APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1994, p. 2584–2592
0099-2240/94/$04.00+0

Vol. 60, No. 7

Pulsed-Field Fingerprinting of Listeriae: Identification of Genomic Divisions for *Listeria monocytogenes* and Their Correlation with Serovar

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Received 7 March 1994/Accepted 7 May 1994

Clamped homogeneous electric field (CHEF) electrophoresis was optimized for genomic analyses of *Listeria monocytogenes*. Various human, animal, food, and environmental isolates, as well as strains representing other *Listeria* species, were separately digested with rarely cutting endonucleases. Of 176 *L. monocytogenes* strains analyzed, the enzymes *Asc*I and *Apa*I established 63 and 72 unique restriction endonuclease digestion profiles (REDP), respectively. The 22 non-*L. monocytogenes* strains exhibited 18 *Asc*I and 19 *Apa*I unique REDP. Statistical analyses of REDP information using the Dice coincidence index and principal component analysis revealed two distinct genomic divisions of *L. monocytogenes* that also correlated with the flagellar (H) antigen type: division I contained serovar 1/2a, 1/2c, 3a, and 3c strains, and division II contained serovar 1/2b, 3b, 4b, 4d, and 4e strains. Division I isolates digested with *Apa*I were further grouped into cluster IA (serovar 1/2c and 3c) and cluster IB (serovar 1/2a and 3a) strains. Likewise, division II isolates digested with *Apa*I were further grouped into cluster IIA (serovar 1/2b and 3b) and cluster IIB (serovar 4b, 4d, and 4e) strains. These data indicate that genotypic data generated by CHEF can be directly related to phenotypic data generated by serotyping for establishing the overall relatedness of isolates. Moreover, these data further substantiate that CHEF analysis is a reproducible and highly discriminating method for characterizing *L. monocytogenes* strains at the molecular level.

Although cases of food-related listeriosis in the United States have decreased by half since 1986 (1), as evidenced by the appreciable increase in product recalls between 1988 and 1992 (2), *Listeria monocytogenes* remains a serious threat to human health. Therefore, it is not surprising that considerable efforts are continually directed to develop rapid, reproducible, and discriminating methods to characterize this important food-borne pathogen. Several phenotype- and genotype-based typing strategies, each with their intrinsic advantages and shortcomings, are available for differentiating listeriae (for examples, see references 3, 5, 10, 13, 15, 18 to 22, 24, 25, and 27). Genomic fingerprinting via pulsed-field gel electrophoresis (PFGE) is one of the most, if not the most, discriminating typing method available for *L. monocytogenes* (3–6, 15). Thus, one aim of the present study was to substantiate the discriminating powers of PFGE by using a larger and more diverse collection of strains than previously examined. A second aim of this study was to associate, if possible, pulsed-field restriction endonuclease digestion profiles (REDP) with relevant phenotypic typing (i.e., serovar) criteria. A third aim was to apply a computer-based, statistically relevant strategy to evaluate numerous REDP to provide an overview of genetic relatedness among phenotypically similar listeriae.

(Preliminary reports of portions of this work were presented at the 80th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, 1 to 4 August 1993, in Atlanta, Ga.)

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MATERIALS AND METHODS

Listeriae. A total of 176 different *L. monocytogenes* strains from various sources and 22 strains representing other *Listeria* species were analyzed in this study. Isolates were maintained as described previously (7) and transferred at least twice prior to use. Representative strains and their relevant characteristics are listed in Tables 1 and 2. Serovar data were obtained from reference laboratories and/or were provided by culture curators.

Preparation and cleavage of genomic DNA in agarose plugs. Intact, high-molecular-weight genomic *Listeria* DNA was isolated and digested in agarose plugs as described elsewhere (3, 15) with two exceptions. The present study used 10^8 cells (40 μl) per agarose plug and employed a lysis solution containing 0.5 M EDTA, 0.5% *N*-lauroylsarcosine (Sigma Chemical Co., St. Louis, Mo.), 2 mg of deoxycholic acid (Sigma) per ml, and 2.5 mg of lysozyme (egg white; Calbiochem-Novabiochem Co., La Jolla, Calif.) per ml. Genomic DNA within each agarose plug was completely digested with 2 U of *Asc*I (New England Biolabs, Inc., Beverly, Mass.) or 20 U of *Apa*I (Boehringer GmbH, Mannheim, Germany).

Macrorestriction fragments were resolved by the PFGE technique of contour-clamped homogeneous electric field (CHEF) electrophoresis, hereafter referred to as PFGE-CHEF, using a CHEF-DRII (Bio-Rad Laboratories, Richmond, Calif.) apparatus. Restriction fragments were resolved within electrophoresis grade agarose (1%; GIBCO-Bethesda Research Laboratories, Life Technologies, Inc., Gaithersburg, Md.), at 200 V and with pulse times ramped from 1 to 40 s over 23 h while the mixture was maintained at 18°C. The low-range PFGE marker (New England Biolabs) and/or lambda DNA concatamers (Promega Corp., Madison, Wis.) were used as molecular weight size standards. For comparison among different gels, *L. monocytogenes* JBL1434 (serovar 4b, clinical

TABLE 1. Designation, source, and relevant characteristics of representative *L. monocytogenes* strains[a]

Genomic division	JBL strain no.	Other designation	Serovar	Source	Results for endonuclease			
					*Apa*I		*Asc*I	
					REDP no.	No. of strains[b]	REDP no.	No. of strains[c]
I	1451[d,e]	CDC/G-2347	1/2a	Human	1	4	*m*11a	1
I	1562[e]	CDC/G-6847	1/2a	Human	1		*m*11b	2
I	1564[e]	CDC/G-6686	1/2a	Human	1		*m*11c	1
I	1389[d,e]	CDC/F-6854	1/2a	Food	2	10	*m*42a	12
I	1510[d]	CDC/G-5138	1/2a	Human	3	2	*m*42a	
I	1612[d,e]	CDC/F-7269	1/2a	Human	4	5	*m*32a	5
I	1617[d,e]	CDC/G-1127	3a	Human	5	1	*m*30a	1
I	1570[d,e]	CDC/G-4484	1/2a	Food	6	2	*m*40a	1
I	1571[e]	CDC/G-3911	1/2a	Food	6		*m*40b	1
I	1518[d,e]	CDC/G-3562	1/2a	Human	7	2	*m*40c	2
I	2121[d,e]	CLIP 9569	1/2a	Food	8	1	*m*50a	1
I	2122[d,e]	CLIP 5864	1/2a	Food	9	1	*m*50b	1
I	1618[d,e]	CDC/G-5824	3a	Human	10	3	*m*51a	1
I	1568[e]	CDC/G-6647	1/2a	Food	10		*m*51b	4
I	1312[d,e]	ATCC 35152	1/2a	Animal	11	4	*m*51c	1
I	1569[e]	CDC/G-5652	1/2a	Food	10		*m*51d	2
I	1563[d]	CDC/G-6767	1/2a	Human	12	1	*m*51d	
I	1460[d,e]	CDC/G-3857	1/2a	Human	13	3	*m*41a	1
I	1459[e]	CDC/G-3828	1/2a	Human	13		*m*41b	2
I	1566[d,e]	CDC/G-6135	1/2a	Human	14	1	*m*41c	1
I	1615[d,e]	CDC/F-8605	3a	Human	15	1	*m*41d	1
I	1616[d,e]	CDC/F-8705	3a	Human	16	1	*m*41e	1
I	1317[d,e]	SLCC 2479	3c	NK	17	11	*m*41f	13
I	1589[d]	CDC/G-5460	1/2c	Human	18	1	*m*41f	
I	1587[d]	CDC/G-5813	1/2c	Food	19	1	*m*41f	
I	1588[d,e]	CDC/G-5661	1/2c	Food	20	1	*m*41g	2
I	1582[d]	CDC/G-7784	1/2c	Human	21	1	*m*41g	
I	1585[d,e]	CDC/G-3321	1/2c	Human	22	1	*m*41h	1
	1320[e]	ATCC 19116	4c	Food		1	*m*35a	1
II	1530[d,e]	27[f]	1/2b	Human	23	2	*m*13a	2
II	1574[d,e]	CDC/G-6592	1/2b	Human	24	1	*m*13b	1
II	2123[e]	CLIP 10456	3b	Human	25		*m*23j	1
II	1313[d,e]	SLCC 2755	1/2b	Animal	25	2	*m*23a	6
II	1620[d]	CDC/F-4540	3b	Food	26	2	*m*23a	
II	1465[d]	CDC/F-4278	1/2b	Food	27	2	*m*23a	
II	1577[d,e]	CDC/G-7419	1/2b	Food	28	2	*m*23b	1
II	1506[d,e]	CDC/G-3310	1/2b	Human	29	2	*m*23c	2
II	1576[d,e]	CDC/G-6346	1/2b	Human	30	1	*m*23d	2
II	1580[d]	CDC/G-5805	1/2b	Food	31	1	*m*23d	
II	1572[d,e]	CDC/G-6853	1/2b	Human	32	1	*m*23e	1
II	1610[d,e]	CDC/F-5899	1/2b	Human	33	4	*m*23f	4
II	1573[d,e]	CDC/G-6805	1/2b	Food	34	1	*m*23g	1
II	1579[d,e]	CDC/G-6378	1/2b	Food	35	1	*m*23h	1
II	1581[d,e]	CDC/G-5397	1/2b	Food	36	1	*m*23i	1
II	1316[d,e]	SLCC 2540	3b	Human	37	1	*m*24a	1
II	1614[d,e]	CDC/G-5777	1/2b	Human	38	1	*m*24b	1
II	1578[d,e]	CDC/G-6961	1/2b	Food	39	1	*m*12a	1
II	1503[d,e]	CDC/G-2405	1/2b	Human	40	6	*m*22a	1
II	1502[d,e]	CDC/G-2311	1/2b	Human	41	2	*m*22b	4
II	1515[d]	CDC/G-3288	1/2b	Human	42	2	*m*22b	
II	1575[d]	CDC/G-6544	1/2b	Human	43	1	*m*22b	
II	1516[e]	CDC/G-3410	1/2b	Human	40		*m*22c	6
II	1504[d,e]	CDC/G-2594	4b	Human	44	4	*m*13d	6
II	1597[d]	CDC/G-7800	4b	Food	45	1	*m*13d	
II	1598[d]	CDC/G-7614	4b	Food	46	1	*m*13d	
II	1595[d,e]	CDC/G-6768	4b	Human	47	1	*m*22d	1
II	1231[d,e]	FRI/*Lm* RS	4b	Human	48	2	*m*22e	2
II	1596[d,e]	CDC/G-6541	4b	Human	49	1	*m*22f	1
II	1431[d]	CDC/G-3205	4b	Human	50	4	*m*22g	
II	1613[d]	CDC/G-5470	4b	Human	51	1	*m*22g	
II	1520[e]	CDC/G-4088	4b	Human	52		*m*22g	5
II	1321[d,e]	ATCC 19117	4d	Animal	52	6	*m*22h	6
II	1529[d,e]	26[f]	4b	Human	53	1	*m*22i	1

Continued on following page

TABLE 1—*Continued*

Genomic division	JBL strain no.	Other designation	Serovar	Source	Results for endonuclease			
					*Apa*I		*Asc*I	
					REDP no.	No. of strains[b]	REDP no.	No. of strains[c]
II	1244[d,e]	CLIP 154	4b	Human	54	1	*m*22j	1
II	1000[d,e]	ScottA	4b	Human	55	15	*m*22k	19
II	1421[d]	CDC/G-4264	4b	Human	56	3	*m*22k	
II	1527[d]	18[f]	4b	Human	57	1	*m*22k	
II	1599[d,e]	CDC/G-6257	4b	Food	58	2	*m*22l	2
II	1594[d,e]	CDC/G-6777	4b	Human	59	1	*m*22m	1
II	1434[d,e]	CDC/F-4565	4b	Human	60	8	*m*21a	8
II	1425[d,e]	CDC/G-3638	4b	Human	61	3	*m*21b	3
II	1419[d,e]	CDC/G-4061	4b	Human	62	1	*m*21c	1
II	1420[d,e]	CDC/G-4063	4b	Human	63	1	*m*21d	1
II	1174[d,e]	ATCC 19118	4e	Food	64	1	*m*21e	1
II	2124[d,e]	CLIP 891	4b	Animal	65	1	*m*21f	1
II	1603[d,e]	CDC/G-1964	4b	Human	66	2	*m*21g	2
II	1455[d,e]	CDC/G-3056	4b	Human	67	1	*m*21h	11
II	1464[d]	CDC/F-4275	4b	Food	68	1	*m*21h	
II	1462[d]	CDC/G-3073	4b	Human	69	7	*m*21h	
II	1532[d]	33[f]	4b	Food	70	2	*m*21h	
II	1429[d,e]	CDC/G-2090	4b	Human	71	4	*m*21i	4
II	1522[d,e]	3[f]	4b	Human	72	3	*m*21j	3

[a] Abbreviations: NK, not known; ATCC, American Type Culture Collection; CDC, culture collection of the Centers for Disease Control and Prevention; CLIP, *Listeria* collection of the Pasteur Institute; FRI, culture collection of the Food Research Institute; JBL, culture collection of J. B. Luchansky, Food Research Institute; SLCC, special *Listeria* culture collection.
[b] Total number of strains with the particular *Apa*I REDP. The total number of all *Apa*I-tested strains was 176.
[c] Total number of strains with the particular *Asc*I REDP. The total number of all *Asc*I-tested strains was 176.
[d] Strain representative of the particular *Apa*I REPD depicted in Fig. 1.
[e] Strain representative of the particular *Asc*I REDP depicted in Fig. 2 or 3.
[f] Strain received from S. Kathariou, University of Hawaii.

TABLE 2. Designations and relevant characteristics of representative *Listeria* sp. strains

Species	JBL strain no.	Other designation[a]	Serovar	Results for endonuclease			
				*Apa*I		*Asc*I	
				REDP no.	No. of strains[b]	REDP no.	No. of strains[c]
L. ivanovii	1673[d,e]	CLIP 12065	5	73	1	*iv*11a	1
	1674[d,e]	CLIP 2737	5	74	1	*iv*11b	1
	1672[d,e]	CLIP 2300	5	75	1	*iv*01a	2
	1676[d]	CLIP 257	5	76	1	*iv*01a	
L. welshimeri	1677[d,e]	CLIP 11633	NK[f]	77	1	*w*01a	1
	1681[d,e]	CLIP 20280	NK	78	1	*w*01b	1
	1679[d,e]	CLIP 19889	NK	79	1	*w*01c	1
	1683[d,e]	CLIP 19732	NK	80	1	*w*01d	1
	1669[d,e]	ATCC 35897	6b	81	1	*w*01e	1
	1678[d,e]	CLIP 81	NK	82	1	*w*01f	1
L. grayi	1671[d,e]	CLIP 12518	NK	83	1	*g*23a	1
L. seeligeri	1685[d,e]	CLIP 9529	NK	84	1	*s*12a	1
	1665[d,e]	SLCC 3990	NK	85	1	*s*13a	1
	1667[d,e]	SLCC 3502	NK	86	1	*s*12b	1
L. innocua	1220[d,e]	FRI/306	6b	87	1	*in*11a	1
	1225[d,e]	FRI/501	6a	88	1	*in*11b	1
	1325[d,e]	ATCC 33090	6a	89	1	*in*21a	1
	1218[d,e]	FRI/109	6a	90	2	*in*31a	2
	1221[d,e]	FRI/308	6a	91	3	*in*21b	3

[a] ATCC, American Type Culture Collection; CLIP, *Listeria* collection of the Pasteur Institute; FRI, culture collection of the Food Research Institute; JBL, culture collection of J. B. Luchansky, Food Research Institute; SLCC, special *Listeria* culture collection.
[b] Total number of strains with the particular *Apa*I REDP. The total number of all *Apa*I-tested strains was 22.
[c] Total number of strains with the particular *Asc*I REDP. The total number of all *Asc*I-tested strains was 22.
[d] Strain representative of the particular *Apa*I REPD depicted in Fig. 1.
[e] Strain representative of the particular *Asc*I REDP depicted in Fig. 2 or 3.
[f] NK, not known.

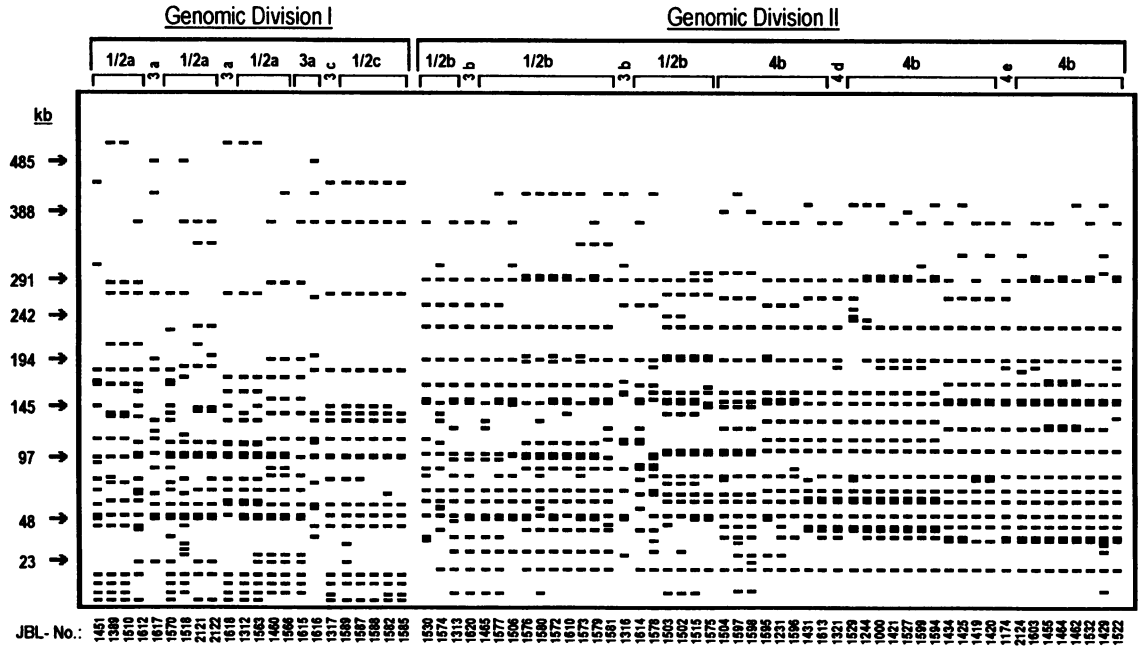

FIG. 1. Diagram of 72 representative *Apa*I REDP of 175 *L. monocytogenes* isolates. The identities and/or origins of strains are listed sequentially in Table 1.

isolate) was included on each gel as an internal standard. Gels were stained in ethidium bromide, and banding patterns were visualized on a shortwave UV transilluminator and photographed.

Data management. Representative strains displaying unique *Apa*I REDP via visual inspection of gel photographs were delineated numerically (1 through 72 [Table 1]). Representative strains displaying unique *Asc*I REDP were designated by an alphanumeric code (e.g., *m*11a [Table 1]) based on the species name (e.g., "*m*" denotes *monocytogenes*), the number of bands within the first two arbitrary fields (e.g., "11" indicates the presence of a single band in both arbitrary field 1 [≤48.5 kb] and arbitrary field 2 [48.5 to 97 kb]), and the number of strains with equivalent bands in both arbitrary fields (e.g., "a" denotes a unique REDP displaying a single band in fields 1 and 2). Although a specific file management program (PC-File 6.5; ButtonWare, Bellevue, Wash.) was used to organize strain and REDP information, any generic database package would suffice. The presence or absence of macrorestriction fragments for each strain was then transcribed into binary scores to diagram and manage REDP data with the ELBAMAP (16) software program. The ELBAMAP program determines the largest and smallest bands of the database and plots within these limits. In addition, similarity matrices were created from the entire *Asc*I and *Apa*I REDP database by pairwise comparisons of REDP. The similarity among profiles was calculated by using the Dice coincidence index (12), which is calculated as follows: DNA fingerprint similarity (S_{xy}) for strains x and y is the number of common bands in both DNA profiles (n_{xy}) divided by the average number of bands exhibited by both strains [$S_{xy} = 2n_{xy}/(n_x + n_y)$]. To more clearly visualize genomic relationships among *L. monocytogenes* strains, principal-component analysis of Dice similarity coefficients was performed, using the SAS/ETS system (SAS Institute, Cary, N.C.) as reported by Chen et al. (8).

RESULTS

Preliminary screening by PFGE-CHEF and numerical codification of *L. monocytogenes* strains. With the exception of JBL1320 (ATCC 19116, serovar 4c, food isolate), all 176 strains tested displayed *Apa*I REDP. Visual comparisons of macrorestriction patterns revealed 72 different *Apa*I REDP (Fig. 1; Table 1). At present, it is not known why *Apa*I did not digest genomic DNA from the serovar 4c isolate.

Inspection of all fragments within and among REDP was admittedly tedious and labor intensive, and probably imprecise, because of the overabundance of strains and restriction fragments analyzed. To facilitate more exact and less subjective comparisons of REDP among strains, the number of macrorestriction fragments within 13 arbitrary fields (≤48.5, 48.5 to 97, 97 to 588 in 48.5-kb increments, and >588 kb) was recorded for individual strains. These experiments were conducted primarily with *Asc*I to simplify record keeping of REDP data, since this enzyme consistently generated the smallest number of discernible fragments while maintaining discriminatory power (for example, see Fig. 2). Also, by determining the number of *Asc*I fragments within field 1 (≤48.5 kb) and field 2 (48.5 to 97 kb) it was possible to assign an alphanumeric code to each strain (Table 1) and readily identify related strains with the PC-File 6.5 file management program. Strains displaying the same number of *Asc*I fragments within each arbitrary field were subsequently compared in adjacent lanes by PFGE-CHEF to determine more precisely if their REDP were identical or simply related. CHEF analyses of *Asc*I REDP by this approach identified 63 distinct REDP groupings among the 176 strains tested (Fig. 3; Table 1). In addition, genomic DNA from the serovar 4c strain (JBL1320) that was not amenable to digestion with *Apa*I generated 12 fragments following digestion with *Asc*I (Fig. 3, JBL1320). It was also observed that the majority (97%) of *L. monocytogenes* strains displayed an *Asc*I fragment about 245 kb long (Fig. 3). Further

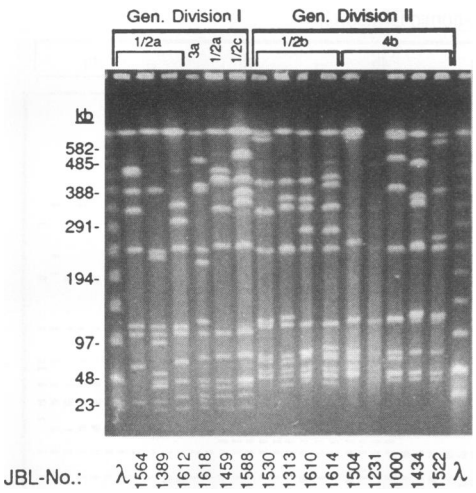

FIG. 2. *Asc*I PFGE-CHEF gel of *L. monocytogenes* isolates. The identities and/or origins of strains are listed sequentially in Table 1. Gen., genomic.

studies to establish whether the 245-kb *Asc*I fragment is specific for *L. monocytogenes* in size and/or nucleotide sequence are warranted.

Comparison of low-molecular-weight *Asc*I restriction fragments of *L. monocytogenes*. Closer scrutiny of *Asc*I fragments about 23 to 60 kb long (Fig. 2 and 3) revealed that *L. monocytogenes* isolates presenting type AB or BD flagellar (H) antigens (serovars 1/2a, 1/2c, 3a, or 3c [24]) exhibited similar REDP and comprised one genomic division, whereas isolates presenting type ABC flagellar (H) antigens (serovars 1/2b, 3b, 4b, 4d, or 4e [24]) displayed similar REDP and constituted another genomic division. These data indicate that visual analyses of low-molecular-weight macrorestriction fragments displayed on gels (Fig. 2) or interpretive diagrams (Fig. 3) may prove useful for confirming and identifying the serovar of *L. monocytogenes* isolates.

Principal-component analyses of *L. monocytogenes* REDP data. To further confirm genetic relatedness among strains, similarity matrices were calculated (data not shown) by pairwise comparisons of REDP data using the Dice coincidence index. Because of the magnitude and complexity of the REDP and Dice similarity information collected, principal-component analysis was conducted to more clearly expose genomic relationships among the strains tested. For the *Apa*I REDP data, the first component of principal-component analysis (Fig. 4, *x* axis), which accounted for 72% of the total variance, identified two primary divisions among the 175 *L. monocytogenes* isolates tested: division I comprised serovar 1/2a, 1/2c, 3a, and 3c strains, whereas division II comprised serovar 1/2b, 3b, 4b, 4e, and 4d strains. Analysis of the second component of principal-component analysis (Fig. 4, *y* axis), which accounted for 10% of the total variance, identified two subdivisions within each primary division: cluster IA (serovar 1/2c and 3c) and IB (serovar 1/2a and 3a) strains and cluster IIA (serovar 1/2b and 3b) and IIB (serovar 4b, 4d, and 4e) strains. Similar results were obtained from principal-component analyses using *Asc*I REDP similarity data (Fig. 5). The first component (*x* axis) accounted for 60% of the total variance, and the second component (*y* axis) accounted for 10% of the total variance. Although the *Asc*I genomic divisions are clearly delineated, the *Asc*I clusters and/or subdivisions within each genomic division are not apparent.

CHEF analyses of genomic DNA from other *Listeria* species. Genomic fingerprinting was also conducted on strains of *Listeria* species other than *L. monocytogenes*. The enzymes *Apa*I and *Asc*I generated 19 and 18 distinct REDP (Fig. 6 and 7, respectively) for the 22 strains tested. There were no REDP common among the different non-*L. monocytogenes* species tested, and genomic fingerprints of the 22 non-*L. monocytogenes* strains examined did not match fingerprints displayed by any of the 176 *L. monocytogenes* isolates analyzed by PFGE-

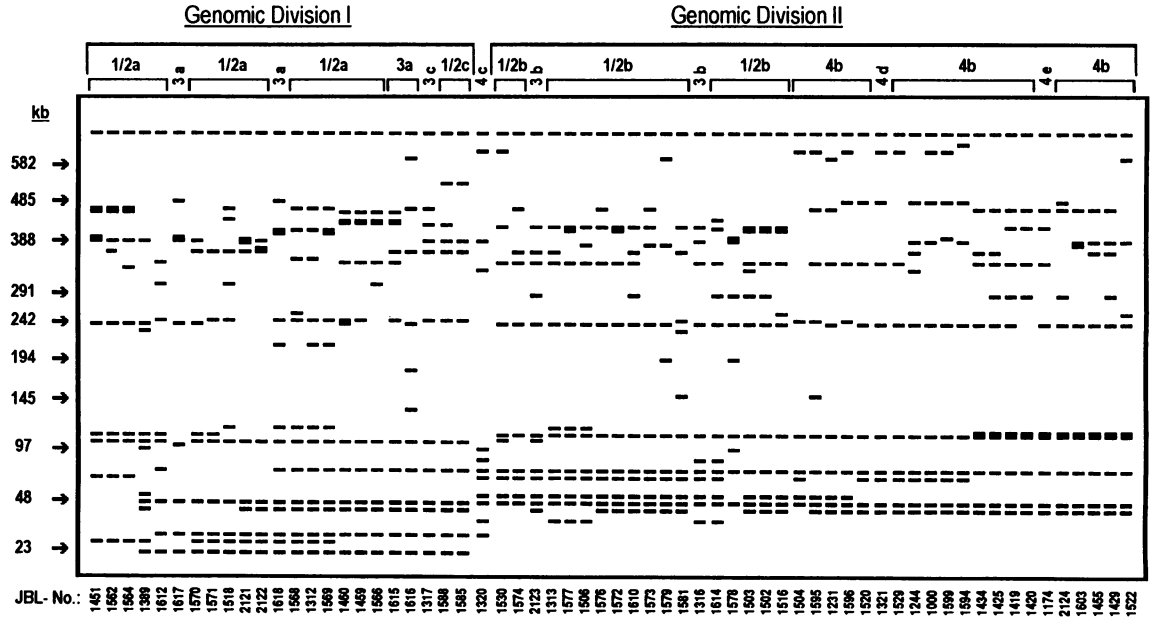

FIG. 3. Diagram of 63 representative *Asc*I REDP of 176 *L. monocytogenes* isolates. The identities and/or origins of strains are listed sequentially in Table 1.

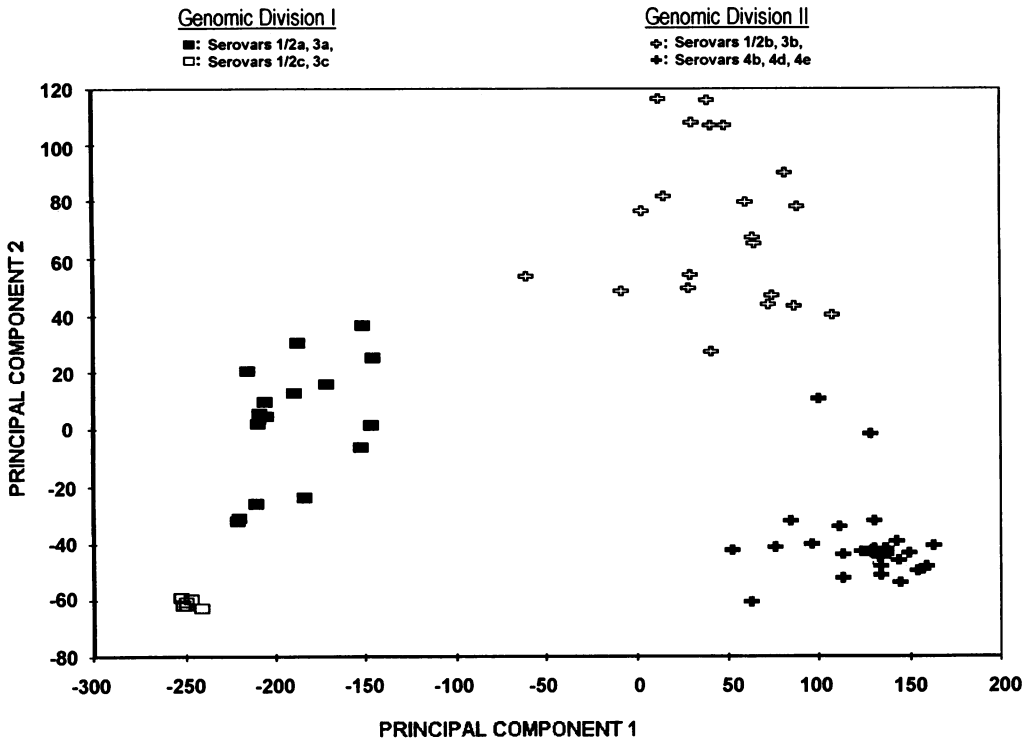

FIG. 4. Plot of the first and the second principal components obtained by principal-component analysis of Dice similarity data of 72 representative *Apa*I REDP of *L. monocytogenes*.

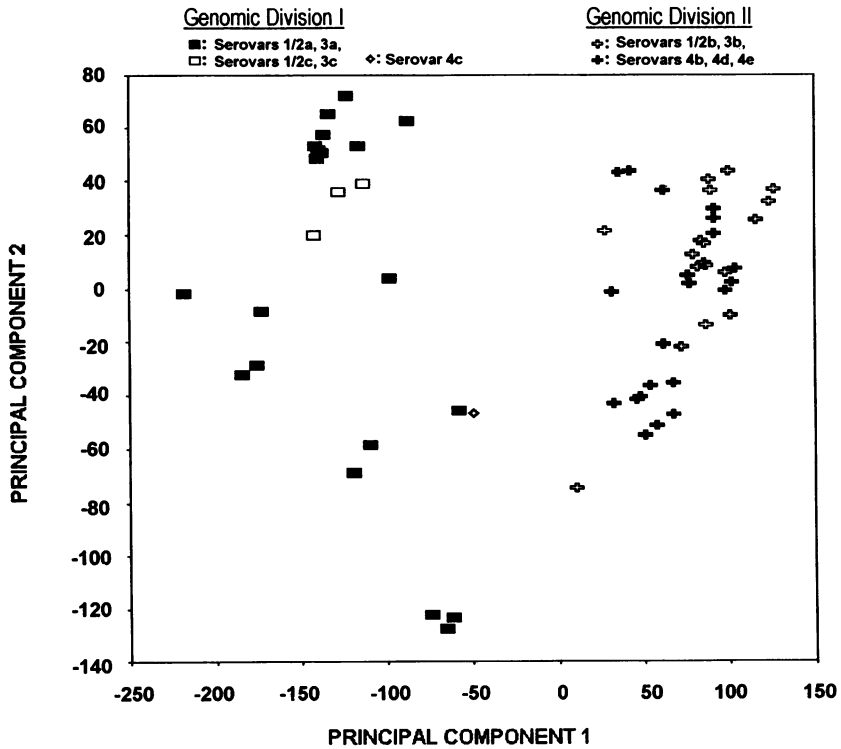

FIG. 5. Plot of the first and the second principal components obtained by principal-component analysis of Dice similarity data of 63 representative *Asc*I REDP of *L. monocytogenes*.

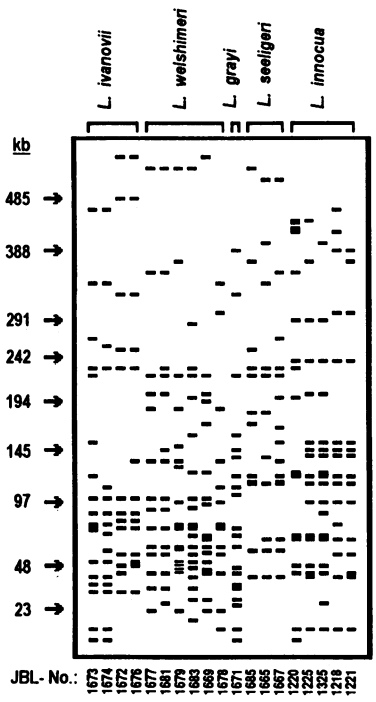

FIG. 6. Diagram of representative *Apa*I REDP of 22 non-*L. monocytogenes* isolates of *Listeria* species. The identities and/or origins of strains are listed sequentially in Table 2.

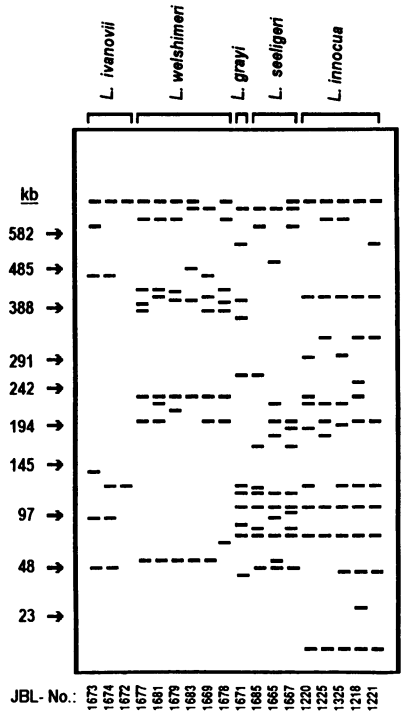

FIG. 7. Diagram of representative *Asc*I REDP of 22 non-*L. monocytogenes* isolates of *Listeria* species. The identities and/or origins of strains are listed sequentially in Table 2.

CHEF. Also, it was not useful to perform principal-component analyses on non-*L. monocytogenes* species of *Listeria* because their similarity coefficients were too dissimilar (data not shown). However, as observed with *L. monocytogenes*, the absence of low-molecular-weight bands for *Listeria innocua*, *Listeria ivanovii*, *Listeria seeligeri*, and *Listeria welshimeri* correlated with the ABC flagellar (H) antigens of these species. Additionally, the eight *L. innocua* strains examined displayed a common *Asc*I fragment about 15 kb in length. Further studies using a greater number of strains are required to determine if the 15-kb *Asc*I fragment is unique to *L. innocua*.

DISCUSSION

As reported previously (15), the enzymes *Asc*I and *Apa*I were most useful for generating a convenient number of readily discernible macrorestriction fragments of listeriae. Digestion of 176 *L. monocytogenes* DNAs with these two enzymes generated 87 genomically distinct groups. Although *Asc*I was generally less discriminatory than *Apa*I for subtyping *L. monocytogenes*, the *Asc*I data were easier to interpret, since *Asc*I typically generated fewer bands (8 to 14 fragments) than *Apa*I (18 to 23 fragments). Our results also revealed that with few exceptions strains of different serovars and strains within each serovar displayed different REDP. Another observation was that some strains with different serovars displayed identical REDP. For example, a serovar 1/2a strain (JBL1648) displayed *Apa*I and *Asc*I REDP characteristic of serovar 1/2c and 3c strains. As another example, a serovar 4d strain (JBL1321) displayed the same genomic fingerprint as several serovar 4b strains. Although our results are in close agreement with results already published, most strains analyzed in this study displayed 18 to 23 bands following digestion with *Apa*I, compared with the 15 to 18 *Apa*I bands reported elsewhere (3, 4, 6). Our ability to discern additional fragments is attributed, in part, to the use of higher DNA concentrations, which in turn allowed for better visualization of lower-molecular-weight fragments. It was also interesting that genomic DNA from *L. monocytogenes* ATCC 19116 was amenable to digestion with *Asc*I but, despite repeated attempts, was not restricted with *Apa*I. Daniellsson-Tham et al. (9) made a similar observation that some strains of *L. innocua* were not restricted with *Apa*I but were restricted with *Sma*I. With the exception of these two reports, there have been no previous indications that listeriae were nontypeable by PFGE. In our experience in subtyping over 450 strains of *Listeria* by PFGE, to date, only 3 strains were found to be nontypeable. Thus, the occurrence of *L. monocytogenes* and *L. innocua* isolates that are not typeable by using a single enzyme for PFGE is extremely rare and, as is the case with the serovar 4c strain used in this study, such isolates may be amenable to digestion by other enzymes. Experiments are under way to confirm the serovar of our presumed ATCC 19116 clone and to assess why *Apa*I does not restrict genomic DNA from this isolate.

The results herein corroborate our previous findings (15) and highlight the usefulness of PFGE-CHEF for elucidating species-specific differences for *Listeria* strains. With the exception of the potentially unique 15-kb *Asc*I fragment found in all *L. innocua* strains tested, only one strain of *L. innocua* displayed an *Asc*I fragment smaller than 40 kb. The general absence of low-molecular-weight *Asc*I fragments for *L. innocua*, as well as *L. ivanovii*, *L. seeligeri*, and *L. welshimeri* (species all characterized by type ABC flagellar [H] antigens [24]), was in agreement with the observation that serovar 1/2b, 3b, 4b, 4d, and 4e strains of *L. monocytogenes* (ABC flagellar antigenic structure) were also missing low-molecular-weight fragments. This observation demonstrates that *Listeria* strains

with flagellar antigens of the ABC type can clearly be distinguished from strains displaying other flagellar antigens on the presence or absence of low-molecular-weight *Asc*I restriction fragments.

The conversion of banding patterns on photographs into computer-readable binary scores and the subsequent use of the ELBAMAP software program enabled us to better track strains in our database, as well as diagram REDP, calculate Dice similarity matrices, and conduct principal-component analyses. As an aside, the Dice index also facilitated genomic comparisons among outbreak-related *L. monocytogenes* isolates (4) and strains of *Staphylococcus* (11) and *Rickettsia* (23). Two-dimensional plotting of the first and second components of principal-component analyses of *Apa*I and *Asc*I REDP data clearly demonstrated the existence of two distinct genomic divisions within the species *L. monocytogenes*. The further delineation of the two genomic divisions into two clusters within each division was observed with *Apa*I but not *Asc*I, presumably because *Apa*I generated more fragments and, as such, identified more subtle differences among strains. It is noteworthy that the two primary divisions identified by principal-component analyses (Fig. 4 and 5) corresponded with the two primary divisions established by visual inspection of the lower-molecular-weight *Asc*I and *Apa*I fragments (Fig. 1 to 3). It was also significant that the genomic divisions delineated through REDP analyses correlated directly with the flagellar (H) antigen types and, therefore, the serovars of listeriae. It should be noted that in a previous study (5) field inversion gel electrophoresis failed to establish any clear division between serovar 1/2a, 1/2c, 3a, 3c strains and serovar 1/2b and 3b strains, primarily because the serovar designations of several strains as received were incorrect. The present study also established the absence of any clear correlation(s) between REDP and somatic (O) antigens of listeriae (data not shown). For example, some serovar 3a and 1/2a strains, 3c and 1/2c strains, or 3b and 1/2b strains exhibited identical REDP but the REDP of serovar 1/2a and 1/2b strains were very different.

The identification of two major genomic divisions of *L. monocytogenes* by PFGE-CHEF corresponds with results obtained by other investigators using surface protein profiles, nucleotide (*lisA*) probes, and/or multilocus enzyme electrophoresis to type listeriae (20, 21, 25). However, serovar 1/2b and 4b strains were not always distinguished by multilocus enzyme electrophoresis (19, 20), whereas PFGE-CHEF consistently revealed distinct differences between serovar 1/2b and 4b strains. Similarly, analysis of sodium dodecyl sulfate-extracted surface proteins of *L. monocytogenes* was useful for discriminating between strains of serogroup 1 and 4 but the protein clusters did not match the clusters established with multilocus enzyme electrophoresis or PFGE-CHEF, particularly with respect to the placement of serotype 1/2b isolates (25). Rasmussen et al. (21) identified two types of *L. monocytogenes* according to the sequence of the *lisA* gene that correlated with flagellar antigens and, as such, coincided with results obtained with multilocus enzyme electrophoresis and PFGE-CHEF. However, the flagellar antigen type was not determined for 21 of 38 strains (55%) screened with the *lisA* probe sequence (21). Lastly, in related studies, correlations were not observed between serovar and monoclonal antibody reactivity patterns (26) or serovar and random amplified polymorphic DNA profiles (18). Despite the ability of several typing strategies to classify *L. monocytogenes* into two broad groups, our results substantiate that PFGE-CHEF identifies more subtle differences among isolates and, thus, approximates a more accurate reflection of genomic groupings and relatedness.

In summary, codification of REDP information greatly simplified the initial screening and comparison of strains, and computer management of REDP allowed for more exacting comparisons among isolates. The ability to statistically analyze numerous REDP obtained by PFGE-CHEF was also critical for establishing the relationship between serovars and genomic fingerprints, a finding not previously reported for *L. monocytogenes* but observed with some (14), though not all (17), pathogens. From a more pragmatic viewpoint, having established a fairly extensive REDP database, it is now possible to predict and/or confirm the serovars of strains of interest. As another application, when specific antisera are not available, it may be possible to use PFGE-CHEF in combination with commercially available polyvalent antisera to provide information similar to that provided by the serovar for typing purposes. Our findings also confirm that PFGE-CHEF represents a facile and highly discriminatory method for fine structure comparisons for epidemiologic investigations and molecular characterization of listeriae.

ACKNOWLEDGMENTS

We extend our sincere appreciation to the following colleagues for providing and/or typing isolates: Jacques Bille (W.H.O. Reference Laboratory, Lausanne, Switzerland), Donald Conner (Auburn University), Pascale Cossart (Institut Pasteur, Paris, France), Susan K. Harlander (Land O' Lakes), Sophia Kathariou (University of Hawaii, Honolulu), James McLauchlin (P.H.L.S., Colindale, United Kingdom), Peter M. Muriana (Purdue University), Michael Reeves (Centers for Disease Control and Prevention, Atlanta, Ga.), Jocelyne Rocourt (Institut Pasteur), Marc Tabouret (INRA, Paris, France), and Robert Weaver (Centers for Disease Control and Prevention). We are also grateful to Peter Crump and Elisa Santos (University of Wisconsin—Madison) for their assistance with some of the statistical analyses.

This project was supported in part by a grant from the International Life Sciences Institute (ILSI) North America, by NRICGP grant 91-37201-6762 from the U.S. Department of Agriculture, and by contributions to the Food Research Institute (Madison, Wis.).

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