Intragenetic Relatedness of *Listeria* Pirie

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Deoxyribonucleic acid (DNA) base composition, differential biochemical properties, and DNA:DNA hybridization patterns of listeriae were studied. DNA from *Listeria monocytogenes*, *L. grayi*, and *L. murrayi* contained 38 to 40 mole percent guanine plus cytosine (% GC); however, DNA from *L. denitrificans* contained 56% GC. Hybridization studies indicated that *L. murrayi* and *L. grayi* were closely related and formed a homogeneous genetic group distinct from *L. monocytogenes*. *L. monocytogenes* strains could be separated into two groups on the basis of percent reassociation values with *L. monocytogenes* 19303, the reference strain. One group of six strains showed 80 to 100% relative reassociation with strain 19303; four strains showed only 40 to 70% relative reassociation. There was no correlation between virulence, origin, or serotype and the degree of molecular relatedness to the reference strain. *L. denitrificans* was distinct from the other three species of *Listeria* in biochemical properties and DNA composition, and the relative reassociation values for DNA from *L. denitrificans* with DNA from other listeriae were very low. It is suggested that consideration be given to the reclassification of *L. denitrificans*.

Listeria monocytogenes (Murray et al.) Pirie was first described in 1926 (11) and until relatively recently was the only recognized species of the genus *Listeria* Pirie. Since 1961, three species, *L. denitrificans* Prévot (12), *L. grayi* Larsen and Seeliger (8), and *L. murrayi* Welshimer and Meredith (15), which to date appear apathogenic, have been added to the genus. Most studies have been related to the role of *L. monocytogenes* in infections, and although the isolation, identification, and immunological properties have been investigated, little comparative information concerning the physiology and metabolic characteristics of *Listeria* is available. The four species of *Listeria* bear a superficial, morphological resemblance to the *Corynebacteriaceae*, the family to which *L. monocytogenes* was assigned in the seventh edition of *Bergey's Manual of Determinative Bacteriology* (1). The inclusion of *Listeria monocytogenes* in the family *Corynebacteriaceae* warrants reexamination in view of numerical taxonomy studies based on Adansonian principles (2, 5, 6). One species, *L. denitrificans*, is quite different from the other listeriae (12).

Our studies have been directed toward defining the intra- and intergeneric relatedness of strains which by present taxonomic criteria are classified as belonging to *Listeria*. As a

prelude to examination of the taxonomic placement of the genus, a comparison of the four species of *Listeria* with respect to differential biochemical properties, deoxyribonucleic acid (DNA) composition and molecular relatedness as assessed by DNA:DNA duplex formation on nitrocellulose membrane filters was made.

MATERIALS AND METHODS

Bacterial strains, media, and biochemical tests. The bacterial strains used in this study are listed in Table 1. All listeriae were maintained on slants of Tryptose blood agar base (Difco) to which 1% glucose had been added. Tryptose-phosphate broth (TD, Tryptose [Difco], 20 g; NaCl, 10 g; glucose, 2 g; Na₂HPO₄, 3.5 g; distilled water, 1 liter) was used to cultivate organisms for DNA extraction and for isotopic labeling. *Escherichia coli* CSH-2 was maintained on nutrient agar slants and grown in volume in tryptic soy broth (Difco).

The methods for biochemical characterization followed standard bacteriological techniques as presented in the *Manual of Microbiological Methods* (14).

DNA isolation. The method of Marmur (9) was used for all DNA extractions except strains of *Listeria monocytogenes*. Cells from *L. monocytogenes* strains were killed by heating at 70 °C for 10 min before centrifugation and then were suspended in 25 ml of 0.005 M CaCl₂ containing 100 µg of lipase (type 448,

TABLE 1. *Bacterial strains*^a

Strain	Origin	Source
<i>Listeria monocytogenes</i> 19303	Human	Ft. Detrick, Md.
<i>L. monocytogenes</i> A4413	Human	Ft. Detrick, Md.
<i>L. monocytogenes</i> JC	Human	HJW
<i>L. monocytogenes</i> HBr	Human	HJW
<i>L. monocytogenes</i> HD	Human	HJW
<i>L. monocytogenes</i> T ₃ b	Human	CDC (KC224)
<i>L. monocytogenes</i> T ₄ b	Human	CDC (KC226)
<i>L. monocytogenes</i> V-7	Vegetation	HJW
<i>L. monocytogenes</i> V-8	Vegetation	HJW
<i>L. monocytogenes</i> V-11	Vegetation	HJW
<i>L. monocytogenes</i> V-12	Vegetation	HJW
<i>L. grayi</i> ATCC 19120	Chinchilla feces	ATCC
<i>L. grayi</i> V-1	Vegetation	HJW
<i>L. murrayi</i> F-2	Vegetation	HJW
<i>L. murrayi</i> F-6 (ATCC 25402)	Vegetation	HJW
<i>L. murrayi</i> F-9 (ATCC 25401)	Vegetation	HJW
<i>L. murrayi</i> F-11	Vegetation	HJW
<i>L. murrayi</i> F-12 (ATCC 25403)	Vegetation	HJW
<i>L. denitrificans</i> ATCC 14870	Ox blood	ATCC
<i>Escherichia coli</i> CSH-2		MCV

^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.

Nutritional Biochemical Co.) per ml. The mixture was incubated with shaking for 1 h at 37 C, centrifuged, and suspended in saline-EDTA (0.15 M sodium chloride, 0.1 M disodium ethylenediaminetetraacetate) with 800 µg of lysozyme (A grade, Calbiochem) per ml. After incubation for 1 h at 37 C, 100 µg of Pronase (B grade, Calbiochem) per ml was added, and incubation continued for 1 to 3 h. The remainder of the purification technique followed the procedure outlined by Marmur. DNA samples were stored in 1 × SSC (0.15 M sodium chloride, 0.015 M trisodium citrate) over chloroform at 4 C.

T_m determination. The thermal melting point (T_m) of DNA was determined in 0.1 × SSC using a Gilford recording spectrophotometer (model 2400, Gilford Instrument Laboratories, Inc.) as described by Marmur and Doty (10). Mole percent guanine plus cytosine (% GC) was calculated by using the following equation of Marmur and Doty (10): $GC_x = GC_{known} + 1/0.41 (T_{m_x} - T_{m_{known}})$. DNA from *E. coli* CSH-2 was used as the internal reference in each T_m determination.

Buoyant density determination. The buoyant density of the DNA was determined by isopycnic centrifugation in CsCl at 25 C in a Spinco model E analytical ultracentrifuge as described by Schildkraut et al. (13). Densitometer tracings of the ultraviolet films were made on a model 2400 Gilford recording spectrophotometer. The % GC was calculated from the buoyant density according to DeLey (3): % GC = $1038.47 (\rho - 1.6616)$. *E. coli* CSH-2 was used as standard reference DNA. The buoyant density of the reference DNA was taken to be 1.710 g/cm³ (13).

Preparation of radioactive DNA. Radioactive DNA was produced in *L. monocytogenes* 19303 and *L.*

grayi ATCC 19120 by incubation with [8-¹⁴C] adenine as follows. A 0.1-ml amount of a 24-h TD broth culture was added to 500 ml of TD broth and incubated without shaking for 17 h at room temperature (23 C). Fresh medium (100 ml) containing 200 µCi of [8-¹⁴C] adenine (56 mCi/mmol; 100 µCi/ml, Schwarz-Mann) was added, and stationary incubation continued for 3 h. The cells were harvested by centrifugation and washed with saline-EDTA. DNA samples isolated from *L. grayi* ATCC 19120 and *L. monocytogenes* 19303 had specific activities ranging from 4,000 to 7,000 counts per min per µg of DNA.

Radioactive DNA from *L. murrayi* F-9 was obtained by incubation of log-phase cells grown in TD broth containing 100 µCi of [8-¹⁴C] adenine (50 mCi/mmol; 100 µCi/ml, Schwarz-Mann) and 2 × 10⁻⁴ M deoxyguanosine. Label and deoxyguanosine were added simultaneously to a 12-h shake culture, and incubation was continued at room temperature with shaking for 8 h. The cells were harvested by centrifugation and washed in saline-EDTA. DNA isolated from *L. murrayi* F-9 had a specific activity of 3,600 counts per min per µg of DNA.

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

Immobilization of DNA on filters. Approximately 50 µg of unlabeled DNA dissolved in 3 ml of 0.1 × SSC were denatured by holding in a boiling-water bath for 10 min. After immediate cooling in ice, an equal volume of 12 × SSC was added to give a final concentration of 6 × SSC. A modification of the

general procedure of Gillespie and Spiegelman (4) was used for immobilization of single-stranded DNA on nitrocellulose membrane filters (type B-6, 25 mm, Schleicher and Schuell Co.). Immobilized DNA was prepared by gravity filtration of the denatured DNA in $6 \times \text{SSC}$. The amount of DNA not retained by the membrane was calculated from the A_{260} of the filtrate. The filters were allowed to air-dry overnight and then were heated for 8 h at 80 C. The loaded filters were stored in screw-capped scintillation vials at room temperature.

Hybridization and thermal elution. Filters loaded with 40 to 55 μg of unlabeled, single-stranded DNA were placed individually in scintillation vials containing 1.5 ml of $1.25 \times \text{SSC}$ plus 0.02 M tris (hydroxymethyl)aminomethane-hydrochloride, pH 8, and 1 μg of sheared, denatured labeled DNA. Vials were incubated at 65 C for 20 h without shaking. After incubation the filters were removed and washed in 10 ml of $0.1 \times \text{SSC}$ for 10 min at 55 C. Thermal elution profiles were obtained by washing the filters in 2.5 ml of $0.1 \times \text{SSC}$ for 15 min at 5 C temperature increments from 60 to 85 C. Each aqueous sample and the filter were placed in 15 ml of Triton X-100-toluene base scintillation fluid (666 ml of toluene, 333 ml of Triton X-100, 5.5 g of 2,5-diphenyl-oxazol, and 0.125 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene] and counted in a Packard Tri-Carb liquid scintillation spectrometer (model 3320, Packard Instrument Co.).

Thermal stability of hybrid duplexes was determined from thermal elution profiles. The mid-point of the thermal elution profile (T_{me}) is the temperature at which half of the reference DNA reassociated with test DNA bound to the filter has become dissociated and eluted into the wash solution. In each case, the T_{me} of the reference system matched the T_m of native DNA from that organism. Percent base mispairing was calculated according to Laird et al. (7) from the change in T_{me} 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 labeled reference DNA.

RESULTS

Biochemical differentiation of listeriae. The biochemical reactions which serve to distinguish between the four species of *Listeria* are given in Table 2.

Determination of base composition of DNA. The % GC of DNA extracted from each species of *Listeria* calculated from both T_m and buoyant density determinations are given in Table 3. Although the values for % GC calculated from buoyant density (38-42) were somewhat higher than those calculated from T_m (37-39), *L. murrayi*, *L. grayi*, and *L.*

monocytogenes form a homogeneous group on the basis of DNA composition. DNA from *L. denitrificans* had a notably different composition (56-57% GC).

DNA:DNA hybridizations. The results of DNA:DNA hybridizations using labeled *L. monocytogenes* 19303 DNA as reference are shown in Tables 4 and 5. *L. monocytogenes* strains HD, HBr, JC, and A4413 showed a high degree of relatedness to *L. monocytogenes* 19303 (Table 4). These strains are all virulent isolants from human cases of listeriosis. Both *L. monocytogenes* T₃b and T₄b had fewer nucleotide sequences in common with strain 19303, and the duplexes formed were somewhat less stable. The *L. monocytogenes* strains designated V were isolated from vegetation in Hanover county, Va. *L. monocytogenes* V-7, a virulent isolant, appears to be identical to the reference strain. Strain V-12, also very closely related to 19303, is an avirulent isolant. The other two isolants, V-8 and V-11, had fewer nucleotide sequences in common with 19303; both strains are avirulent.

The molecular relatedness of the other species of *Listeria* to *L. monocytogenes* 19303 is shown in Table 5. *L. grayi* and *L. murrayi* were considerably less related to *L. monocytogenes* 19303 than were isolants identified as *L. monocytogenes*. *L. denitrificans* shared only 20% of its nucleotide sequences with *L. monocytogenes* 19303.

Table 6 shows the relative reassociation of labeled DNA from *L. murrayi* F-9, the type strain (15), with DNA from other listeriae. All strains of *L. murrayi* were closely related. Strains F-6 and F-11 gave consistently high percent reassociation values with *L. murrayi* F-9 with near perfect duplex formation; *L. murrayi* F-2 and F-12 exhibited fewer nucleotide sequences in common with the type strain. Strains F-2 and F-12 are distinguishable from other isolants identified as *L. murrayi* by their inability to produce acid from rhamnose. Hybrid duplexes formed between *L. murrayi* F-9 and *L. grayi* ATCC 19120 indicated 71% of the nucleotide sequences were shared by both species, although the duplexes were slightly less stable than those formed by the homologous system. V-1, to our knowledge the only other isolant of *L. grayi*, gave somewhat lower numerical value, but the duplexes were perfectly matched. The *L. monocytogenes* strains exhibited relative reassociation values with *L. murrayi* which ranged from 9 to 30%, and the decreased thermal stabilities indicated considerable base mispairing. *L. denitrificans* was least related to *L. murrayi* F-9.

TABLE 2. *Differential properties of Listeria species*^a

Species	Biochemical tests					
	Voges-Proskauer	Nitrate reduction	Acid from mannitol	Acid from arabinose	Acid from xylose	Metachromatic granules
<i>Listeria monocytogenes</i>	+	—	—	—	—	—
<i>L. grayi</i>	+	—	+	—	—	—
<i>L. denitrificans</i>	—	+	—	+	+	+
<i>L. murrayi</i>	+	+	+	—	—	—

^a All listeriae are catalase positive, methyl-red positive, and produce acid from glucose, salicin, aesculin, and trehalose; these reactions serve to differentiate listeriae from other *Corynebacteriaceae*.

TABLE 3. *DNA composition of listeriae*

Strain	% GC	
	From <i>T_m</i> ^a	From buoyant density ^a
<i>Listeria monocytogenes</i> 19303	38	38
<i>L. monocytogenes</i> HD	37	39
<i>L. monocytogenes</i> V-11	37	38
<i>L. grayi</i> ATCC 19120	39	41
<i>L. grayi</i> V-1	39	41
<i>L. murrayi</i> F-6	38	42
<i>L. murrayi</i> F-9	38	42
<i>L. murrayi</i> F-12	39	42
<i>L. denitrificans</i> ATCC 14870	56	57

^a The % GC values given are averages of at least three determinations using different DNA extractions.

Table 7 shows relative reassociation values for labeled DNA from *L. grayi* ATCC 19120, the type strain (8), with DNA from other listeriae. As expected, *L. grayi* V-1 was most closely related to the type strain. *L. murrayi* strains showed relatively high reassociation values with only slight base mispairing. *L. monocytogenes* strains were much less related, and the hybrid duplexes formed were, in general, less well matched. Again, *L. denitrificans* was the least related to the reference strain.

Results of reciprocal binding reactions between *L. monocytogenes* 19303 and *L. grayi* ATCC 19120, *L. monocytogenes* 19303 and *L. murrayi* F-9, and *L. grayi* ATCC 19120 and *L. murrayi* F-9 were nearly identical in thermal stability; however, nonreciprocal binding appeared to occur in reactions between *L. murrayi* F-9 and *L. monocytogenes* 19303 and *L. grayi* ATCC 19120 and *L. monocytogenes* 19303. *L. grayi* ATCC 19120 showed 9% less

binding to *L. monocytogenes* 19303 than the reciprocal reaction, and *L. monocytogenes* 19303 showed 5% less reassociation with *L. murrayi* F-9 than the reciprocal reaction. *L. grayi* ATCC 19120 bound only 2% less to *L. murrayi* than the reciprocal case. These apparent differences may reflect inherent differences in purity of certain of the DNA preparations used for hybridization, unknown differences in experimental method, or a difference in genome size of these organisms. Genome size determinations have not yet been made.

DISCUSSION

With the exception of *L. denitrificans*, listeriae form a homogeneous group with respect to composition of DNA. *L. denitrificans* also differs from the other listeriae in numerous taxonomically important reactions. It is the only species of *Listeria* which produces acid from xylose and arabinose and does not produce acetylmethylcarbinol. Furthermore, *L. denitrificans* is the sole member of the genus to produce metachromatic granules, accumulate fat droplets, and give a positive benzidine test.

Based on hybridization studies, *L. monocytogenes* strains may be separated into two easily distinguishable groups (Table 8). *L. monocytogenes* strains HD, HBr, JC, A4413, V-7, and V-12 that comprise the first group are all closely related to *Listeria monocytogenes* 19303, although they represent both virulent and avirulent strains of diverse origin and serotype. These strains form a compact group which exhibits little relatedness to either *L. murrayi* or *L. grayi*. *L. monocytogenes* strains in the second group, T₃b, T₄b, V-8, and V-11, exhibited lower reassociation values with *L. monocytogenes* 19303, ranging from 40 to 70%. The degree of relatedness of these strains

TABLE 4. Molecular relatedness of *Listeria monocytogenes* strains

Source of unlabeled DNA	Relative reassociation with <i>L. monocytogenes</i> 19303 (%)	ΔT_{me}^a	Base mispairing ^b (%)
<i>Listeria monocytogenes</i> 19303	100	0	0
<i>L. monocytogenes</i> HD	90	0	0
<i>L. monocytogenes</i> HBr	90	0	0
<i>L. monocytogenes</i> JC	85	2	3
<i>L. monocytogenes</i> A4413	82	0	0
<i>L. monocytogenes</i> T ₃ b	40	2	3
<i>L. monocytogenes</i> T ₄ b	69	2	3
<i>L. monocytogenes</i> V-7	100	1	1.5
<i>L. monocytogenes</i> V-8	60	0	0
<i>L. monocytogenes</i> V-11	59	0	0
<i>L. monocytogenes</i> V-12	97	2	3

^a Difference in degrees C between the T_{me} of the reference system and the T_{me} of the test system.

^b Calculated according to Laird (7); 1.5% bases mispaired per degree C ΔT_{me} .

TABLE 5. Molecular relatedness of *Listeria monocytogenes* 19303 to other listeriae

Source of unlabeled DNA	Relative reassociation with <i>L. monocytogenes</i> 19303 (%)	ΔT_{me}^a	Base mispairing ^b (%)
<i>Listeria monocytogenes</i> 19303	100	0	0
<i>L. grayi</i> ATCC 19120	21	2	3
<i>L. grayi</i> V-1	40	1	1.5
<i>L. murrayi</i> F-2	44	2	3
<i>L. murrayi</i> F-6	25	2	3
<i>L. murrayi</i> F-9	20	3	4.5
<i>L. murrayi</i> F-11	33	3	4.5
<i>L. murrayi</i> F-12	36	2	3
<i>L. denitrificans</i> ATCC 14870	20	2	3

^a See Table 4.

^b See Table 4.

TABLE 6. Relative reassociation of *Listeria murrayi* F-9 DNA with DNA from other listeriae

Source of unlabeled DNA	Relative binding with <i>L. murrayi</i> F-9 (%)	ΔT_{me}^a	Base mispairing ^b (%)
<i>Listeria murrayi</i> F-9	100	0	0
<i>L. murrayi</i> F-2	82	2	3
<i>L. murrayi</i> F-6	99	0	0
<i>L. murrayi</i> F-11	96	1	1.5
<i>L. murrayi</i> F-12	71	0	0
<i>L. grayi</i> ATCC 19120	71	0	0
<i>L. grayi</i> V-1	61	0	0
<i>L. monocytogenes</i> 19303	25	3	4.5
<i>L. monocytogenes</i> JC	16	2	3
<i>L. monocytogenes</i> HD	9	6	9
<i>L. monocytogenes</i> HBr	11	3	4.5
<i>L. monocytogenes</i> A4413	14	2	3
<i>L. monocytogenes</i> T ₃ b	12	3	4.5
<i>L. monocytogenes</i> T ₄ b	17	2	3
<i>L. monocytogenes</i> V-7	14	3	4.5
<i>L. monocytogenes</i> V-8	30	3	4.5
<i>L. monocytogenes</i> V-11	12	2	3
<i>L. monocytogenes</i> V-12	15	2	3
<i>L. denitrificans</i> ATCC 14870	8	1	1.5

^a See Table 4.

^b See Table 4.

TABLE 7. *Relative reassociation of Listeria grayi* ATCC 19120 DNA with DNA from other listeriae

Source of unlabeled DNA	Relative binding to <i>L. grayi</i> ATCC 19120 (%)	ΔT_{me}^a	Base mispairing ^b (%)
<i>Listeria grayi</i> ATCC 19120	100	0	0
<i>L. grayi</i> V-1	84	2	3
<i>L. murrayi</i> F-2	58	1.4	2.1
<i>L. murrayi</i> F-6	68	1	1.5
<i>L. murrayi</i> F-99	69	2.5	3.7
<i>L. murrayi</i> F-11	76	6	8.5
<i>L. murrayi</i> F-12	75	1	1.5
<i>L. monocytogenes</i> 19303	12	0.4	0.6
<i>L. monocytogenes</i> T ₃ b	10	2	3
<i>L. monocytogenes</i> T ₄ b	12	0.4	0.6
<i>L. monocytogenes</i> HD	13	3	4.5
<i>L. monocytogenes</i> HBr	13	3	4.5
<i>L. monocytogenes</i> A4413	18	7	10.0
<i>L. monocytogenes</i> JC	13	3	4.5
<i>L. monocytogenes</i> V-7	10	2	3
<i>L. monocytogenes</i> V-8	7	2	3
<i>L. monocytogenes</i> V-11	10	4	6
<i>L. monocytogenes</i> V-12	9	0	0
<i>L. denitrificans</i> ATCC 14870	5	4	6

^a See Table 4.^b See Table 4.TABLE 8. *Intragenetic relatedness of listeriae*

Source of unlabeled DNA	Relative binding to <i>L. monocytogenes</i> 19303 (%)	Relative binding to <i>L. murrayi</i> F-9 (%)	Relative binding to <i>L. grayi</i> ATCC 19120 (%)
<i>Listeria monocytogenes</i> group I (19303, HD, HBr, JC, A4413, V-7, V-12)	80-100	10-25	9-18
<i>L. monocytogenes</i> group II (V-11, V-8, T ₃ b, T ₄ b)	40-70	12-30	10-15
<i>L. grayi</i> (ATCC 19120, V-1)	21,40	71,61	100,84
<i>L. murrayi</i> (F-2, F-6, F-9, F-11, F-12)	20-44	71-100	58-76
<i>L. denitrificans</i> ATCC 14870	20	8	5

to *L. murrayi* and *L. grayi*, however, is similar to that of the first group.

The two strains of *L. grayi* showed relatively high and comparable degrees of relatedness to *L. murrayi* F-9 but far fewer nucleotide sequences in common with *L. monocytogenes*. *L. grayi* is distinguished from *L. murrayi* only by its ability to reduce nitrate (Table 2). The ability to produce acid from mannitol is shared by both of these species and differentiates them from *L. monocytogenes*. Both *L. grayi* and *L. murrayi* lack H factors in common with *L. monocytogenes* (8, 15). Although the serotypes of *L. grayi* and *L. murrayi* have not yet been fully elucidated, some flagellar antigens are shared (15).

The *L. murrayi* strains showed 20 to 40% relative reassociation with *L. monocytogenes* 19303 and 71 to 100% relative reassociation with *L. murrayi* F-9. The strains more closely related to *L. monocytogenes* had fewer nucleotide sequences in common with *L. murrayi*.

Reassociation values for DNA from the sole strain of *Listeria denitrificans* studied with any of the other species of *Listeria* were quite low.

These studies indicate that the two avirulent species, *L. murrayi* and *L. grayi*, are quite closely related. Further, our hybridization results suggest that *L. murrayi* and *L. grayi* comprise a homogeneous genetic group distinct from *L. monocytogenes*. Although there is no notable demarkation in the classical taxonomic

sense, *L. monocytogenes* strains fall into two distinct groups: those closely related to the reference strain 19303 and those which showed only 40 to 70% relative reassociation. In some *L. monocytogenes* strains, as much as 60% of the DNA has diverged to a point where it no longer reassociates with the reference strain under stringent conditions. There is no apparent correlation between the source of isolation, serotype, or virulence of these strains and their molecular relatedness.

On the basis of differences in taxonomically important biochemical reactions, growth characteristics, DNA base composition, and low molecular relatedness to any of the other species of *Listeria*, strong consideration should be given to reclassification of *L. denitrificans*.

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