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Amplified Intergenic Locus Polymorphism as a Basis for Bacterial Typing of *Listeria* spp. and *Escherichia coli*†

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DNA-based methods are increasingly important for bacterial typing. The high number of polymorphic sites present among closely related bacterial genomes is the basis for the presented method. The method identifies multilocus genomic polymorphisms in intergenic regions termed AILP (amplified intergenic locus polymorphism). For each locus, a pair of unique PCR primers was designed to amplify an intergenic sequence from one open reading frame (ORF) to the adjacent ORF. Presence, absence, and size variation of the amplification products were identified and used as genetic markers for rapidly differentiating among strains. Polymorphism was evaluated using 18 AILP sites among 28 strains of *Listeria monocytogenes* and 6 strains of *Listeria* spp. and 30 AILP markers among 27 strains of *Escherichia coli*. Up to four alleles per locus were identified among *Listeria* strains, and up to six were identified among *E. coli* strains. In both species, more than half of the AILP sites revealed intraspecies polymorphism. The AILP data were applied to phylogenetic analysis among *Listeria* and *E. coli* strains. A clear distinction between *L. monocytogenes* and *Listeria* spp. was demonstrated. In addition, the method separated *L. monocytogenes* into the three known lineages and discriminated the most common virulent serotypic group, 4b. In *E. coli*, AILP analysis separated the known groups as well as the virulent O157:H7 isolates. These findings for both *Listeria* and *E. coli* are in agreement with other phylogenetic studies using molecular markers. The AILP method was found to be rapid, simple, reproducible, and a low-cost method for initial bacterial typing that could serve as a basis for epidemiological investigation.

Bacterial strain typing has several important applications in microbiology. In clinical practice, strain typing is useful for diagnosis and determining treatment strategy and is essential for rapid identification of disease outbreaks and new virulent strains. In the food industry, strain typing is necessary to ensure food safety and for linking cases of food-borne infections to suspected items in the food chain. Classical bacterial identification is based on selective enrichment, followed by plating on selective media. Species identification is mainly by biochemical characterization, and strain identification is primarily based on serology. These methods do not meet the requirement for rapid identification and typing in clinical, epidemiological, and food industry applications. Recent advances in biotechnology have resulted in the development of numerous methods for detection and typing of microorganisms (11–13, 19, 25) which differ in their sensitivity, rapidity, labor intensiveness, complexity, discriminatory power, reproducibility, and cost (5, 32, 43, 49). In principle, by screening a large number of polymorphic sites, genomic methods should be able to provide very accurate discrimination among closely related strains. The total multilocus output of these methods is often termed "DNA fingerprints" or a "DNA bar code."

In the present study, we present a new method (amplified intergenic locus polymorphism [AILP]), based on the above principles, which is specifically useful for generating DNA bar codes for discrimination among bacterial strains. The method is based on the finding that whole-genome sequence comparisons within and between closely related bacterial species show the presence of numerous single nucleotide polymorphic sites (SNPs) and genome rearrangements (e.g., see references 15, 17, 20, 28, 33, and 36). This implies that a pair of PCR primers designed to amplify a randomly selected genomic fragment in one strain will often produce different fragment sizes in other strains or may fail to amplify the genomic fragment altogether due to sequence mismatch, insertion/deletions, and other variation at the priming site. A major advantage of the proposed method is that, given complete or partial genome sequences, no additional prior information is required to identify informative AILP markers. The experiments described here were carried out to evaluate the potential of this new strain typing methodology for representative gram-positive and gram-negative bacterial species. *Listeria* spp. are gram-positive bacteria that include seven classified species, among which only *Listeria monocytogenes* is pathogenic to humans and responsible for listeriosis (14, 39). *Escherichia coli* is a gram-negative bacterium composed of numerous strains and serotypes. The species includes commensal strains and a variety of pathogenic strains, such as enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and enterotoxigenic *E. coli* (ETEC) (1, 30, 31, 34, 42, 47). The availability of the complete genome sequences for *E. coli* K-12 (4) and *L. monocytogenes* (17) provided the basis to designing primers for PCR amplification of random genomic targets in these organisms. The results showed the ability of the AILP method to discriminate within and between

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† Supplemental material for this article may be found at http://aem.asm.org/.

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TABLE 1. *Listeria* sp. strains screened in the present study

Organism	Description and source
Listeria monocytogenes	
LM6 DA3	Serotype 4b[a]
LM8 Scott A	Serotype 4b[a]
LM26H	Serotype 4b, ATCC 19115
LM28/2	Serotype 4b, food isolate[b]
LM54	Serotype 4b, human isolate[b]
LM21/1	Serotype 1/2b, human isolate[b]
LM25/2	Serotype 1/2b, food isolate[b]
LM31	Serotype 1/2b, human isolate[b]
LM1	Serotype 1/2b, human isolate[b]
LM14 EGD	Serotype 1/2a[a]
LM16	Serotype 1/2a, SLCC5764
LM17/3	Serotype 1/2a, food isolate[b]
LM19/1	Serotype 1/2a, food isolate[b]
LM10	Serotype unknown[a]
LM11	Serotype unknown[a]
LM17	Serotype 4c[a]
LM25H	Serotype 4a, ATCC 19114
LM15 LO28	Serotype 1/2c[a]
LM24H	Serotype 1/2c,[b] ATCC 7644
WHO/1	Serotype 3a[b]
WHO/16	Serotype 3a[b]
WHO/28	Serotype 3a[b]
WHO/11	Serotype 3b[b]
WHO/14	Serotype 3b[b]
WHO/19	Serotype 3b[b]
WHO/33	Serotype 3c[b]
WHO/52	Serotype 3c[b]
WHO/60	Serotype 3c[b]
Listeria spp.	
L. innocua	ATCC 33090
L. ivanovii	ATCC 19119
L. seeligeri	ATCC 35967
L. welshimeri	ATCC 35897
L. grayi	ATCC 19120
L. murrayi	ATCC 25401

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species in *Listeria* spp. and *E. coli*. The uncovered genetic variation data were used for phylogeny analysis in both species.

MATERIALS AND METHODS

Bacterial strains. The study included a set of 34 *L. monocytogenes* strains, six strains of the remaining species of the *Listeria* genus (Table 1), and two sets of *E. coli* strains: a set of 27 pathogenic and nonpathogenic strains of *E. coli* (Table 2) (12) and a set of 72 wild-type *E. coli* strains from a reference collection (30).

DNA preparation. A modified procedure of Jersek et al. (22) was used for DNA extraction from pure cultures. Cultures of listeriae and *E. coli* were grown for 24 h at 37°C on brain heart infusion and Luria agar plates, respectively. A loop was transferred from the plate to a microcentrifuge tube containing 1 ml of SSC buffer (0.15 M NaCl, 15 mM sodium citrate, pH 8.0) and vortexed thoroughly. The suspension was centrifuged for 1 min at 21,000 × *g*.

For listeriae, the pellet was resuspended in 100 μl lysozyme solution (4 mg/ml in 20% sucrose–1 mM sodium phosphate) and incubated for 1 h at 37°C. To this were added 200 μl TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0), 100 μl *N*-lauryl-sarcosine solution (5% in TE), and 100 μl proteinase K solution (20 mg/ml in TE). The mixture was incubated overnight at 50°C, 500 μl EZ-DNA solution (Biological Industries, Beit-Haemek, Israel) was added, followed by incubation at 60 °C for 1 h and ethanol precipitation according to the manufacturer's instructions. The DNA extract was treated with 0.1 mg/ml RNase, followed by extraction with phenol chloroform and ethanol precipitation. The DNA was stored at −20°C.

For *E. coli*, the pellet was resuspended in 200 μl TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0)–100 μl proteinase K solution (20 mg/ml in TE) and incubated overnight at 50°C. Two percent sodium dodecyl sulfate was added, and the mixture was incubated at 55 °C for 1 h, followed by extraction with phenol chloroform and ethanol precipitation. The DNA was stored at −20°C.

In addition, standard boiling for rapid DNA purification was used with equal success for both *E. coli* and *Listeria*.

Locus selection and primer construction. The complete genomic sequence of *L. monocytogenes* (EGD-e, serotype 1/2a) was obtained from http:/www.ncbi.nlm.nih.gov/, and that of *E. coli* (K-12) was obtained from http://mol.genes.nig.ac.jp/ecoli/. With the exceptions noted below, genomic loci were randomly selected along the bacterial chromosome. PCR primers were usually selected to amplify a specific intergenic locus, from an open reading frame (ORF) to the adjacent ORF. Primers were selected using the Gene Runner (version 3.05) with up to 3°C melting temperature (T_m) difference between the primers. In the case of *Listeria*, the study began before the complete sequence was available. Consequently, the first nine markers were based on various GenBank sequences of *L. monocytogenes*; two markers were derived from the unfinished genome sequence of *L. monocytogenes* (serotype 4b [The Institute for Genomic Research]), six were from the published genome sequence (EGD-e, serotype 1/2a) (17), and one was from the published genome sequence of *L. innocua* (Clip11262) (17; Table S1 in the supplemental material). All loci, except *abc* and *Lin0694*, were found in the published genome of EGD-e, serotype 1/2a. The *E. coli* markers were based on the finished K-12 genome sequence (GenBank accession no. U00096) (4; Table S2 in the supplemental material). Loci were named after the downstream ORF.

PCR amplification. The PCR mixture contained 0.2 mM deoxynucleoside triphosphates, 0.4 μM each forward and reverse primer, 0.5 U *Taq* polymerase (Super Nova; JMR Holding, Kent, England), 1× buffer (1.5 mM $MgCl_2$), and 50 ng template DNA in a total volume of 25 μl. The reaction was carried out in a PCR thermocycler (HYBAID Omn-E; Hybaid, Ashford, United Kingdom) as follows: 95°C for 5 min; five cycles of 45 s at 95°C, 45 s at the T_m (Tables S1 and S2 in the supplemental material), and 45 s at 72°C; 20 cycles of 45 s at 95°C, 45 s at the T_m minus 5°C, and 45 s at 72°C; and a final step of 72°C for 7 min. PCR amplification products were analyzed by agarose gel (2%) electrophoresis and observed by UV fluorescence.

Data analysis. Two classes of polymorphism were observed: class I, presence or absence of amplification product, and class II, presence of a product different in size from the expected product, based on the published sequence. In some instances, class II polymorphisms included two bands, the expected product and an additional product. Allele designations for each locus were as follows: allele 1, the obtained product with the expected length according to the GenBank sequence (Tables S1 and S2 in the supplemental material); allele 2, absence of product; allele 3, two amplification products, the expected product and an additional product different in size; alleles 4 to 7, all products present different in length from the expected product and from each other. For further analysis, fragment data for all genotypes of a specific locus were scored as 1 (present) or 0 (absent) for each of the alleles. Where two fragments were obtained (allele 3), each was scored as 1. Using SAS version 8.02 (38), the data were used to calculate the simple matching coefficients of association (41) and to generate two corresponding genetic distance matrices, one for the 34 *Listeria* isolates and the other for the 27 *E. coli* isolates. These matrices were used to determine the relationships among strains. The dendrograms were constructed by means of the unweighted-pair group method using average linkages (UPGMA) with MEGA version 2.1 (24).

RESULTS

A set of distributed intergenic loci were selected along the published genomes of *L. monocytogenes* and *E. coli* for AILP analysis. Eighteen sites were chosen along the genome of *L. monocytogenes* (EGD-e 1/2a), and 30 sites were chosen along the genome of *E. coli* (K-12) (Tables S1 and S2, respectively, in the supplemental material). Analysis of the PCR amplification products by gel electrophoresis showed considerable polymorphism among the tested strains of *Listeria* and *E. coli* (Fig. 1). To ensure reproducibility, only the major bands were considered as products. Results were verified by at least three independent PCRs for each strain. In addition, identical results were obtained in our lab by different personnel/staff using various thermal cyclers, as well as in other labs (e.g., David

TABLE 2. *E. coli* strains screened in the present study

E. coli group and strain serotype	Description and source[a]
EHEC	
O22:H8	*E. coli* Reference Center, 90.0327
O42:H2	*E. coli* Reference Center, 88.0501
O111:NM	*E. coli* Reference Center, 88.0015
O113:H2	*E. coli* Reference Center, 88.0632
O26:H11	Centers for Disease Control and Prevention CDC 2239-69
O157:NM	USDA-FSIS, MF7123A
O157:H7	USFDA, SEA13B88, Odwalla cider outbreak strain
O157:H7 HER phage type 1057	Ontario Public Health Laboratory; 1
O157:H7 HER phage type 1058	
O157:H7 HER phage type 1261	
O157:H7 HER phage type 1265	
O157:H7 HER phage type 1266	
ETEC	
O78:NM	Haifa Public Health Department, Rowe no. E10407
O8:H9	Central Laboratories, Ministry of Health, Jerusalem, Israel
O9:H33	
O86:H10	
O86:H18	
O153:H	
EPEC	
O111ac:NM	Haifa Public Health Department, Rowe no. E639616
O26:H	Central Laboratories, Ministry of Health, Jerusalem, Israel
O55:H7	
O127:H21	
K-12	
DH5α	Technion Faculty of Food Engineering & Biotechnology collection
W3110	
W4100	
B	
SR9b, SR9c	
Wild type	
Reference collection strains 1–72	Michigan State University; 30

[a] USDA, U.S. Department of Agriculture; FSIS, Food Safety and Inspection Service; USFDA, U.S. Food and Drug Administration.

Walt, Tufts, University, Boston, Mass.) (40). Figure 1 depicts the intraspecies polymorphism at both the *abc* and *gbuA* loci among 15 strains of *L. monocytogenes*.

AILP in *Listeria* spp. Eighteen loci were used to assess the variation among 28 *L. monocytogenes* strains (including human pathogenic isolates) and 6 strains of other *Listeria* spp. (Table 3). The other *Listeria* species served as control isolates presenting the ability of the method to distinguish between the pathogenic *L. monocytogenes* isolates and the other *Listeria* species. All loci were polymorphic, having 2 to 4 alleles with an average of 2.61 alleles per locus. Nine (50%) of the loci showed class I polymorphism, possessing two alleles (product or no product); the remaining loci showed class II polymorphism, seven loci showing three alleles, and two showing four alleles. Using the information from all tested loci, the AILP method can assign the 34 *Listeria* strains to 18 different AILP types (Table 3). Five of the loci (*clpE*, *ATTM*, *cheR*, *Lmo0196*, and *gid*), clearly differentiated between *L. monocytogenes* and other *Listeria* species, presented the same allele in all *L. monocytogenes* strains. Ten (56%) loci were polymorphic within *L. monocytogenes*, thereby facilitating differentiation among strains. Furthermore, a combination of two loci, *abc* and *gbuA*, enabled the identification of *L. monocytogenes* from the other *Listeria* spp., discriminated the three known *L. monocytogenes* lineages, and differentiated the 4b serotypic group from the other serotypic groups in lineage I (with the exception of strain LM17; Table 3).

The AILP data were also used for analysis of phylogenetic relationships among the 34 *Listeria* isolates. A genetic-distance matrix was generated based on 47 polymorphic points (18 loci by the number of alleles in each locus), followed by cluster analysis using the UPGMA. The resulting dendrogram showed five main branching nodes (Fig. 2). There was clear and deep separation of the *L. monocytogenes* strains from the other six *Listeria* species (node 1; average genetic distance, 0.615 ± 0.099). Genetic distances among *L. monocytogenes* strains ranged from 0.000 to 0.383 (average genetic distance, 0.150 ± 0.10), with strains from serotypes 4a and 4c being the most distant (average genetic distance, 0.325 ± 0.079). Strains of *L. monocytogenes* were clustered in the three known lineages. Strains of serotypes 4a and 4c comprised a separate node (lineage III, node 2). Strains from serotypes 1/2a, 1/2c, 3a, and 3c were grouped together (lineage II, node 4), separately from strains belonging to serotypes 1/2b, 4b, and 3b (lineage I). Lineage I serotypes, in turn, were divided into two nodes (nodes 3 and 4). Although some of the lineage I strains clustered closely to lineage II, the genetic distance between lineage

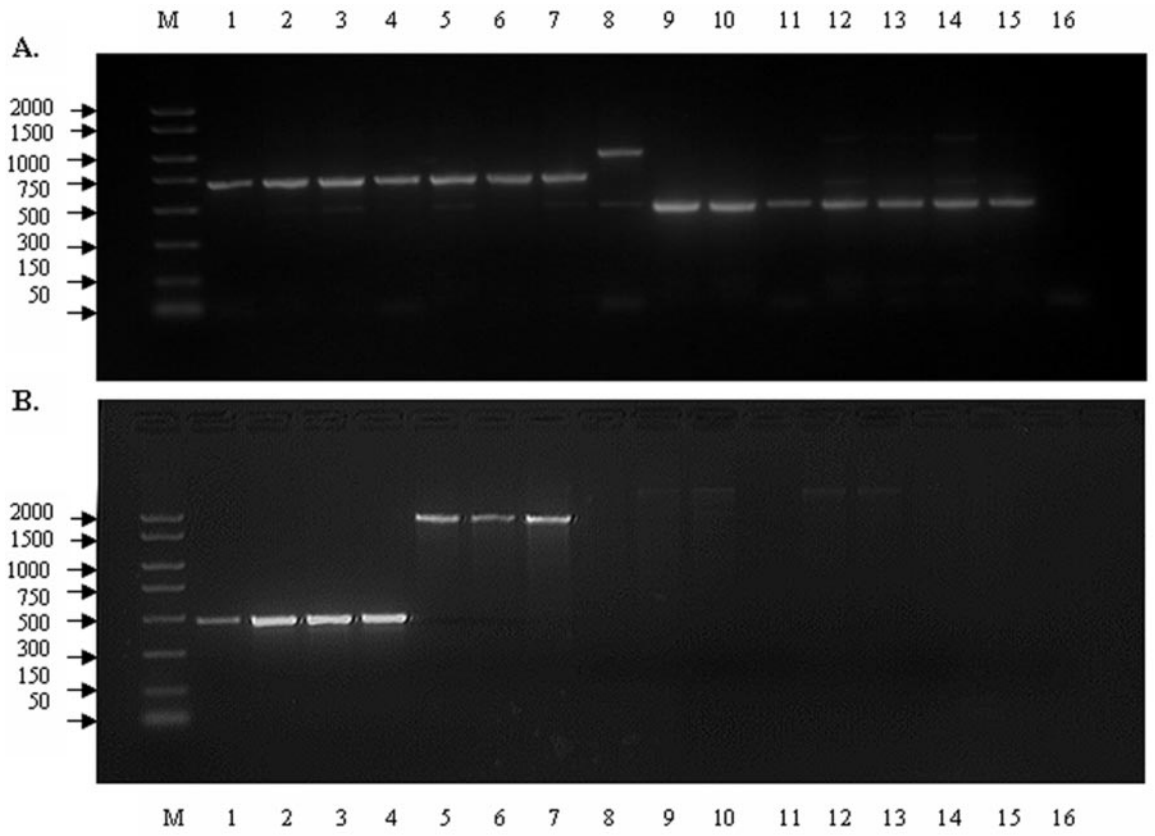

FIG. 1. AILP analysis of 15 *L. monocytogenes* strains at the *gbuA* (a) and *abc* (b) loci. Amplification products were separated on a 2% agarose gel. Lanes were as follows: M, size standards (bp); 1, LM8:4b; 2, LM26H:4b; 3, LM6:4b; 4, LM54:4b; 5, LM21/1:1/2b; 6, LM25/2:1/2b; 7, LM31:1/2b; 8, LM25H:4a; 9, LM15:1/2c; 10, LM14:1/2a; 11, LM16:1/2a; 12, LM17/3:1/2a; 13, LM19/1:1/2a; 14, WHO/1:3a; 15, WHO/33:3c; 16, no DNA (for details, see Table 1).

II and lineage I was significant (average of 0.155 ± 0.056). Two pairs of *Listeria* species gave identical AILP fingerprint patterns: *L. grayi*-*L. murrayi* and *L. innocua*-*L. seeligeri*. The latter pair was separated from the other *Listeria* species (node 5).

AILP in *E. coli*. Thirty loci were used to assess the variation among a set of 27 *E. coli* isolates (Tables 2 and 4). Sixteen loci (53%) were polymorphic, having 2 to 6 alleles (with an average of 2.62 alleles per locus); 14 loci were monomorphic, presenting only a single allele. Eleven (69%) of the polymorphic loci showed class I polymorphism, possessing two alleles; five loci showed class II polymorphism, presenting two to six alleles. Using the 16 informative loci, the AILP method can assign the 27 strains to 19 AILP types. The AILP data were used for analysis of genetic relationships among the 27 isolates. A genetic-distance matrix was generated based on 64 polymorphic points (total number of alleles in the 30 loci). Genetic distances among *E. coli* isolates ranged from 0.000 for the very close isolates to 0.27 for the most distant isolates, with a mean genetic distance of 0.162 ± 0.06. Cluster analysis of the distance matrix was performed using the UPGMA. The resulting dendrogram presented in Fig. 3, shows 10 main branching nodes. As expected, all O157:H7 isolates exhibited similar patterns and clustered together (node 10). Similarly, isolates SR9b and SR9c of *E. coli* group B (node 7) had the same AILP pattern. Isolates from *E. coli* K-12 were grouped together (node 9).

In addition, the *E. coli* reference collection of 72 strains (30) was analyzed at a subset of five AILP markers (*yaiN*, *ycgW*, *serW*, *b2345*, and *ykgE*), yielding 15 polymorphic points (total number of alleles across the five loci). The genetic distance between the most distant isolates (ECOR23 and ECOR66) was 0.73, and the average genetic distance was 0.24 ± 0.18. Cluster analysis, presented in Fig. 4, revealed that all B2 group ECOR isolates (21) were grouped to a distinct node (node 1), followed by a cluster of most of the group D ECOR isolates (node 2) and a cluster consisting of most of the group A and B1 isolates (node 3).

DISCUSSION

This study presents a new, simple, DNA-based bacterial typing method, AILP. The method is based on PCR amplification of a randomly chosen intergenic locus. The typing is determined by presence, absence, or size variation in the amplified products. Specific strain typing is achieved by multilocus analysis. The power of the method derives from the large number of polymorphic sites that are found across the whole genome (2, 15, 20, 33, 35, 36). These numerous polymorphic

TABLE 3. AILP types based on electrophoretic profiles of PCR amplification products for 28 *L. monocytogenes* strains and 6 *Listeria* spp. at 18 sites

Strain or species	Allele[a] at following locus:																			AILP type
	Lmo1430	*clpE*	*BetL*	*ATTM*	*lrrC*	*cheR*	*lisR*	*Lmo0672*	*Lmo0196*	*Lmo0075*	*Lin0694*	*Lmo0023*	*gid*	*fhuB*	*Lmo0042*	*Lmo0176*	*abc*	*gbuA*	
LM6-4b	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	4	1
LM8-4b	1	1	1	1	1	1	1	1	1	2	1	1	1	3	1	1	1	4	2
LM26H-4b	1	1	1	1	1	1	1	1	1	2	1	1	1	3	1	1	1	4	2
LM28/2-4b	1	1	1	1	2	1	1	1	1	2	1	1	1	3	1	1	1	2	3
LM54-4b	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	4	1
LM21/1-1/2b	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	4	4	4
LM25/2-1/2b	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	4	4	5
LM31-1/2b	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	4	4	5
LM1-1/2b	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	4	2	6
LM14-1/2a	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	7
LM16-1/2a	1	1	1	1	2	1	1	1	1	1	2	1	1	1	1	1	2	1	8
LM17/3-1/2a	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	7
LM19/1-1/2a	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	7
LM10	2	1	2	1	2	1	1	4	1	2	2	1	1	1	2	1	2	3	9
LM11	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	4	10
LM17-4c	2	1	1	1	2	1	1	4	1	4	2	1	1	1	2	1	2	1	11
LM25H-4a	2	1	2	1	2	1	1	2	1	2	2	1	1	1	2	1	2	3	12
LM15-1/2c	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	1	2	1	13
LM24H-1/2c	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	7
WHO/1-3a	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	7
WHO/16-3a	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	7
WHO/28-3a	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	7
WHO/11-3b	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	4	4	14
WHO/14-3b	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	4	4	14
WHO/19-3b	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	4	4	14
WHO/33-3c	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	7
WHO/52-3c	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	7
WHO/60-3c	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	1	7
L. innocua	2	2	2	2	2	2	1	2	3	2	1	1	4	1	2	1	2	2	15
L. ivanovii	2	2	2	2	2	2	2	2	2	2	2	4	2	2	2	4	2	2	16
L. seeligeri	2	2	2	2	2	2	1	2	3	2	1	1	4	1	2	1	2	2	15
L. welshimeri	2	2	2	2	2	2	1	2	2	2	2	3	2	2	2	2	2	2	17
L. grayi	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	18
L. murrayi	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	18

[a] Allele designations: 1, amplification product at the expected size according to GenBank sequence; 2, absence of product; 3, one product in addition to the expected product; 4 to 7, products differing in length from one another and from the expected product.

sites make it likely that a pair of PCR primers that amplify a specific, randomly chosen intergenic locus will produce polymorphic products when screened across a set of closely related species or strains within a species. Yet, prior information of variation among strains is not required, only the primary genome sequence. The observed polymorphisms may be caused by SNPs at the primer sites, as well as insertions or deletions (20). Recent findings of serotype- or strain-specific genes and many SNPs among three *L. monocytogenes* 4b and 1/2a strains (28) support the basis for the AILP typing approach. Similar approaches of presence and absence variations have been used in binary typing based on probe hybridization (44, 45). Recently, Nekrutenko et al. (27) discussed the possible use of an in silico screen for the identification of presence/absence variation among strains, as well as the use of PCR amplification.

In the present study, we found that more than half of the randomly chosen loci in both *L. monocytogenes* and *E. coli* were polymorphic within a representative set of strains, revealing intraspecies polymorphism. Interspecies polymorphism was found in *Listeria* as well. Up to four alleles for a given locus were identified among 34 *Listeria* strains and up to six alleles were identified among 27 *E. coli* strains.

Reliability of a typing method is crucial for accurate distinction among different bacterial isolates (e.g., see references 32 and 49). The AILP method uses unique primers for PCR amplification under high-stringency conditions, providing reliable and reproducible results. In order to compare the results obtained by AILP analysis with those obtained using other typing methods, sets of AILP data were applied to phylogenetic analyses of a set of *Listeria* and *E. coli* strains. Phylogenetic analyses using AILP data were consistent with other studies. In *Listeria*, five of the loci clearly differentiated between the pathogenic species *L. monocytogenes* and the other *Listeria* species. Ten (56%) loci were polymorphic within *L. monocytogenes*, thereby facilitating assignment of the 28 *L. monocytogenes* strains to 14 different AILP types (Table 3). AILP phylogenetic analysis divided *L. monocytogenes* strains into three distinct genetic lineages (Fig. 2, nodes 2, 3, and 4). This accords closely with other DNA subtyping methods, including pulsed-field gel electrophoresis (PFGE), ribotyping,

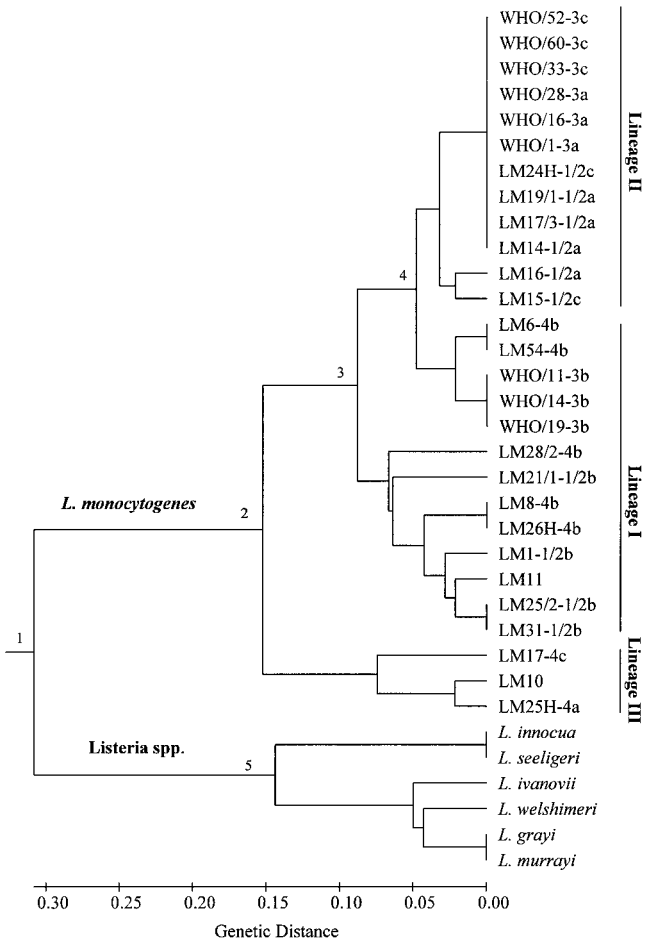

FIG. 2. Dendrogram presenting the genetic relationships among *Listeria* isolates using UPGMA cluster analysis of the AILP data (for details, see Table 1).

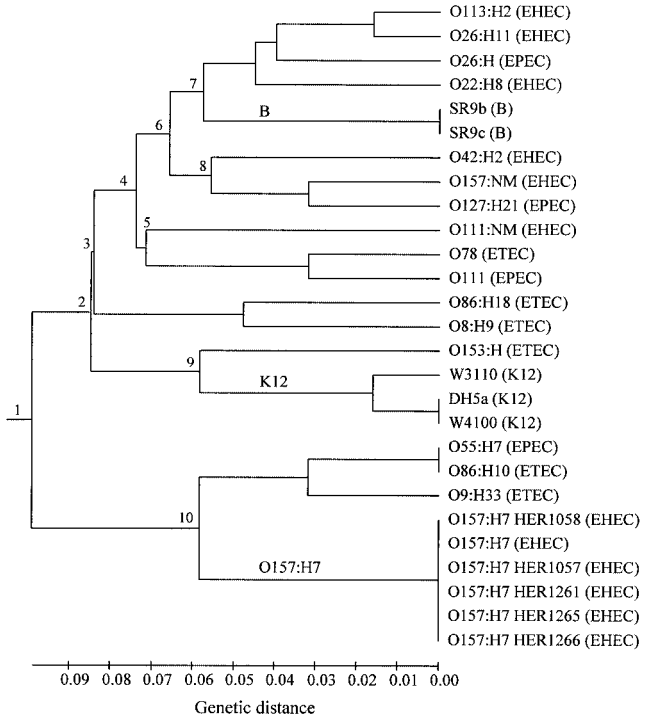

FIG. 3. Dendrogram presenting the genetic relationships among *E. coli* isolates using UPGMA cluster analysis of the AILP data (for details, see Table 2).

mixed-genome microarray, and multilocus sequence typing (MLST) (e.g., see references 6, 10, 26, 37, and 48). Serotypes 4a and 4c (lineage III) were found to be the most genetically distant strains (average genetic distance of 0.325 ± 0.079), in accordance with previous studies suggesting that lineage III represents a unique subset of *L. monocytogenes* characterized by reduced virulence for humans and by other genetic features (48). Similar lineage clustering but higher discrimination was achieved by MLST analysis of simple sequence repeat (SSR) loci (19 sequence types) and by PFGE (24 PFGE profiles) in the same set of 28 *L. monocytogenes* strains (L. Somer, Y. Danin-Poleg, L. Valinsky, and Y. Kashi, unpublished data). In conclusion, AILP analysis separated *L. monocytogenes* from the other *Listeria* species, divided *L. monocytogenes* isolates among the three known lineages comparable to the serological division, and discriminated the most virulent 4b serotypic group. These results support the efficiency of the AILP method for rapid strain typing of *Listeria*.

In *E. coli*, the 16 informative loci facilitated the assignment of the 27 strains to 19 AILP types. Similar discrimination ability was achieved (17 sequence types) with the same bacterial set using MLST analysis of SSR loci (12). Phylogenetic analysis of AILP data separated the B, K-12, and O157:H7 serological groups into distinct clusters. O157:H7 isolates were clustered together with the O55:H7 isolate (Fig. 3, node 10), supporting the findings that O55:H7 and O157:H7 have recently evolved from a common ancestor (46). The six O157:H7 isolates exhibited the same pattern, indicating close genetic relations and low diversity, in agreement with other methods (23, 29). However, due to wide genome rearrangements reported between O157:H7 and K-12 (20, 33), it is likely that AILP analysis with primers designed on the basis of the O157:H7 genome (rather than the K-12 genome) would facilitate discrimination between O157:H7 isolates. Indeed, in silico variation was found in the studied *serW* AILP site between the two published genomes of O157:H7 (33). In addition, analysis of a second *E. coli* set consisting of the *E. coli* reference collection strains (30) was performed at five AILP markers. The analysis showed increased genetic distance among strains (average distance of 0.24 ± 0.18 compared to 0.162 ± 0.06 in the first set of 27 strains) as a result of the higher genetic diversity in the wild-type ECOR collection. The phylogenetic analysis clearly separated B2 strains from the D strains and from A and B1 strains (Fig. 4) (21). Parallel clustering was achieved with the same bacterial set using MLST analysis of SSR loci (12).

Comparing the AILP results for *Listeria* and *E. coli* revealed that in *L. monocytogenes* a high correlation was found between serologic profiles and other genetic typing methods (3), providing initial typing comparable to serology (48). In contrast, in *E. coli*, only a low correlation was found between the serologic profiles or pathogenic groups (e.g., EPEC, ETEC, and EHEC)

TABLE 4. AILP types based on electrophoretic profiles of PCR amplification products of 27 *E. coli* strains at 30 sites

Group and strain	Allele[a] at following locus:																												AILP type
	hisC	*vitaB*	*ftsZ*	*b1688*	*aidB*	*b1284*	*yaiN*	*ycgW*	*caiF*	*yacA*	*serW*	*dsrB*	*yaaH*	*molR-1*	*yjiD*	*b0829*	*yibA*	*b1031*	*mhpR*	*folA*	*pyrD*	*b2345*	*ykgE*	*osmB*	*gutP*	*b1248*	*galS*	*uvrB*	*yafY*	*pepD*	
EHEC																														
O22:H8	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	6	1	1	1	1	1	1	4	1	1	1	2	1	1
O42:H2	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	1	5	1	1	1	1	1	1	4	1	1	2	2	1	2
O111:NM	2	4	1	1	1	1	1	1	1	1	5	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	2	1	3
O113:H2	3	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	2	2	1	4
O26:H11	3	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	1	1	2	1	5
O157:NM	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	4	1	1	1	1	1	1	1	1	1	2	2	1	6
O157:H7	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	2	1	7
O157:H7-1057	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	2	1	7
O157:H7-1058	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	2	1	7
O157:H7-1261	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	2	1	7
O157:H7-1265	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	2	1	7
O157:H7-1266	1	1	1	1	1	1	2	2	1	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	2	1	7
ETEC																														
O78	1	2	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	4	1	1	1	1	1	1	1	1	1	1	2	1	8
O8:H9	1	1	1	1	1	1	1	1	1	1	5	1	1	1	1	2	1	7	1	1	1	1	1	1	1	1	1	1	2	1	9
O9:H33	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	5	1	1	1	1	1	1	1	1	1	2	2	1	10
O86:H10	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	2	5	1	1	1	1	1	1	1	1	1	2	2	1	11
O86:H18	1	1	1	1	2	1	1	1	1	1	6	1	1	1	2	2	1	7	1	1	1	1	1	1	1	1	1	1	2	1	12
O153:H	1	1	1	1	1	1	1	1	1	1	7	1	1	1	1	2	1	1	1	1	1	1	1	1	4	1	1	1	1	1	13
EPEC																														
O111	2	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	2	4	1	1	1	1	1	1	1	1	1	1	2	1	14
O26:H	3	1	1	1	1	1	1	1	1	1	2	1	1	2	1	1	1	2	1	1	1	1	1	1	4	1	1	1	2	1	15
O55:H7	1	1	1	1	1	1	2	1	1	1	1	1	1	1	1	2	2	5	1	1	1	1	1	1	1	1	1	2	2	1	11
O127:H21	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	4	1	1	1	1	1	1	1	1	1	2	2	1	16
K-12																														
DH5a	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	17
W3110	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	18
W4100	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1	1	1	17
B																														
SR9b	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	2	1	1	1	1	1	1	1	2	1	1	2	1	19
SR9c	1	1	1	1	1	1	1	1	1	1	4	1	1	1	1	1	1	2	1	1	1	1	1	1	1	2	1	1	2	1	19

[a] Allele designations: 1, amplification product at the expected size according to GenBank sequence; 2, absence of product; 3, one product in addition to the expected product; 4 to 7, products differing in length from one another and from the expected product.

and AILP typing. This is expected as in general there is a low correlation between serological profiles and genetic relationships in *E. coli* (3, 42, 46). The higher rates of horizontal gene transfer reported in *E. coli* (46) compared to the clonal structure characterization of *L. monocytogenes* (23, 50) could explain these differences. This is further documented by the whole-genome comparison between *L. monocytogenes* and *L. innocua* showing that no large genome rearrangements have occurred between these species (8, 17, 28). In contrast, genome-wide rearrangements were described between *E. coli* strains K-12 and O157:H7 (20, 33).

The AILP typing method was found to be more efficient in bacterial species with a clonal structure, such as *Listeria* species. Its efficiency is demonstrated by the fact that using only two AILP sites, *abc* and *gbuA*, enabled the discrimination of the three known *L. monocytogenes* lineages (Fig. 2; Table 3) and distinguished the most common virulent serotypic group, 4b, from the other serotypic groups of lineage I. Similar differentiation was achieved using infrequent restriction site PCR (16). Furthermore, similar to the AILP analysis, only 4 probes (out of 29) of the mixed-genome microarray were needed for the same differentiation (10).

Compared to other typing methods, AILP is a rapid, low-cost, and simple typing method. It provides discrimination power comparable to serology but lower than MLST (12, 37) and PFGE (7, 18). However, AILP is much faster (a few hours compared to a few days) and less expensive, provides results that are simple to analyze, and requires distinctly less experienced manpower. Equipment and infrastructure requirements for AILP are minimal, consisting of basic laboratory equipment, thermocycler and minigel apparatus. Thus, this method is suitable for initial rapid bacterial typing scanning. Following this, detailed strain typing can be done as a second step by using typing methods such as MLST (9, 37) or PFGE (7, 18), which provide higher discrimination but are labor intensive and expensive.

Due to the high stringency of the reaction conditions and the unique set of primers, the efficiency of AILP analysis can be increased by multiplexing a number of loci in the same reaction mixture. A rapid DNA preparation (such as standard boiling) is suitable for routine high-throughput identification, as similar results were obtained using rapid DNA purification methods (data not shown). However, the same DNA purification method should be applied to all isolates. High-throughput strain identification with the AILP method could be achieved by technologies such as microarrays (40) or real-time PCR. AILP analysis can be applied to any microorganism with prior knowledge of part or all of its genome sequence but does not

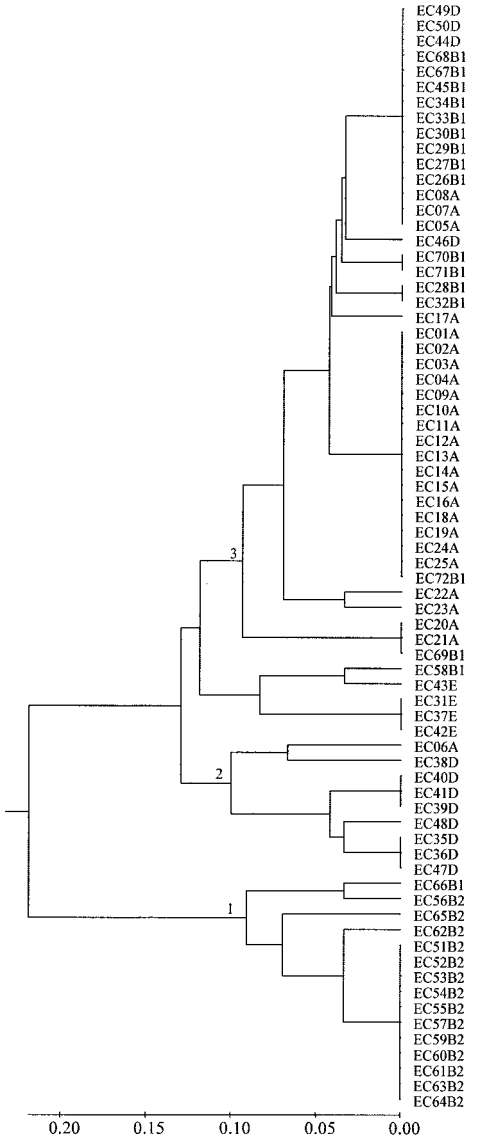

FIG. 4. Dendrogram presenting the genetic relationships among 72 strains of an *E. coli* reference collection using UPGMA cluster analysis of five AILP markers (*yaiN*, *ycgW*, *serW*, *b2345*, and *ykgE*).

require prior knowledge of sequence variation among species or strains. In cases where genomes of different strains are available, in silico selection of AILP primers directed to variable (multiallelic) chromosomal sites is possible.

In conclusion, the AILP method provides rapid and simple initial identification of isolates as a basis for epidemiological investigation, clearly discriminating between different strains or revealing similarities that can be further tested using high discriminatory power typing methods. Thus, the AILP method should be a useful addition to the available methodologies for rapid initial microbial strain typing.

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