NOTES

Listeria welshimeri sp. nov. and *Listeria seeligeri* sp. nov.

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The names *Listeria welshimeri* sp. nov. and *Listeria seeligeri* sp. nov. are proposed for two groups of gram-positive, asporogenous, motile, aerobic bacilli that were previously classified as nonpathogenic *Listeria monocytogenes*. The separation of both of these new species from *L. monocytogenes* is supported by the results of deoxyribonucleic acid relatedness studies, determinations of biochemical characteristics, and studies of pathogenicity for adult mice. The new species differ from each other and from the recently proposed species *Listeria innocua* by deoxyribonucleic acid relatedness and biochemical features. The type strain of *L. welshimeri* is strain CIP 8149, and the type strain of *L. seeligeri* is strain CIP 100100.

Deoxyribonucleic acid (DNA) relatedness studies have shown the heterogeneity of *Listeria monocytogenes* sensu lato (7, 13). A recent study has demonstrated five genomic groups among a collection of 66 strains labeled *L. monocytogenes* (7) in the Special Listeria Culture Collection (SLCC) of the Institute of Hygiene and Microbiology, University of Würzburg, Würzburg, Federal Republic of Germany. Genomic group 1 contains the type strain of *L. monocytogenes* and 29 strains of serovars 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and "7" (a provisional serovar). Thus, genomic group 1 corresponds to *L. monocytogenes* sensu stricto. Genomic group 2 contains nine strains of serovar 5 and corresponds to "*Listeria bulgarica*" (4). The name "*L. bulgarica*" does not appear on the Approved Lists of Bacterial Names (12) and has not been validated by announcement or publication; thus, at present "*L. bulgarica*" has no nomenclatural standing. Genomic group 3 contains the type strain of *Listeria innocua* (10) and 10 strains of serovars 6a and 6b and undesignated serovars.

Genomic group 4 contains 6 strains of serovars 6a and 6b, and genomic group 5 contains 10 strains of serovars 1/2b, 4c, 4d, and 6b and undesignated serovars. In this paper, genomic groups 4 and 5 are named *Listeria welshimeri* and *Listeria seeligeri*, respectively. A type strain is designated and a description is provided for each of these two new species.

Genomic groups 4 and 5 represent two new *Listeria* species. The levels of DNA relatedness (as determined by the S1 nuclease method) within the genus *Listeria* are summarized in

Table 1 (7). Strain SLCC 5334^T (T = type strain) (genomic group 4) and strain SLCC 3990 (genomic group 5) were 42 and 24% related, respectively, to strain SLCC 53 (= ATCC 15313), the type strain of the type species of the genus *Listeria*, *L. monocytogenes* (7). The levels of relatedness among the five genomic groups were 18 to 58%, and the differences between the thermal denaturation midpoints of the homoduplexes and the thermal denaturation midpoints of the heteroduplexes (ΔT_m values) were more than 7.1°C. The levels of DNA relatedness between genomic groups 4 and 5 and *Listeria grayi* and *Listeria murrayi* were very low (less than 11%). ΔT_m values of more than 7°C are generally taken to indicate that strains do not belong to the same species (1). Thus, genomic groups 1 through 5 should be considered different species (genomic species).

Biochemical tests can differentiate genomic species 4 and 5 from other *Listeria* genomic species (Table 2). This differentiation rests on acid production from D-xylose, L-rhamnose, and α -methyl-D-mannoside and the results of two CAMP tests, one using *Staphylococcus aureus* and the other using *Rhodococcus equi* (*Corynebacterium equi*). The CAMP phenomenon is an enhancement of hemolysis (by the tested bacterium) in the vicinity of beta-hemolytic *S. aureus* or *R. equi*. (C, A, M, and P are the initials of the authors who first described this phenomenon [2]). The detailed biochemical procedures used for the characterization of *Listeria* species have been described previously (8). Genomic groups 3 through 5 are experimentally nonpathogenic for mice (6).

TABLE 1. DNA relatedness among *Listeria* genomic species^a

Genomic group	Species	Avg % relatedness (relative binding ratio) with reference DNA from strain:				
		<i>L. monocytogenes</i> SLCC 5329	" <i>L. bulgarica</i> " SLCC 2379	<i>L. innocua</i> SLCC 3379	<i>L. welshimeri</i> SLCC 5334	<i>L. seeligeri</i> SLCC 3990
1	<i>L. monocytogenes</i>	80 (4.1–6.1) ^b	39 (7.1–7.8)	50 (7.5–10.6)	43 (10.9–11.8)	34 (12–15.4)
2	" <i>L. bulgarica</i> "	30 (11.5–12.5)	100 (0–1.2)	32 (11.5–14.8)	30 (13–14.5)	28 (8.9–10)
3	<i>L. innocua</i>	52 (8.8–10.1)	38 (10.2–10.8)	93 (1.9–3.3)	45 (10.9–11.5)	26 (10.6–11.4)
4	<i>L. welshimeri</i>	43 (9.7–10.2)	31 (10–11.3)	43 (9.2–13.1)	92 (0.2)	24 (10–10.6)
5	<i>L. seeligeri</i>	33 (10.5–11.7)	42 (7.1–11.7)	30 (9.6–13.7)	32 (14–14.4)	83 (2.7–2.9)
	<i>L. grayi</i>	16	6	14	11	4
	<i>L. murrayi</i>	7	2	6	4	1

^a Data from reference 7.^b The numbers in parentheses are ΔT_m values.

In the last 10 years a number of apathogenic *L. monocytogenes* (*sensu lato*) strains have been isolated from the environment and from human and animal feces (5, 14, 15). Experimentally, these strains are devoid of pathogenicity for mice and often differ from pathogenic strains by antigenic characters (11), hemolytic properties (10), or production of acid from xylose (3). Some workers have expressed concern that the specific designation of these apathogenic *Listeria* strains as *L. monocytogenes* may be misleading and may result in erroneous interpretation of the role of *L. monocytogenes* in human and veterinary medicine and epidemiology (3, 9). Thus, in addition to the scientific reasons expressed above, there is a practical need for a subdivision of *L. monocytogenes sensu lato* into several homogeneous species.

The description of *L. innocua* (10) did not resolve all of the heterogeneity of *L. monocytogenes sensu lato*.

We propose the names *Listeria welshimeri* sp. nov. for genomic species 4 and *Listeria seeligeri* sp. nov. for genomic species 5.

***Listeria welshimeri* sp. nov. (i) Description.** *Listeria welshimeri* (wel.shi'mer.i, M. L. gen. n. *welshimeri* of Welshimer, honoring Herbert J. Welshimer, American bacteriologist) cells are small (0.4 to 0.5 by 0.5 to 2.0 μ m), asporogenous, gram-positive rods which are motile at 28°C by means of peritrichous flagella. Colonies on tryptose agar (Difco Laboratories) are small (1 to 2 mm in diameter after 1 or 2 days of incubation at 37°C), regular, and smooth with a blue-green color when they are examined with obliquely transmitted light. Growth occurs at 4°C within 5 days. Metabolism is facultatively anaerobic, catalase is produced, the oxidase test is negative, and nitrates are not reduced to nitrite. Acid, but no gas, is produced from D-glucose, D-xylose, and α -methyl-D-mannoside. Acid may or may not be produced from L-

TABLE 2. Differentiation of *Listeria* genomic species^a

Genomic group	Serovars	CAMP test with:		Acid from:			Virulence for mice (CFU/ml) ^b	G + C content (mol%) ^c
		<i>S. aureus</i>	<i>R. equi</i>	D-Xylose	L-Rhamnose	α -Methyl-D-mannoside		
1	1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, "7"	+ ^d	–	–	+	+	1×10^5 – 5×10^5	39 (38) ^e
2	5	–	+	+	–	–	1×10^6 – 3×10^6	38
3	6a, 6b, 4ab, Un ^f	–	–	–	D	+	$>10^8$	38
4	6a, 6b	–	–	+	D	+	$>10^8$	36
5	1/2b, 4c, 4d, 6b, Un	+	–	+	–	– ^g	$>10^8$	36

^a Data from reference 8 unless stated otherwise. +, All strains positive; –, all strains negative; D, different reactions observed.^b Data from reference 6. The data are expressed as 50% lethal doses (in colony-forming units [CFU] per milliliter).^c Determined by the spectrophotometric method (thermal denaturation), using *Escherichia coli* K-12 DNA with a guanine-plus-cytosine content of 50.6 mol% (unpublished data).^d Of 30 strains tested, 1 (strain ATCC 15313⁷) did not give a positive reaction.^e The value in parentheses is from reference 13.^f Un, Undesignated serovars.^g Of 10 strains tested, 1 gave a positive reaction.

FIG. 1. Electron micrograph of *L. welshimeri* SLCC 5334^T negatively stained with 2% (wt/vol) phosphotungstic acid. Cell size, 1.5 by 0.5 μm .

rhamnose. Acid is not produced from D-mannitol. 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, and CAMP tests with *S. aureus* CIP 5710 and *R. equi* CIP 5869 are negative. Gelatin is not hydro-

lyzed, and H_2S is not produced. The strains presently assigned to *L. welshimeri* belong to serovars 6a and 6b (these serovars also occur in *L. innocua*) and are nonpathogenic for holoxenic pathogen-free mice (50% lethal dose, more than 10^8 colony-forming units per ml). The guanine-plus-cytosine content of the DNA is 36 mol%.

FIG. 2. Electron micrograph of *L. seeligeri* SLCC 3954^T negatively stained with 2% (wt/vol) phosphotungstic acid. Cell size, 1.5 by 0.5 μm .

Isolated from decaying plants and soil in the United States. The type strain, strain SLCC 5334 (= CIP 8149 = Welshimer V8), was isolated in the United States by H. J. Welshimer from decaying vegetation.

(ii) **Description of the type strain of *L. welshimeri*.** The description of the type strain is the same as the description for the species. In addition, this strain does not produce acid from L-rhamnose and belongs to serovar 6b. Figure 1 shows an electron micrograph of strain SLCC 5334^T.

Listeria seeligeri sp. nov. (i) **Description.** *Listeria seeligeri* (see.li.ger'i. M. L. gen. n. *seeligeri* of Seeliger, honoring Heinz P. R. Seeliger, German bacteriologist) cells are small (0.4 to 0.8 by 0.5 to 2.5 μ m), asporogenous, gram-positive rods which are motile at 28°C by means of peritrichous flagella. Colonies on tryptose agar are small (1 to 2 mm diameter after 1 or 2 days of incubation at 37°C), regular, and smooth with a blue-green color when they are examined with obliquely transmitted light. Growth occurs at 4°C within 5 days. Facultatively anaerobic. Catalase is produced, the oxidase test is negative, and nitrates are not reduced to nitrite. Acid, but no gas, is produced from D-glucose and D-xylose. Acid is not produced from D-mannitol or L-rhamnose. Most strains do not produce acid from α -methyl-D-mannoside. The Voges-Proskauer and methyl red tests are positive. Esculin is hydrolyzed in 1 day. Urea is not hydrolyzed, and indole is not produced. The CAMP test is positive when *S. aureus* CIP 5710 is used and negative when *R. equi* CIP 5869 is used. Gelatin is not hydrolyzed, and H₂S is not produced. The strains presently assigned to *L. seeligeri* belong to serovars 1/2b, 4c, and 4d (these serovars also occur in *L. monocytogenes*), 6b (this serovar also occurs in *L. innocua* and *L. welshimeri*), and undesignated serovars. These strains are nonpathogenic for holoxenic pathogen-free mice (50% lethal dose, more than 10⁸ colony-forming units per ml). The guanine-plus-cytosine content of the DNA is 36 mol%. Isolated from plants, soil, and animal feces (sheep) in Europe. The type strain, strain SLCC 3954 (= CIP 100100 = Weis 1120), was isolated in Germany from soil.

(ii) **Description of the type strain of *L. seeligeri*.** The description of the type strain is the same as

the description for the species. In addition, this strain does not produce acid from α -methyl-D-mannoside and belongs to serovar 1/2b. Figure 2 shows an electron micrograph of strain SLCC 3954^T.

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