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Assignment of *Listeria grayi* and *Listeria murrayi* to a Single Species, *Listeria grayi*, with a Revised Description of *Listeria grayi*

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The genomic relatedness between *Listeria grayi* and *Listeria murrayi* was reevaluated by using DNA-DNA hybridization, multilocus enzyme electrophoresis, and rRNA restriction fragment length polymorphism techniques. The high levels of similarity observed between the strains of these two species confirmed the data published since 1973 and indicated that they should be considered members of a single species. On grounds of priority, the species should be named *L. grayi*.

DNA-DNA hybridization and 16S rRNA sequencing results have shown that the genus *Listeria* consists of two distinct groups of genomically related species; *Listeria monocytogenes*, *Listeria innocua*, *Listeria ivanovii*, *Listeria welshimeri*, and *Listeria seeligeri* are in one group, and *Listeria grayi* and *Listeria murrayi* are in the second group (3, 13, 15).

L. grayi Errebo Larsen 1966 (11) and *L. murrayi* Welshimer and Meredith 1971 (25) are isolated very rarely (14) and are nonpathogenic (5). The status of these organisms as two distinct species is unclear, and their status in the genus was left as species incertae sedis by Seeliger and Jones (18). In 1973, Stuart and Welshimer observed a high level of DNA-DNA homology between strains of *L. grayi* and *L. murrayi* (21) and subsequently proposed that they should be placed in a single species (22). However, this proposal was not put into effect on the Approved Lists of Bacterial Names (20), nor was it validated by a subsequent publication, possibly because Stuart and Welshimer (22) proposed exclusion of these two species from the genus *Listeria* and their transfer to a new genus, "*Murraya*," as "*Murraya grayi* subsp. *grayi*" and "*Murraya grayi* subsp. *murrayi*" (22).

The close relationship between these two species has been shown by numerical taxonomic analyses, which grouped them as a single distinct cluster with a level of similarity of 85 to 90% (6, 26), as well as by multilocus enzyme analysis (2). Furthermore, the sharing of specific chemotaxonomic properties distinguish these organisms from other members of the genus *Listeria* and support their close relationship; they have the same DNA base composition values (6, 21), produce similar electropherograms for whole-cell proteins (10), and exhibit identical substitutions of the lipoteichoic acids (16) and a common antigenic structure (17), with small differences in O factors (23). Finally, the two species have been distinguished from each other only on the basis of nitrate reduction data (18).

In this study we thoroughly reexamined the genomic distance between *L. grayi* and *L. murrayi* in order to evaluate whether these two taxa should be retained. To do this, we used the following three methods: DNA-DNA hybridization, multilocus enzyme electrophoresis, and rRNA gene restriction fragment length polymorphism.

MATERIALS AND METHODS

Bacterial strains. The designations and sources of the five strains of *L. grayi* and the five strains of *L. murrayi* used in this study are shown in Table 1. These organisms were identified by using well-recognized biochemical markers (18).

DNA-DNA hybridization. DNA-DNA hybridization experiments were performed as previously described (13), except that we used the procedure described below to lyse the bacteria. The growth from six Roux flasks that were incubated for 48 h at 37°C and contained 150 ml of Columbia agar was harvested, washed in 20 ml of 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate, pH 7.0), and incubated for 1 h at 37°C in 5 ml of a lysozyme solution (0.01 M sodium phosphate–20% sucrose [pH 7] containing 12 mg of lysozyme [Appligène, France]). Then we added 40 ml of a proteinase K solution (10 mM Tris-HCl [pH 8], 1 mM EDTA, 1% sodium dodecyl sulfate, 20 mg of proteinase K [Appligène]), and the mixture was incubated for 2 h at 37°C. The DNA was then sequentially extracted by using phenol and chroroform and hybridized by using the S1 nuclease-trichloroacetic acid method.

Multilocus enzyme electrophoresis. The methods used and results obtained with 18 enzymes have been described previously (2).

rRNA gene restriction pattern. Chromosomal DNAs were extracted and digested with *Eco*RI or *Hin*dIII. The resulting fragments were subjected to electrophoresis, transferred to a nitrocellulose membrane, and hybridized with a cloned rDNA (genes coding for rRNA) from *Bacillus subtilis* that was labeled with [α-^{32}P]dCTP by using previously described methods (4, 9).

RESULTS AND DISCUSSION

The results of the DNA-DNA homology experiments indicated that there were high levels of relatedness among all of the strains which we used. Labeled DNA from *L. grayi* CLIP 12518^T (T = type strain) was 83 to 94% (average, 87%) related to unlabeled DNAs from *L. murrayi* strains; similarly, labeled DNA from *L. murrayi* CLIP 12515^T was 78 to 88% (average, 83%) related to unlabeled DNAs from *L. grayi* strains. The ΔT_m values ranged from 0 to 0.9°C (Table 1). These data are in broad agreement with those obtained by Stuart and Welshimer (58 to 76% DNA homology, deter-

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TABLE 1. Levels of relatedness between *L. grayi* and *L. murrayi* strains

Strain[a]	Origin	% of reassociation with labeled DNA from:		Isoenzyme electrophoretic type	Ribogroup	
		Strain CLIP 12518^{T}	Strain CLIP 12515^{T}		*Eco*RI	*Hin*dIII
L. grayi						
CLIP 12518^{T} (= ATCC 19120^{T})	Chinchilla, Denmark	100 (0)[b]	82	51	EG1	HG1
CLIP 640	Cattle, Brazil	94	81	51	EG1	HG1
CLIP 7353	Human, Italy	92	85	50	EG1	HG1
CLIP 14014	Human food, France	102	88	51	EG1	HG1
SLCC 3322	Dog, Germany	87 (0.3)	78	55	EG2	HG2
L. murrayi						
CLIP 12515^{T} (= ATCC 25401^{T})	Vegetation, United States	87 (0.9)	100	53	EG1	HG1
CLIP 12516 (= ATCC 25402)	Vegetation, United States	87	104	53	EG1	HG1
CLIP 12157 (= ATCC 25403)	Vegetation, United States	83 (0.2)	77	52	EG3	HG1
CLIP 13861 (= CIP 103213)	Cattle, France	85 (0.3)	78	54	EG4	HG1
SLCC 3792[c]	Germany	95	86	56	EG1	HG3

[a] CLIP, *Listeria* Collection, Pasteur Institute, Paris, France; SLCC, Special *Listeria* Culture Collection, Würzburg, Germany; ATCC, American Type Culture Collection, Rockville, Md.; CIP, Collection de l'Institut Pasteur, Paris, France.

[b] The values in parentheses are ΔT_m values (in degrees Celsius).

[c] Previously misidentified as *L. grayi* (12).

mined by using the filter method [22]) and by Bakulov et al. (90% DNA homology, determined by using the filter method [1]). Overall DNA sequence relationships as determined by DNA hybridization have become the standard basis for assessing taxonomic relationships at the species level; a species is now defined as a group of strains that share approximately 70% or greater DNA relatedness with ΔT_m values of less than 5°C (24). Therefore, it appears that *L. grayi* and *L. murrayi* constitute a single species.

On the basis of the results obtained with 18 enzymes, the five *L. grayi* and five *L. murrayi* strains formed a single major division that was distinct from the other divisions found in the genus *Listeria* (2). The members of this group produced seven different isoenzymes patterns, and the greatest genetic distance was 0.34, a value far below the limit of 0.60 to 0.70 that is empirically considered to indicate separate species (Fig. 1).

Multilocus enzyme electrophoresis has been used for some years as a powerful method for evaluating genomic distances between microorganisms that are phenotypically similar (19). A recent reexamination of the taxonomy of the genus *Listeria* in which this method was used resulted in a

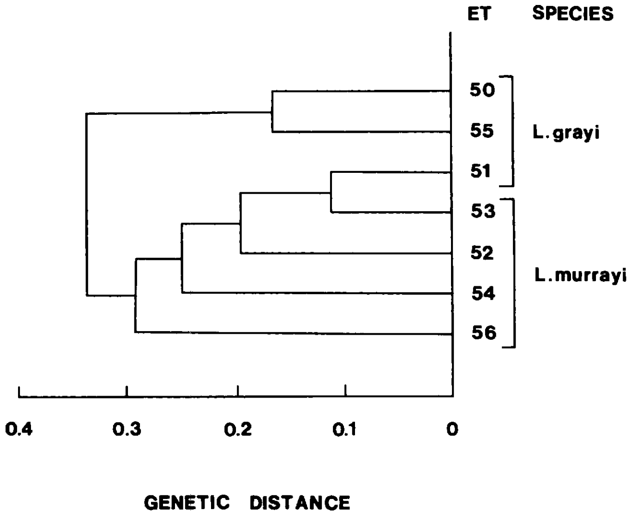

FIG. 1. Genetic relationships among the seven electrophoretic types (ETs) of *L. grayi* and *L. murrayi*. The dendrogram was generated by using the average-linkage method of clustering from a matrix of pairwise coefficients of genetic distances that were based on electrophoretically demonstrable allelic variation at 18 enzyme loci. This dendrogram is part of a dendrogram published elsewhere (2). ET 50, strain CLIP 7353; ET 55, strain SLCC 3322; ET 51, strains CLIP 12518^{T}, CLIP 640, and CLIP 14014; ET 53, strains CLIP 12515^{T} and CLIP 12516; ET 52, strain CLIP 12517; ET 54, strain CLIP 13861; ET 56, strain SLCC 3792.

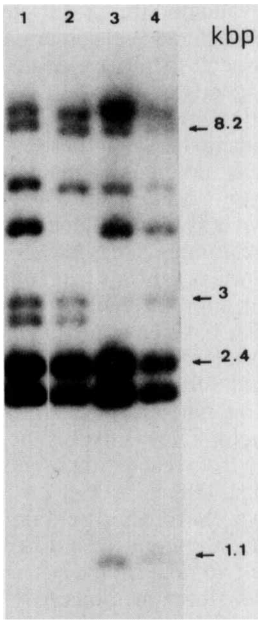

FIG. 2. Autoradiogram produced after hybridization of the rDNA probe with the following Southern-blotted *Eco*RI restriction fragments: lane 1, strain CLIP 640 (profile EG1); lane 2, strain SLCC 3322 (profile EG2); lane 3, strain CLIP 12517 (profile EG3); lane 4, strain CLIP 13861 (profile EG4).

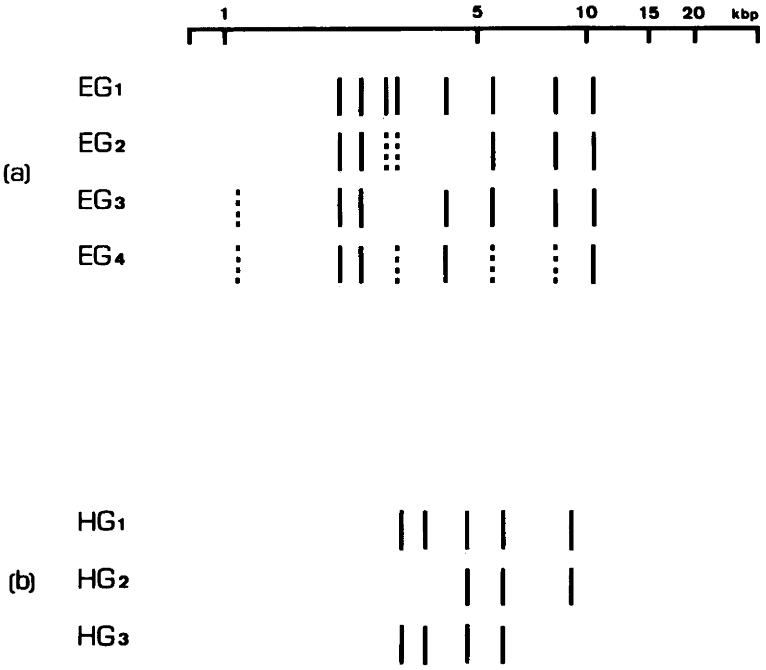

FIG. 3. Schematic representation of rRNA gene restriction patterns of *L. grayi* and *L. murrayi* after digestion with *Eco*RI (a) and *Hin*dIII (b). The dotted lines indicate weakly labeled bands.

complete correlation with a previous classification of *Listeria* strains as determined by DNA-DNA hybridization (2, 13). The data which we obtained reinforced the usefulness of this method for assessing closely related species.

rDNA fingerprinting is used increasingly for classification (7, 8, 12). Our results provided further evidence of the high level of homology between *L. grayi* and *L. murrayi*. The *Eco*RI restriction patterns of highly conserved rRNA genes obtained from the representatives of *L. grayi* and *L. murrayi* revealed four different groups (Fig. 2 and 3), which were different from the groups determined for the other *Listeria* species (9). Interestingly, profile EG1 was found in five *L. grayi* and three *L. murrayi* isolates, including the type strains of both species. Only three groups were detected when DNAs were digested with *Hin*dIII; one of these groups (group HG1) contained both the *L. grayi* type strain and the *L. murrayi* type strain (Fig. 2).

On the basis of DNA homology values, isoenzymes patterns, and rRNA genes restriction patterns, *L. grayi* and *L. murrayi* constitute a homogenous taxon, confirming the significant genomic and phenotypic similarities that have been reported since 1973 (1, 2, 9, 10, 16, 21, 22, 26). On grounds of priority, this taxon should be named *L. grayi*.

Emended description of *L. grayi*. Detailed descriptions of the two former species that form *L. grayi* have been published previously in *Bergey's Manual of Systematic Bacteriology* (18). Briefly, cells are small (0.4 to 0.5 by 0.5 to 2 μm), asporogenous, gram-positive, peritrichous rods which are motile at 28°C. Colonies on tryptose agar are small (1 to 2 mm in diameter after 1 to 2 days of incubation at 37°C), regular, and smooth. Growth occurs at 4°C within 5 days. Metabolism is facultatively anaerobic, catalase is produced, the oxidase test is negative, and reduction of nitrates to nitrites is variable. Acid, without gas, is produced from glucose, mannitol, and other sugars. Voges-Proskauer and methyl red tests are positive. Esculin is hydrolyzed in 1 day. Urea is not hydrolyzed, and indole is not produced. Sheep erythrocytes are not hemolyzed. Gelatin is not hydrolyzed, and H_2S is not produced. The guanine-plus-cytosine content of the DNA is 41 mol%.

The type strain is strain ATCC 19120 (= NCTC 10815 = CIP 6818).

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