPD type, and enzyme profile for *L. monocytogenes* strains of RAPD types A through R isolated from raw poultry products and the poultry-processing environment over a 6-month period

ET	RAPD type	Relative mobility of enzyme[a]:										
		6PD	G6D	GP1	ALD	CAT	PGM	ES1	ES2	ACP	FUM	MPI
1	B, C, D, E	6	4	4	5	5	7	5	0	7	4	5
2	K	6	4	6	5	5	7	5	7	7	4	5
3	A	6	6	5	5	5	7	5	3	5	5	7
4	H	7	6	5	5	5	8	5	7	6	5	6
5	M	7	6	5	5	5	8	5	7	6	5	7
6	G, L	7	6	5	5	5	8	5	7	5	5	6
7	O	7	5	5	5	5	8	5	7	5	5	6
8	P	7	5	5	5	5	8	5	7	6	5	6
9	Q	7	5	5	5	5	7	5	0	6	5	5
10	A	7	6	5	5	4	8	5	4	5	5	7
11	F	6	6	5	5	6	7	5	7	6	5	6
12	N	6	4	5	5	4	6	5	5	5	5	5
13	J	7	5	5	4	6	6	5	5	5	5	6
14	R	6	4	5	3	5	7	5	6	6	5	4
15	I	7	6	6	5	6	6	6	0	5	5	6

[a] 6PD, 6-phosphogluconate dehydrogenase; G6D, glucose-6-phosphate dehydrogenase; GP1, NAD-dependent glyceraldehyde-3-phosphate; ALD, alanine dehydrogenase; CAT, catalase; PGM, phosphoglucomutase; ES1 and ES2, α-naphthyl propionyl esterases 1 and 2; ACP, acid phosphatase; FUM, fumarase; MPI, mannose phosphate isomerase. Mobilities are numbered in increasing order of anodal migration.

casion (May), RAPD type A was not detected. Typical sites within the cooked-poultry-processing environment from which the genotype was isolated included food contact surfaces, floor drains, floors, and floor squeegees.

The second most common RAPD profile, RAPD type B, which consisted of a PCR product of around 400 bp, accounted for a further 14% (42 of 289) of all isolates. However, type B appeared to be more prevalent in the cooked-poultry-processing environment, where it constituted 28% (30 of 106) of strains from this source compared with 7% (6 of 90) and 6% (6 of 93) from the raw-poultry-processing environment and raw poultry products, respectively. Again, floor drains were one of the main sources from which this genotype was isolated.

The remaining 16 RAPD types (types C through R), which accounted for only 24% of all the strains, occurred only sporadically throughout the sampling period and were found to be confined to either the raw product/processing environment or the cooked-poultry-processing environment. Types G, F, I, J, Q, R, M, N, and O were isolated only from the raw product/processing environment, whereas types C, D, E, H, K, and L were found only in the cooked-poultry-processing environment.

In general terms, the distribution of *L. monocytogenes* RAPD types isolated from the raw-poultry-processing environment and from raw poultry products was more homogeneous than the distribution of those isolated from the cooked sector. In most cases, the RAPD types found within raw poultry products reflected those found within the raw-poultry-processing environment on that sampling occasion. RAPD analysis of a further 17 *L. monocytogenes* strains isolated from cooked poultry products and the cooked-poultry-processing environment up to 1 year after the 6-month survey revealed that 15 of 17 (88%) isolates produced a RAPD type A profile, while 2 of 17 (12%) produced a RAPD type B profile. No other genotypes were detected in these samples.

Further characterization of *L. monocytogenes* strains by MEE. (i) Designation of ETs. In the 136 strains isolated from the poultry-processing environment over a 6-month period, a total of 15 unique combinations of electromorphs or ETs, as determined by MEE, were identified. These were arbitrarily designated ETs 1 through 15 (Table 2) and separated into two distinct groups at a genetic distance of 0.52 (Fig. 2). One group contained ETs 1 and 2 (corresponding to RAPD types B, C, D, E, and K), while the second group encompassed ETs 3 through 15 (corresponding to RAPD types A, F, G, H, I, J, L, M, N, O, P, Q, and R).

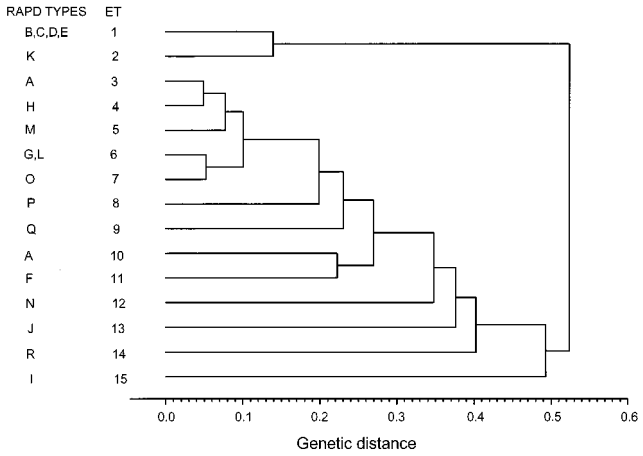

FIG. 2. Dendrogram showing the genetic relationships among *L. monocytogenes* strains of RAPD types A through R, isolated from raw poultry products and the poultry-processing environment.

Within the 31 RAPD type A strains, two ETs (ETs 3 and 10), separated by a genetic distance of 0.27, were produced, indicating two distinct groups of *L. monocytogenes* within this genotype. Eighteen strains displayed the ET 3 profile, while the remaining 13 produced that of ET 10. There was no correlation between the source of isolate or date of isolation and the ET, with each ET containing strains from various locations and isolated over the entire 6-month period of the study. In some cases, strains which originated from the same sample on the same sampling occasion, e.g., floor and floor squeegee, displayed different ETs. Serotyping confirmed two distinct groups within this collection of strains, with strains of ET 3 being of serotype 1/2a and those of ET 10 being of serotype 1/2c.

Within RAPD types B through R, no further differentiation of isolates was obtained by MEE, and in some cases, strains which produced distinct RAPD profiles produced identical ETs. For example, RAPD types B, C, D, and E displayed a single profile, ET 1, while RAPD types G and L displayed the enzyme profile of ET 6 (Table 2).

(ii) Genetic diversity. All of the enzymes were polymorphic for this group of strains. The number of alleles at each enzyme locus ranged between 2 and 5, with the greatest diversity being found in the α-naphthyl propionyl esterase 2 (Table 3). The mean genetic diversity averaged over all the enzymes was found to be 0.462.

TABLE 3. Genetic diversity at 11 enzyme loci among 15 ETs of *L. monocytogenes* isolated from raw poultry products and the poultry-processing environment

Enzyme locus[a]	Genetic diversity
6PD	0.514
G6D	0.686
GP1	0.362
ALD	0.257
CAT	0.533
PGM	0.686
EST1	0.133
EST2	0.762
ACP	0.648
FUM	0.248
MPI	0.714
Mean	0.462

[a] See Table 2, footnote *a*, for information on enzyme abbreviations.

DISCUSSION

Although previous studies have demonstrated systematic contamination of various food-processing environments by *L. monocytogenes* (3, 4, 12, 16, 32), the detection of endemic strains within a poultry-processing environment over an extended period has hitherto not been reported. In this study, the application of RAPD to *L. monocytogenes* strains isolated from a poultry-processing plant and poultry products identified the predominance of a single genotype (RAPD type A) over a 6-month period. This particular RAPD profile has also been found to be common within *L. monocytogenes* strains isolated from a range of nondairy foods and food environmental sources within the United Kingdom (17), thus emphasizing its importance within food and food-related strains.

With respect to sources or critical control points for *L. monocytogenes* within the raw-poultry-processing environment, RAPD type A was first isolated from the incoming birds, which suggested that this was the origin of contamination at this stage. However, the predominance of this genotype within raw poultry products and on various food contact surfaces further along the processing line indicates either that the product acts as a continuous source of inoculation or that the genotype is persisting on equipment surfaces, which then ultimately act as a source of cross-contamination. The latter view is supported by the consistent finding of RAPD type A strains in environmental samples, e.g., floors and drains, which were not in direct contact with product. Furthermore, the finding of continuous environmental contamination, albeit at a reduced level, of RAPD type A strains in the cooked-poultry environment supports the view that contamination is not entirely via the product, since at this stage the product has been cooked to an internal temperature of 85°C and cannot therefore act as a vehicle for transfer of the genotype into this environment. This therefore suggests the establishment and persistence of these strains within the wider processing environment and supports the view that certain clones of *L. monocytogenes* may be adapted to this specific niche (12, 24). The persistence of these strains within this niche may also be enhanced by their ability to attach to surfaces and form biofilms, giving them added protection against biocidal agents used for their control (19). The isolation of RAPD type A strains from a number of locations within the cooked-poultry environment suggests that cross-contamination from the raw-poultry environment has occurred at some stage, allowing the genotype to become established. The finding of a second genotype (RAPD type B), albeit at lower levels, within both the raw- and cooked-poultry-processing environments would support this view.

Although *L. monocytogenes* RAPD types A and B existed in the cooked-poultry-processing environment during the 6-month sampling period, the organism was not isolated from cooked products immediately after heat processing during this time. However, RAPD type A and B strains were subsequently identified in the cooked-poultry-processing environment and cooked poultry products up to 1 year after the initial study, demonstrating the potential for environmental strains to persist for long periods, survive the cleaning schedules in place, and ultimately contaminate the final product.

The occurrence of RAPD types C through R appeared to be more sporadic and relatively short-lived compared with that of RAPD types A and B. Since the former genotypes were confined to either the raw- or the cooked-poultry-processing en-

vironment and were not found on the incoming birds, it is likely that these strains originated from a variety of sources within their respective environments. The association between the genotypes found on raw poultry products further along the processing line and those found in the immediate processing environment also indicates that cross-contamination of the product from the environment was probable. The findings also demonstrate that further processing and handling of poultry products play a major role in spreading contamination, since it can be seen that a relatively wide range of RAPD types was present on raw portioned poultry products (RAPD types A, B, F, G, I, J, and O), compared with a single type on the raw whole bird (RAPD type A).

The finding that RAPD type A strains could be further differentiated into two distinct groups by MEE and serotyping emphasizes the importance of using a combination of typing techniques to gain maximum discrimination between strains. Isolates from both groups were found throughout the entire sampling period, from raw poultry products and from within the raw- and cooked-poultry-processing environments, indicating that each group was equally persistent and widespread. The occurrence of strains representing each ET and serotype together on environmental samples suggests that these types may coexist within this environment. It is not known why these two phenotypically distinct types gave the same RAPD profile, but it is possible that the PCR product amplified is associated with a phenotypic characteristic, which in some way enhances the ability of a strain to colonize a food contact surface. It is also interesting that within RAPD type A, all strains displayed the serogroup 1/2 somatic antigen type. This observation supports previous findings that recurrent strains isolated from a variety of foods from individual processors over the period of 1 year were of serogroup 1/2 (12) and raises the question of the role of surface antigens in the ability of *L. monocytogenes* to colonize surfaces within the food-processing environment. The persistence of serogroup 1/2 strains in the processing environment may also be a possible explanation for the prevalence of *L. monocytogenes* of this serogroup in foods.

The finding that the *L. monocytogenes* strains examined fell into two main groups when analyzed by MEE agrees with previous findings (2, 3, 12, 24). The occurrence of RAPD type A strains of serotypes 1/2a and 1/2c in the second group indicates that this large group (ETs 3 through 15) corresponds to strains with flagellar antigen types a and c, while the first group (ETs 1 and 2) corresponds to strains of flagellar type b (2). Since it is mainly *L. monocytogenes* of the first group (ETs 1 and 2) which are associated with human listeriosis outbreaks, it is anticipated that strains of ETs 1 and 2, although representing only 17% of the total number of strains isolated, may be of more significance from an epidemiological point of view. The widespread finding of RAPD type B strains within the cooked-poultry-processing environment and the occurrence of these strains in cooked poultry products further emphasize the potential threat to human health imposed by the ability of these strains to contaminate ready-to-eat foods.

Although MEE was found to be less discriminatory than RAPD for this group of isolates in that it did not further differentiate RAPD types B through R and produced identical enzyme profiles for strains of RAPD types B, C, D, E, and G and L, the method further differentiated RAPD type A strains and provided a measure of the genetic distance separating strains within this group. This information helped to confirm that the group contained two genotypes which were genetically distinct, demonstrating the value of using more than one typing technique in epidemiological investigations. The mean genetic diversity within this group of strains, as determined by MEE, also demonstrated that although these strains were isolated from a single processing environment and there was a predominance of one or two types within this environment, there was almost as much genetic diversity within the entire group, as was previously found in strains isolated from various sources (12). This may be a reflection of the fact that most of the strains belong to serogroup 1/2, in which the genetic diversity tends to be greater than among strains of serogroup 4.

Although RAPD type A strains were further differentiated by alternative typing techniques in this study, RAPD proved to be a rapid and valuable technique for screening large numbers of strains isolated from foods and the food-processing environment. Meunier and Grimont (22) have expressed some concern about the reproducibility of RAPD profiles but concluded that reproducibility was excellent as long as the methodology was standardized. This was confirmed in this study, in which *L. monocytogenes* strains examined on different occasions gave identical RAPD profiles and, indeed, strains isolated from foods and the cooked-poultry-processing environment up to 1 year after the main study gave profiles identical to those of some of the isolates examined earlier. Therefore, it can be concluded that the application of RAPD in the characterization of *L. monocytogenes* isolated from the food-processing environment can provide a valuable insight into the presence of endemic strains and can provide valuable information on potential sites of cross-contamination.

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