INTERNATIONAL JOURNAL of SYSTEMATIC BACTERIOLOGY
April 1974, p. 177-185

Vol. 24, No. 2
Printed in U.S.A.

Taxonomic Reexamination of *Listeria* Pirie and Transfer of *Listeria grayi* and *Listeria murrayi* to a New Genus, *Murraya*

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The intergeneric relationships of *Listeria monocytogenes* have been examined by both classic taxonomic criteria and deoxyribonucleic acid (DNA) polynucleotide sequence homology. Analysis of characters according to Adansonian principles indicated that *Listeria monocytogenes* 19303 was phenotypically most closely related to streptococci, *Staphylococcus aureus*, and *Diplococcus pneumoniae*. DNA:DNA hybridization studies, however, failed to reveal significant relatedness between the listeriae and *Corynebacteriaceae*, *Micrococcaceae*, or *Lactobacillaceae*. It is recommended, on the basis of the results reported here and the results of a previous hybridization study, that *Listeria grayi* and *Listeria murrayi* be transferred to a new genus, *Murraya*, as *M. grayi*, and *M. grayi* subsp. *murrayi*, respectively. A new family, *Listeriaceae*, is proposed for the genera *Listeria* and *Murraya*.

For many years *Listeria monocytogenes*, first described by Murray, Webb, and Swann in 1924, constituted a monotypic genus which was placed in the family *Corynebacteriaceae*. Within recent years three avirulent species, *L. grayi* (1), *L. murrayi* (7), and *L. denitrificans* (27, 31), have been added to the genus; however, the taxonomic placement of these species has not been reexamined.

Previous comparisons of listeriae with other bacteria have provided only superficial evidence of their relationship to other organisms. Sneath (32) and Sneath and Cowan (33) placed *L. monocytogenes* in a group with *Streptococcus*, *Micrococcus*, *Staphylococcus*, *Aerococcus*, *Erysipelothrix*, and *Corynebacterium pyogenes* by numerical methods based on Adansonian principles. Dendrograms generated by phenetic classification prepared by DaSilva and Holt (7) showed *Kurthia*, currently placed in *Brevibacteriaceae*, and *Listeria* at the same phenon level with respect to strains of *Corynebacterium*. Jones et al. (16) asserted a relationship between *L. monocytogenes* and *Erysipelothrix* on the basis of similarities in morphology, growth requirements, and disease syndrome produced but noted significant differences in serology and sensitivity to certain inhibitors. On the other hand, Gray and Killinger (12) pointed out that there was no reason for confusion in the separation and identification of *Erysipelothrix* and *L. monocytogenes*. No close relationship between these two genera was demonstrable by the methods of numerical taxonomy. Most recently, Stuart and Pease (34) have shown in a computer comparison of selected general and physiological characters that *Listeria* and *Erysipelothrix* are distinct genera and that listeriae most closely resemble fecal streptococci. Davis et al. (8) concluded from a computer study of 199 characteristics that *Listeria*, *Erysipelothrix*, and *Microbacterium thermosphactum* were more closely related to the *Lactobacillaceae* than to the *Corynebacteriaceae*. Jones and Sneath (15) have indicated that *L. monocytogenes* may be related to *Staphylococcus* and *Peptococcus* and somewhat less related to *Streptococcus* and *Diplococcus*.

Deoxyribonucleic acid (DNA):DNA hybridization has become an important tool in bacterial taxonomy for assessing the genetic and molecular relatedness of organisms. This method offers the bacterial taxonomist a direct method of comparing the genetic composition of organisms without relying on the phenotypic expression of their potential.

The intrageneric relatedness of the four species of *Listeria* has been described previously (35). Examination of the taxonomic placement of the listeriae has been extended here to include intergeneric relationships based on phenotypic similarity and molecular relatedness assessed by DNA:DNA hybridization.

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MATERIALS AND METHODS

Bacterial strains. The bacterial strains and cultural conditions used in this study are listed in Tables 1 and 2. Table 1 lists the bacteria tested for similarity to *Listeria monocytogenes* 19303, and Table 2 lists the strains of *Listeria* used and their origins.

Media. All listeriae were maintained on slants of tryptose blood agar base to which 1% glucose had been added. With the exception of *Diplococcus pneumoniae, Branhamella catarrhalis, Erysipelothrix rhusiopathiae,* and *Propionibacterium freudenreichii,* all bacterial strains were grown in Tryptic-Soy broth (Difco, TSB) for the extraction of DNA. *Diplococcus pneumoniae, Erysipelothrix rhusiopathiae,* and *Branhamella catarrhalis* were grown in volume in brain heart infusion (Difco, BHI) for the extraction of DNA. *Propionibacterium freudenreichii* was grown in peptone-yeast extract broth (peptone, 5 g; yeast extract, 5 g; glucose, 5 g; distilled water, 1 liter). Tryptose phosphate broth (TP, tryptose, 20 g; NaCl, 2 g; Na_2HPO_4, 3.5 g; distilled water, 1 liter) was used to cultivate *Listeria* strains for DNA extraction and isotopic labeling.

Phenotypic characterization. The methods for biochemical characterization followed standard bacteriological techniques as presented in the *Manual of Microbiological Methods* (30). Purple broth base (Difco) supplemented with 1% carbohydrate (autoclaved separately) was used for fermentation tests. The ability of test organisms to produce acid within 14 days from glucose, lactose, sucrose, salicin, maltose, rhamnose, melezitose, mannitol, aesculin, dextrin, galactose, fructose, mannose, sorbitol, trehalose, starch, inulin, cellobiose, adonitol, dulcitol, melibiose, arabinose, and raffinose was tested. Motility was tested by the hanging-drop technique during log-phase growth. Hucker's modification of the Gram stain was used to determine Gram reaction both in the early log-phase and in the stationary phase of growth. Albert's stain was used to detect the presence of metachromatic granules after 24- and 48-h growth on Pai medium (whole egg, 690 ml; saline or distilled water, 230 ml; glycerine, 80 ml; inspissated for 90 min). Lipid droplets were visualized by Burdon's staining technique. A complete test for nitrate reduction was performed according to the method described by Wallace and Neave (30) after growth for 48 h in nitrate broth (beef extract, 3 g; peptone, 5 g; potassium nitrate, 1 g; distilled water, 1 liter). The Voges-Proskauer test for acetylmethylcarbinol and the methyl red test were performed on 24-h growth in

TABLE 1. *Strains other than listeriae used in this study*

Species name	Strain designation	Maintenance medium[a]	Incubation temp (C)
Actinobacillus lignieresii	ATCC 19393	BA	37
Arthrobacter globiformis	ATCC 8010	NA	26
Bacillus cereus	ATCC 14579	NA	30
Bacillus megaterium	ATCC 14581	NA	30
Beneckea hyperoptica	ATCC 15803	MA	26
Branhamella catarrhalis[b]	ATCC 8176	BHI	37
Brevibacterium linens	ATCC 9172	NA	30
Cellulomonas biazotea	ATCC 486	NA	30
Chromobacterium violaceum	ATCC 12472	NA	26
Corynebacterium diphtheriae	ATCC 19409	BA	37
Diplococcus pneumoniae	ATCC 6301	BA	37
Erysipelothrix rhusiopathiae	ATCC 11916	BA	37
Erysipelothrix rhusiopathiae	ATCC 19414	BA	37
Escherichia coli	MCV-CSH-2	NA	37
Flavobacterium aquatile	ATCC 11947	FM	30
Haemophilus influenzae	ATCC 19418	CSB	37
Lactobacillus plantarum	ATCC 8014	TJA	30
Leuconostoc mesenteroides	ATCC 12291	BeM	26
Microbacterium lacticum	ATCC 8180	NA	26
Micrococcus luteus	ATCC 398	NA	26
Pediococcus cerevisiae	ATCC 10791	TJA	26
Propionibacterium freudenreichii	ATCC 6207	TJA	26
Staphylococcus aureus	ATCC 6538P	NA	26
Streptococcus faecalis	ATCC 19433	BHI	37
Streptococcus lactis	ATCC 11454	BHI	37
Proteus mirabilis	MCV	BHI	37

[a] All media were prepared according to ATCC specification (1). Abbreviations: BA, blood agar; NA, nutrient agar; MA, marine agar; BHI, brain heart infusion agar; FM, *Flavobacterium* medium; CSB, chocolate agar with supplement B (Difco); TJA, tomato juice agar; BeM, *Betabacterium* medium; ATCC, American Type Culture Collection, Rockville, Md.; MCV, Culture Collection, Medical College of Virginia.

[b] *Branhamella catarrhalis* was formerly designated *Neisseria catarrhalis.*

TABLE 2. *Listeria strains used in this study*

Strains	Origin	Source[a]
L. monocytogenes 19303	Human	Ft. Detrick
L. monocytogenes A4413	Human	Ft. Detrick
L. monocytogenes JC	Human	HJW
L. monocytogenes HBr	Human	HJW
L. monocytogenes HD	Human	HJW
L. monocytogenes T_3b	Human	CDC (KC224)
L. monocytogenes T_4b	Human	CDC (KC226)
L. monocytogenes V-7	Vegetation	HJW
L. monocytogenes V-8	Vegetation	HJW
L. monocytogenes V-11	Vegetation	HJW
L. monocytogenes V-12	Vegetation	HJW
L. grayi ATCC 19120	Chinchilla feces	ATCC
L. grayi V-1	Vegetation	HJW
L. murrayi F-2	Vegetation	HJW
L. murrayi F-6 (ATCC 25402)	Vegetation	HJW
L. murrayi F-9 (ATCC 25401)	Vegetation	HJW
L. murrayi F-11	Vegetation	HJW
L. murrayi F-11	Vegetation	HJW
L. murrayi F-12 (ATCC 25403)	Vegetation	HJW
L. denitrificans (ATCC 14870)	Ox blood	ATCC

[a] Abbreviations: HJW, Culture Collection of H. J. Welshimer, Virginia Commonwealth Univ., Richmond, Va.; ATCC, American Type Culture Collection, Rockville, Md.; CDC, Center for Disease Control, Atlanta, Ga.; MCV, Culture Collection of the Department of Microbiology, Virginia Commonwealth Univ., Richmond, Va.; Ft. Detrick, Frederick, Md.

MRVP medium (Difco). The benzidine test for cytochrome-containing respiratory systems was performed according to the method of Deibel and Evans (9). Simmons' citrate agar (Difco) was employed to test the ability to utilize citrate as the sole carbon and energy source. The presence of catalase was demonstrated by the evolution of gas from 10% hydrogen peroxide placed directly on colonies of the test organism.

The antibiotic-sensitivity pattern for each strain was determined by a qualitative disc diffusion method. The culture was grown for 24 h in Penassay broth (Difco); 0.1 ml of a 10^{-2} dilution of this culture was added to 9.9 ml of Penassay agar tempered to 45 C. After thorough mixing, the contents were poured into a petri dish (100 by 15 mm) and allowed to solidify. Antibiotic disks were then spaced at intervals on the plate. After 24 h of incubation at the appropriate temperature, the cultures were scored for resistance or sensitivity to the antibiotics. All cultures were tested for sensitivity to tetracycline, kanamycin, neomycin, gentamicin, erythromycin, sulfadiazine, chloramphenicol, bacitracin, ampicillin, streptomycin, methicillin, penicillin, and naladixic acid.

The acid and alcohol products of growth in glucose-containing media were analyzed by the chromatographic procedure outlined in the *Anaerobe Laboratory Manual* (13). Alcohol and volatile-acid products were detected in acidified cultures extracted with ether. Forty-eight-h-old cultures in TSB supplemented with 1% glucose were acidified to pH 2 or below by the addition of 50% H_2SO_4 (vol/vol). A 2-ml amount of acidified culture in a conical centrifuge tube was extracted with 1 ml of ethyl ether by inversion of the stoppered tube 20 times. The ether layer was separated by centrifugation and withdrawn. Anhydrous magnesium sulfate was added to one-half the volume of the ether. The samples were allowed to stand at room temperature at least 10 min. A 14-μliter sample of each extract was injected into a Beckman GC-2A gas chromatograph with an aluminum column (6 ft by 0.25 inch) packed with Resoflex LAC-1-R-296 (standard concentration, Burrell Corp.). The resulting tracings were compared with that of an acidified standard which contained 1 meq each of acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic and heptanoic acids per 100 ml of aqueous solution. The alcohol standard contained 1.7 mM ethanol, 0.5 mM propanol, 0.05 mM isobutanol, 0.1 mM butanol, and 0.05 mM pentanol.

Nonvolatile acids were detected by gas chromatography after methylation. A 1-ml amount of an acidified 48-h culture was placed in a small test tube, and 1 ml of BF_3-methanol (boron trifluoride methanol, 14% wt/vol, Applied Science Labs) was added. This mixture was held overnight at room temperature before extraction with 0.5 ml of chloroform. The chloroform layer was separated by centrifugation, withdrawn from under the aqueous layer, and 14 μliters was applied to the chromatographic column. Tracings from each sample were compared to the tracing of a standard solution containing 1 meq each of pyruvic, lactic, oxaloacetic, oxalic, methylmalonic, malonic, fumaric, and succinic acids per 100 ml of water. A 1-ml amount of the standard solution was methylated and extracted under the same conditions as the samples.

An acidified sample of sterile TSB containing 1% glucose was extracted with ether, or methylated and then extracted with chloroform according to the above procedure, and 14 μliters was chromatographed. No significant background concentration of any of the alcohols or acids was detected. All tracings obtained were interpreted only in a qualitative manner. No effort was made to quantitate alcohol or acid products of any of the strains.

DNA isolation. The method of Marmur (23) was used for all DNA extractions except in the cases of *Listeria monocytogenes* and *Staphylococcus aureus*. *L. monocytogenes* strain 19303 cells were extracted as described previously (35). Cells from *Staphylococcus aureus* were suspended in 25 ml of 0.15 M NaCl with 800 μg of lysostaphin/ml (240 U/mg, Schwarz-Mann). After 1 to 2 h of incubation with shaking, 100 μg of Pronase (B grade, Calbiochem) was added per ml, and incubation with shaking was continued for 1 to 3 h. Marmur's procedure was followed after incubation with Pronase. All DNA samples were stored in 1 SSC (0.15 M sodium chloride, 0.015 M trisodium citrate, pH 7.0) over chloroform at 4 C.

Determination of DNA base composition. The mole percent guanine plus cytosine (%GC) of DNA extracted from the cultures was calculated from buoyanti density determinations. The buoyant density of DNA was determined by isopycnic centrifugation in CsCl at 25 C in a Spinco model E analytical ultracentrifuge. Densitometer tracings of the ultraviolet (UV) films were made on a model 2400 Gilford recording spectrophotometer. The %GC was calculated from the buoyant density according to DeLey (10) by using DNA from *Escherichia coli* CSH-2 as standard reference. The buoyant density of the reference DNA was taken to be 1.710 g/cm^3 (28).

Purity of extracted DNA. Purity of extracted DNA was assessed in four ways: the A_{260}/A_{280} ratio, the diphenylamine reaction (29), the orcinol reaction (36), and the phenol-sulfuric acid test for total carbohydrate (11). The quantity of DNA calculated from the A_{260} the diphenylamine reaction, or the phenol-sulfuric acid test, agreed within 10 μg for all preparations. No significant reaction was observed with the orcinol test.

Preparation of radioactive DNA. Radioactive DNA was produced in *L. monocytogenes* 19303, *L. grayi* ATCC 19120, and *L. murrayi* F-9 by a method described previously (35).

Shearing of DNA. Radioactive DNA was sheared by sonic treatment for 3 min by using a Biosnik IV (Bronwill Scientific) set at 90. To prevent thermal denturation, the DNA sample was held in an ice bath and sonically treated for 10-s intervals 20 s apart.

DNA hybridization. Single-stranded DNA bound to nitrocellulose filters was prepared as described previously (35). Since the specificity of hybridization reactions is affected by incubation temperature (14), hybridization reactions with homologous systems were performed at various temperatures. Incubation at 65 C allowed no detectable mismatching of the homologous system. The retention of single-stranded, unlabeled DNA on nitrocellulose filters was tested by incubation of loaded filters under exacting conditions for 20 h. The filter was then removed, and the quantity of DNA leached from the filter was calculated from the A_{260} of the solution. The average retention of unlabeled DNA was 86%. The standard deviation was 7.3. Hybridization reactions were carried out at 55 or 65 C. The intrageneric hybridization experiments were performed under exacting conditions at 65 C. The intergeneric hybridizations were performed at 55 C.

Thermal elution profiles were obtained by washing the filters in 2.5 ml of 0.1 × SSC for 15 min at 5-degree temperature increments from 60 to 85 C. Each aqueous sample and filter was then counted by liquid scintillation. Thermal stability of hybrid duplexes was determined from thermal elution profiles. The midpoint of the thermal elution (Tme) is the temperature at which half of the reference DNA associated with test DNA bound to the filter has become dissociated and eluted into the wash solution. The percentage of base mispairing was calculated according to the method of Laird et al. (17) from the change in Tme observed in hybrid test systems.

Relatedness was assessed by comparison of the relative counts per minute per microgram of labeled reference DNA bound by heterologous test DNA on filters to that bound by filters loaded with unlabeled test DNA from the same organism as the reference DNA.

TABLE 3. *Phenotypic similarity of test organisms to L. monocytogenes 19303*

Strain	Similarity (%)[a]
Actinobacillus lignieresii ATCC 19393	63
Arthrobacter globiformis ATCC 8010	49
Bacillus cereus ATCC 14579	73
Bacillus megaterium ATCC 14581	72
Beneckea hyperoptica ATCC 15803	57
Branhamella catarrhalis ATCC 8176	66
Brevibacterium linens ATCC 9172	44
Cellulomonas biazotea ATCC 486	56
Chromobacterium violaceum ATCC 12472	60
Corynebacterium diphtheriae ATCC 19409	68
Diplococcus pneumoniae ATCC 6301	76
Erysipelothrix rhusiopathiae ATCC 11916	67
Erysipelothrix rhusiopathiae ATCC 19414	67
Flavobacterium aquatile ATCC 11947	29
Lactobacillus plantarum ATCC 8014	64
Leuconostoc mesenteroides ATCC 12291	60
Microbacterium lacticum ATCC 8180	51
Micrococcus luteus ATCC 398	47
Pediococcus cerevisiae ATCC 10791	54
Propionibacterium freudenreichii ATCC 6207	51
Staphylococcus aureus ATCC 6538P	74
Streptococcus faecalis ATCC 19433	80
Streptococcus lactis ATCC 11454	83
L. monocytogenes 19303	100
L. monocytogenes A4413	93
L. monocytogenes JC	94
L. monocytogenes HBr	93
L. monocytogenes HD	94
L. monocytogenes T_3b	93
L. monocytogenes T_4b	94
L. monocytogenes V-7	94
L. monocytogenes V-8	89
L. monocytogenes V-11	93
L. monocytogenes V-12	90
L. grayi ATCC 19120	83
L. grayi V-1	86
L. murrayi F-2	84
L. murrayi F-6 (ATCC 25402)	83
L. murrayi F-9 (ATCC 25401)	84
L. murrayi F-11	84
L. murrayi F-12 (ATCC 25403)	86
L. denitrificans ATCC 14870	76

[a] Calculated from positive and negative matches to the characteristics of *L. monocytogenes* 19303; *Haemophilus influenzae* did not grow in any of the test media and was not considered further in this study.

RESULTS

Phenotypic similarity of test organisms to

Listeria monocytogenes 19303. The percent similarity of the test organisms to *Listeria monocytogenes* 19303 calculated from examination and comparison of 70 unweighted characters according to Adansonian principles (19, 20, 21) is shown in Table 3. Both positive and negative matches were considered. All strains of *Listeria* were more than 75% similar to *L. monocytogenes* 19303. Strains of *Bacillus cereus, Bacillus megaterium, Diplococcus pneumoniae, Staphylococcus aureus, Streptococcus faecalis,* and *Streptococcus lactis* were greater than 70% similar to *L. monocytogenes* 19303. These strains were selected for hybridization studies with *L. monocytogenes. Corynebacterium diphtheriae, Brevibacterium linens, Erysipelothrix rhusiopathiae,* and *Microbacterium lacticum* were included in hybridization studies only because their relationship to *Listeria* has been implied (2, 7, 8).

DNA composition of test organisms. Table 4 shows the mol %GC of representative strains of the four species of *Listeria* and of the other strains selected for hybridization studies with *L. monocytogenes* 19303. As reported previously, *L. monocytogenes, L. grayi,* and *L. murrayi* form a homogeneous group with respect to DNA nucleotide base composition, but the composition of DNA from *L. denitrificans* was quite different (57% GC) (35). The nucleotide composition of DNA from the strains of *Bacillus cereus, Diplococcus pneumoniae, Erysipelothrix rhusiopathiae, Staphylococcus aureus, Streptococcus lactis,* and *Streptococcus faecalis* was similar to that of the strains of *Listeria monocytogenes,* but the %GC of DNA from *Bacillus megaterium* ATCC 14581 and *Corynebacterium diphtheriae* ATCC 19409 (45 and 55% GC, respectively) was higher. DNA from *Brevibacterium linens* ATCC 9172 and *Microbacterium lacticum* ATCC 8180 was quite high in guanine plus cytosine (68 and 70%, respectively).

Intrageneric relatedness of Listeria. Results of experiments specifically designed to study the intrageneric relatedness of *Listeria* have been reported previously (35). These data are summarized in Table 5.

Intergeneric relatedness of Listeria. Table 6 shows the intergeneric relatedness of *Listeria monocytogenes,* the type species of *Listeria,* to phenotypically related genera as assessed by DNA:DNA hybridization. None of the species tested showed over 19% nucleotide sequence homology with *L. monocytogenes* 19303. The strains of *Bacillus cereus, Bacillus megaterium,*

TABLE 4. *Base composition of DNA from strains tested for molecular relatedness to Listeria*

Strain	GC (%)[a]
Bacillus cereus ATCC 14579	36
Bacillus megaterium ATCC 14581	45
Brevibacterium linens ATCC 9172	68
Corynebacterium diphtheriae ATCC 19409	55
Diplococcus pneumoniae ATCC 6301	41
Erysipelothrix rhusiopathiae ATCC 11916	36
Erysipelothrix rhusiopathiae ATCC 19414	36
Microbacterium lacticum ATCC 8180	70
Staphylococcus aureus ATCC 6538P	34
Streptococcus faecalis ATCC 19433	36
Streptococcus lactis ATCC 11454	35
L. monocytogenes 19303	38
L. monocytogenes HD	39
L. monocytogenes V-11	38
L. grayi ATCC 19120	41
L. grayi V-1	41
L. murrayi F-6 (ATCC 25402)	42
L. murrayi F-9 (ATCC 25401)	42
L. murrayi F-12 (ATCC 25403)	42
L. denitrificans ATCC 14870	57

[a] Calculated from buoyant density in CsCl at 25 C. Average of at least two determinations on different DNA preparations.

TABLE 5. *Intrageneric relatedness of Listeria Pirie*

Source of unlabeled DNA	Relative binding to *L. monocytogenes* 19303 (%)	Relative binding to *L. murrayi* F-9 (%)	Relative binding to *L. grayi* ATCC 19120 (%)
L. monocytogenes Group I (19303, HD, HBr, JC, A4413, V-7, V-12)	80-100	10-25	9-18
L. monocytogenes Group II (V-11, V-8, T_3b, T_4b)	40-70	12-30	10-15
L. grayi (ATCC 19120, V-1)	21, 40	71, 61	100, 84
L. murrayi (F-2, F-6, F-9, F-11, F-12)	20-44	71-100	58-76
L. denitrificans ATCC 14870	20	8	5

TABLE 6. *Intergeneric relatedness of Listeria*

Source of unlabeled DNA	Relative binding to *L. monocytogenes* 19301[a]	ΔTme[b]	Base mispairing (%)[c]
L. monocytogenes 19303	100	0	0
Bacillus cereus ATCC 14579	6	3.0	4.5
Bacillus megaterium ATCC 14581	6	3.0	4.5
Brevibacterium linens ATCC 9172	19	6.6	10.5
Corynebacterium diphtheriae ATCC 19409	19	8.6	13.0
Diplococcus pneumoniae ATCC 6301	7	4.0	6.0
Erysipelothrix rhusiopathiae ATCC 11916	9	4.0	6.0
Erysipelothrix rhusiopathiae ATCC 19414	10	2.0	3.0
Microbacterium lacticum ATCC 8180	18	8.6	13.0
Staphylococcus aureus ATCC 6538P	15	6.0	9.0
Streptococcus faecalis ATCC 19433	15	3.0	4.5
Streptococcus lactis ATCC 11454	16	4.0	6.0
Proteus mirabilis MCV	3	10.0	15.0

[a] Hybridization conditions: 55 C, 20 h.
[b] Difference in degrees C between the Tme of the reference system and the Tme of the test system.
[c] Calculated according to Laird et al. (17); 1.5% bases mispaired per degree C ΔTme.

and *Diplococcus pneumoniae* showed 6 to 7% relatedness to *L. monocytogenes* 19303, but many of the duplexes formed were thermally unstable. The two strains of *Erysipelothrix rhusiopathiae* bound 10% of the labeled reference DNA with 3 to 6% of the bases imperfectly matched. *Staphylococcus aureus* ATCC 6538P and the two strains of *Streptococcus* had 15 to 16% of their nucleotide sequences in common with that of *L. monocytogenes* 19303. The strains of *Brevibacterium linens*, *Corynebacterium diphtheriae*, and *Microbacterium lacticum* showed slightly higher binding values, but with correspondingly greater mismatching. *Proteus mirabilis* MCV, phenotypically unrelated to *Listeria* but similar in DNA base composition (38% GC) (28), was included as a negative control. DNA from the *Proteus* strain bound only 3% of the *L. monocytogenes* 19303 DNA, with 15% of the nucleotide pairs imperfectly matched.

DISCUSSION

In accord with the results of Sneath and Cowan (33) and Stuart and Pease (34), our survey indicated that phenotypically the listeriae most closely resemble the streptococci, *Staphylococcus aureus*, and *Diplococcus pneumoniae*. The metabolic processes of bacteria constitute a classic taxonomic criterion, and it is particularly interesting to note the similarity in fermentation patterns of the strains of *Listeria monocytogenes*, *L. grayi*, and *L. murrayi* with those of the two strains of *Streptococcus*. Further, the alcohols and volatile and nonvolatile acids detected after growth of these three species of *Listeria* on glucose-containing media most closely parallel the production of these substances by the lactic acid bacteria (data not shown). The similarity of listeriae to the two strains of *Bacillus* probably reflects bias introduced into the phenotypic testing procedure by failure to include such characteristics as production of spores, cell wall composition, size of individual cells, pathogenesis, etc. Although some overall phenotypic similarity between *Listeria monocytogenes* and *Erysipelothrix* was observed, the similarity was not nearly as striking as that observed between *L. monocytogenes* and the *Lactobacillaceae*.

DNA nucleotide composition may be considered a cardinal taxonomic criterion (22, 24). DNA base composition is also indicative of phylogenetic relationships since the base composition changes only to a very limited degree with mutation. Differences in nucleotide composition are generally indicative of genomic differences; however, similarity in DNA base composition is not always a measure of genetic relatedness. Some investigators have suggested that a difference in GC of 20 to 30% precludes the possibility of nucleotide sequences in common between two genomes (22, 24). A difference in %GC of 5 denotes "at least" a species difference (24). As previously reported (35), *L. monocytogenes*, *L. grayi*, and *L. murrayi* form a homogenous group on the basis of DNA base composition. DNA from *L. denitrificans*, however, was 16% higher in %GC than that from the other listeriae. DNA from

the strains of *Bacillus cereus, Diplococcus pneumoniae, Streptococcus faecalis, Streptococcus lactis, Staphylococcus aureus,* and *Erysipelothrix rhusiopathiae* was within 5 and 38% GC, strengthening their possible relationship to *L. monocytogenes.*

Molecular homology between macromolecules from different organisms has become a potent method for assessing relationships in bacterial taxonomy. Denaturation of DNA samples and their subsequent annealing provides a method of directly studying base-sequence homology. Although correlations between numerical analysis of phenotypic characteristics and polynucleotide sequence relatedness are generally reasonable for both closely and distantly related organisms, DNA binding can be a more sensitive indicator of genetic similarity and divergence (21, 22). Our hybridization studies with listeriae suggest that *L. murrayi* and *L. grayi,* the two avirulent species, are quite closely related and comprise a genetic group distinct from *L. monocytogenes. L. murrayi* and *L. grayi* shared 60 to 70% of their polynucleotide sequences but bound only 20 to 40% of the labeled DNA from *L. monocytogenes* 19303 (35). Although there is no demarcation in the classical taxonomic sense, *L. monocytogenes* strains fall into two groups: those closely related to the reference strain 19303 and those which showed only 40 to 70% binding to 19303 (see Table 5). Genetic divergence within *L. monocytogenes* was significant. In some strains identified as *L. monocytogenes,* as much as 60% of the DNA has diverged to a point where it no longer associated with the reference DNA under exacting conditions. There is no apparent correlation between the source, serotype, or virulence of these strains and their molecular relatedness.

Hybridization studies with *Enterobacteriaceae* have shown high degrees of polynucleotide sequence relatedness within each genus (3-6). Although direct comparison of numerical values obtained in two different hybridization systems is risky, interpretation of assessed relationships is possible. The hybridization results within *Enterobacteriaceae* lend credence to the conclusion that *L. grayi* and *L. murrayi* are indeed a genetic group distinct from *L. monocytogenes* and, further, that strains presently identified as *L. monocytogenes* may actually comprise two separate genetic groups despite extensive phenotypic similarities. Association values for DNA from the sole strain of *L. denitrificans* with any of the other listeriae were quite low. The low molecular relatedness, differences in taxonomically important biochemical characters, and wide discrepancy in nucleotide composition of DNA from *L. denitrificans* and other listeriae, as previously noted (35), provide the basis for the exclusion of this organism from the genus.

Examination of the intergeneric relatedness of the listeriae, specifically *L. monocytogenes* 19303, indicated that the listeriae are not significantly related to *Corynebacteriaceae, Micrococcaceae* or *Lactobacillaceae.* These hybridization studies indicate that the current inclusion of the listeriae in the family *Corynebacteriaceae* is not tenable on the basis of cardinal phenotypic characters, nor can any significant relatedness be demonstrated by molecular methods. Despite biochemical similarities, extensive polynucleotide sequence homology was not observed between the listeriae and representatives of the family *Lactobacillaceae.*

On the basis of a phenotypic analysis according to Adansonian principles and of extensive intrageneric and intergeneric hybridization studies under both exacting and permissive conditions, a taxonomic reordering of the genus *Listeria* is indicated. We propose that the systematic position of the listeriae be modified as follows.

Family Listeriaceae fam. nov. (Lis.te.ri.a' ce.ae. M.L. fem.n. *Listeria* type genus of the family; *-aceae* ending to denote a family; M.L. fem.pl.n. *Listeriaceae* the *Listeria* family.)

Small (0.4 to 0.8 by 0.5 to 2.5 μm), evenly staining, gram-positive rods with rounded ends ranging from coccoid to elongate rods in young cultures and filamentous forms in the rough state; clubbed cells do not occur. Cells from 18- to 24-h-old colonies occur singly and in pairs or short chains. Capsules and endospores are not produced. Not acid-fast. When grown at 20 to 25 C, cells are motile and peritrichous; few, if any, flagella or motile cells occur when grown at 37 C. Growth occurs between 4 and 38 C, especially in the presence of a small amount of glucose. Acid but no gas produced from glucose and several other carbohydrates. Esculin is hydrolyzed. Usually catalase-positive. Antigenic types are determined by H and O antigens. Isolated from the feces of man and other animals and from soil and vegetation. Some species and strains produce infections in man and other animals varying in severity from mild to fatal and may be isolated from the clinical specimens.

Type genus: *Listeria* Pirie.

Genus I. Listeria Pirie 1940.

Type species: *Listeria monocytogenes* (Mur-

ray et al. 1926) Pirie 1940. Type strain (25): ATCC 15313.

Genus II. Murraya gen. nov. (Mur'ray.a. M.L. fem.n. *Murraya* named for E. G. D. Murray, the co-discoverer of *Listeria monocytogenes.*)

Small (0.4 to 0.8 by 0.5 to 2.5 μm), gram-positive, straight rods with rounded ends; occur singly, in pairs or short chains, and occasionally in a palisade arrangement. Actively motile at 20 to 25 C; cells are peritrichous. Motile cells are rare or absent at 37 C. Colonies on sheep-blood agar are white with no hemolysis, but a slight darkening of the medium surrounding the colony may occur. When grown on tryptose agar at 20 to 25 C, some species, after 48 to 72 h, develop a yellow pigmentation of the colonies; the pigmentation becomes more pronounced with continued holding. Catalase-positive. Mannitol is acidified. Nitrates may or may not be reduced to nitrites. Conjunctivitis is not produced when cells are instilled into the eyes of rabbits. May be toxic to some strains of white mice when administered in large doses intraperitoneally, but essentially apathogenic. Isolated from the feces of chinchillas and from soil and vegetation.

Type species: *Murraya grayi* (Errebo-Larsen and Seeliger) comb. nov. (Syn: *Listeria grayi* Errebo-Larsen and Seeliger 1966.) Type strain (18): ATCC 19120.

Subspecies. (i) **Murraya grayi** subsp. **grayi** (Errebo-Larsen and Seeliger 1966) Stuart and Welshimer 1974. (Syn: *Listeria grayi* Errebo-Larsen and Seeliger 1966.) Type strain (18): ATCC 19120.

(ii) **Murraya grayi** subsp. **murrayi** (Welshimer and Meredith 1971) comb. nov. (Syn: *Listeria murrayi* Welshimer and Meredith 1971.) Type strain: (37): ATCC 25401.

No reorganization of the organisms now designated as *L. monocytogenes* is proposed despite hybridization evidence which suggests that perhaps these organisms represent two genetic groups. A more thorough examination of this particular situation is indicated.

L. denitrificans is not included in the family *Listeriaceae.* This study did not include experiments designed to assess the relatedness of *L. denitrificans* to other species. Based on taxonomically important biochemical reactions and DNA base composition, it is possible that *L. denitrificans* is related to the *Corynebacteriaceae,* particularly the genus *Corynebacterium.*

ACKNOWLEDGMENTS

This investigation was supported by A. D. Williams Institutional Research Support Funds, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University. One of us (S. E. S.) was supported by National Defense Education Administration Title IV Fellowship 437-003725.

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