Listeria floridensis sp. nov., Listeria aquatica sp. nov., Listeria cornellensis sp. nov., Listeria riparia sp. nov. and Listeria grandensis sp. nov., from agricultural and natural environments

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Sampling of agricultural and natural environments in two US states (Colorado and Florida) yielded 18 Listeria-like isolates that could not be assigned to previously described species using traditional methods. Using whole-genome sequencing and traditional phenotypic methods, we identified five novel species, each with a genome-wide average BLAST nucleotide identity (ANIb) of less than 85% to currently described species. Phylogenetic analysis based on 16S rRNA gene sequences and amino acid sequences of 31 conserved loci showed the existence of four well-supported clades within the genus Listeria; (i) a clade representing Listeria monocytogenes, L. marthii, L. innocua, L. welschimeri, L. seeligeri and L. ivanovii, which we refer to as Listeria sensu stricto, (ii) a clade consisting of Listeria fleischmannii and two newly described species, Listeria aquatica sp. nov. (type strain FSL S10-1188T=DSM 26686T=LMG 28120T=BEI NR-42633T) and Listeria floridensis sp. nov. (type strain FSL S10-1187T=DSM 26687T=LMG 28121T=BEI NR-42632T), (iii) a clade consisting of Listeria rocourtiae, L. weihenstephanensis and three novel species, Listeria cornellensis sp. nov. (type strain TTU A1-0210T=FSL F6-0969T=DSM 26689T=LMG 28123T=BEI NR-42630T), Listeria grandensis sp. nov. (type strain TTU A1-0212T=FSL F6-0971T=DSM 26688T=LMG 28122T=BEI NR-42631T) and Listeria riparia sp. nov. (type strain FSL S10-1204T=DSM 26685T=LMG 28119T=BEI NR-42634T) and (iv) a clade containing Listeria grayi. Genomic and phenotypic data suggest that the novel species are non-pathogenic.

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Abbreviations: AAI, average amino acid identity; ANIb, BLAST average nucleotide identity; MR, methyl red; PI-PLC, phosphoinositide phospholipase C; VP, Voges-Proskauer.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains TTU A1-0210T, TTU A1-0212T, FSL S10-1188T, FSL S10-1204T and FSL S10-1204T are JX961634, JX961635, JX961636, JX961637 and JX961638, respectively. The accession numbers for the genome sequences of strains TTU A1-0210T, TTU A1-0212T, FSL S10-1188T, FSL S10-1188T and FSL S10-1204T are XJ961634, XJ961635, XJ961636, XJ961637 and XJ961638, respectively. The accession numbers for the genome sequences of strains TTU A1-0210T, TTU A1-0212T, FSL S10-1188T, FSL S10-1188T and FSL S10-1204T are AODE00000000, AODD00000000, AODF00000000, AOCG00000000 and AODL00000000, respectively; the versions described in this paper are the first versions, AODE01000000, AODD01000000, AODF01000000, AOCG01000000 and AODL01000000.

Five supplementary figures and five supplementary tables are available with the online version of this paper.
The genus *Listeria* was described by Pirie (1940), and at present comprises 10 recognized species, *Listeria monocytogenes* (Pirie, 1940), *Listeria grayi* (Errebo Larsen & Seeliger, 1966), *Listeria innocua* (Seeliger, 1981), *Listeria welschimeri* (Rocourt & Grimont, 1983), *Listeria seeligeri* (Rocourt & Grimont, 1983), *Listeria ivanovii* (Seeliger et al., 1984), *Listeria marthii* (Graves et al., 2010), *Listeria rocourtiae* (Leclercq et al., 2010), *Listeria fleischmannii* (Bertsch et al., 2013) and *Listeria weihenstephanensis* (Lang Halter et al., 2013). Additionally, two subspecies have been recognized within *L. ivanovii* (subsp. *ivanovii* and subsp. *londoniensis*; Boerin et al., 1992), *L. grayi* (subsp. *grayi* and subsp. *murrayi* (Stuart & Welshimer, 1973), though these names are not validly published) and *L. fleischmannii* (subsp. *fleischmannii* and subsp. *coloradonensis*; den Bakker et al., 2013).

During sampling projects of agricultural and natural environments in the US states of Florida and Colorado, we isolated 70 isolates, predominantly from water, with a colony morphology on *Listeria* plating medium (LMPM) reminiscent of strains of *Listeria*. These isolates could be placed in the family *Listeriaceae* by phylogenetic analysis of partial sequences of the 16S rRNA gene, and 52 isolates could be assigned to *L. fleischmannii* (32 isolates from Florida and 20 isolates from Colorado); however, 18 isolates did not cluster phylogenetically within previously described species and therefore could not be classified as members of any of the previously described species. Based on further genotypic characterization of these isolates, including by *sigB* sequencing (Sauders et al., 2012), one or two isolates per putative novel taxon were selected for further phenotypic and genomic characterization (see Fig. S1, available in the online Supplementary Material, for more information).

Draft genome sequences were assembled as detailed by den Bakker et al. (2013) from Ion PGM instrument reads obtained for (i) *Brochothrix thermosphacta* ATCC 11509T and *B. campestris* ATCC 43754T, (ii) the type strains *Listeria monocytogenes* ATCC 15313T, *L. weihenstephanensis* DSM 24698T and *L. rocourtiae* CIP 109804T and *L. grayi* ATCC 25401 (the type strain of *Listeria murrayi*), (iii) an additional strain of *L. fleischmannii* (FSL S10-1203) and (iv) four isolates that showed *Listeria*-like characteristics, but could not be classified to species by traditional approaches. Briefly, *de novo* assembly was performed using the MIRA assembler (Chevreux, 1999), and homopolymers were corrected using a homopolymer correction script (available at http://tinyurl.com/oke6xa); this script uses output from VarScan (Koboldt et al., 2012) and BWA (Li & Durbin, 2009) to obtain a consensus about the homopolymer length, which is then used to correct the homopolymers. *De novo* assemblies (draft genomes) of Ion Torrent reads ranged in total size from 2.37 (*B. campestris*) to 3.37 (*L. weihenstephanensis*) Mbp (see Table S1 for total draft genome lengths and additional genome assembly statistics). Comparison of the draft genome obtained here for *L. monocytogenes* ATCC 15313T and a recently finished genome of *L. monocytogenes* SLCC5850 (Kuenne et al., 2013), a strain closely related to the type strain (McLauchlin & Rees, 2009), showed that the draft genome covers 97.3 % of the finished genome. We therefore conclude that the draft genomes obtained in this study are of high quality and suitable for comparative analysis. These draft genomes will also provide a starting point for future in-depth genomic and evolutionary analyses of the genus *Listeria*.

**BLAST** average nucleotide identities (ANIb) were calculated based on draft genome sequences and publicly available genome sequences using *JSpecies* (Richter & Rosselló-Móra, 2009). Additionally, **BLAST** average amino acid identities (AAI) (Konstantinidis & Tiedje, 2005) were calculated using the AAI.rb script from the Enve-omics package (http://enve-omics.ce.gatech.edu/). Pairwise comparisons between currently recognized species in *Listeria sensu stricto* yielded ANIb and AAI values <95 % for comparisons between different species (Fig. 1; Table S2); an ANIb value of 95–96 % generally corresponds to 70 % DNA–DNA hybridization and is typically used as a cut-off value for species delineation in bacterial taxonomy (Richter & Rosselló-Móra, 2009). Our data also support conclusions from previous studies that ANIb values are robust even with fragmented unfinished draft assemblies. This robustness is demonstrated by an ANIb value of 99.94 % for the comparison between the draft genome for the *L. monocytogenes* type strain, ATCC 15313T (a draft genome obtained in this study), and the finished genome for *L. monocytogenes* SLCC5850. For all genomes representing previously reported species, ANIb values supported prior species classification, despite the mixed use of finished and draft genomes (Fig. 1). Most importantly, our analyses showed that all isolates representing novel species proposed in this study show ANIb values <84 % and AAI values <87.9 % (Table S2) when their genomes are compared to each other and to phylogenetically related, previously described species (*L. fleischmannii*, *L. rocourtiae* and *L. weihenstephanensis*). While the high 16S rRNA gene sequence similarity (>99 %) between *L. rocourtiae* CIP 109804T, *L. weihenstephanensis* DSM 24998T, *L. cornellensis* sp. nov. TTU A1-0210T, *L. grandensis* sp. nov. TTU A1-0212T and *L. riparia* sp. nov. FSL S10-1204T could be interpreted as suggesting that these taxa represent ecotypes of a single species (Cohan, 2001; Konstantinidis et al., 2006), ANIb and AAI values and shared gene content do not support this hypothesis. AAI and ANIb values between these species range from 83.2 to 88.6 % and from 77.7 to 83.2 %, respectively, and the proportion of shared gene content between each species pair ranges from 63 to 75 %, justifying recognition as individual species (Fig. S2).

Phylogenetic analysis of (i) 16S rRNA gene sequences (Fig. S2) and (ii) concatenated sequences of 31 amino acid sequences (Wu & Eisen, 2008) (Fig. 2) was performed as described previously (den Bakker et al., 2013), with the exception that Gblocks version 0.91b (Castresana, 2000) was used to remove ambiguous sites from the amino acid sequence alignment. Maximum-parsimony analyses were performed in *PAUP* version 4.0b10 (Wilgenbusch &
Swofford, 2003). Phylogenies obtained from the maximum-parsimony analysis (data not shown) did not differ from those found by maximum-likelihood-based analyses, and hence the results of the maximum-likelihood analyses are discussed here. Both 16S rRNA gene and amino acid sequence phylogenies confirmed the placement of the novel taxa in the genus Listeria, and revealed four well-supported clades [100% bootstrap support (BS)] within the genus Listeria: (i) a clade containing L. monocytogenes and related species (Listeria sensu stricto: L. marthii, L. innocua, L. welshimeri, L. seeligeri and L. ivanovii), (ii) a clade consisting of L. rocourtiae, L. weihenstephanensis, L. cornellensis sp. nov., L. grandensis sp. nov. and L. riparia sp. nov., (iii) a clade consisting of L. fleischmannii, L. floridensis sp. nov. and L. aquatic sp. nov., and (iv) a clade consisting of L. grayi. The 16S rRNA gene sequence analysis (Fig. S3) also indicates that a previously unidentified Listeria-like bacterium (referred to as 'unidentified bacterium isolate' in the species description) is a putative sister species of L. monocytogenes DSM 24998\(^\text{T}\). Phylogenetic analysis of individual genes, such as iap (Fig. S4), also reveal the subdivision of Listeria into four distinct clades. The phylogenetic relationships between the four clades are unresolved in the 31 amino acid loci tree (Fig. 1; BS values <60%); however, the 16S rRNA gene sequence analysis suggests that the L. rocourtiae clade and the L. grayi clade are sister groups (Fig. S3; 85% BS). AAI values between members of each phylogenetically distinct clade range from 67 to 70% (Fig. S2). While Konstantinidis & Tiedje (2005, 2007) show that AAI between 65 and 72% coincides with traditional genus delimitations in bacterial taxonomy, we do not propose classification of these clades into separate genera at this point, in part because reclassification of L. grayi into a new genus has previously been unsuccessful and controversial (McLauchlin & Rees, 2009).

For methyl red (MR) and Voges–Proskauer (VP) tests, all isolates were cultured aerobically on BHI agar (Becton Dickinson) for 18 h at 30°C. A single colony was suspended in 5 ml MR-VP broth (pH 6.9 ±0.2; Becton Dickinson); inoculated tubes were incubated aerobically for 5 days at 35°C. MR and VP tests were carried out according to the manufacturer’s guidelines (Becton Dickinson; procedure L007474). Results are listed in the species descriptions and Table 1.

Testing for catalase activity was performed according to Bacteriological Analytical Manual (BAM) protocol R12 (US Food and Drug Administration, 2013). An isolated colony from BHI agar was emulsified in a drop of 3% hydrogen peroxide on a glass slide. Bubbling within 15 s was interpreted as a positive result for the catalase test. Results were scored independently by two individuals and are listed in the species descriptions and Table 1.

To test for nitrate reduction, isolates were grown in nitrate broth (BAM medium M108; US Food and Drug Administration, 2013) with Durham tubes for 48 h at 30°C without shaking. Durham tubes allowed detection of gas production at 24 and 48 h, which is indicative of N\(_2\) production. Reduction of nitrate to nitrite was tested by adding 50 μl bacterial culture grown in nitrate broth to 0.9 ml phosphate buffer (50 mM, pH 7.4), followed by the addition of 1 ml sulfanilic acid/HCl (5.0 g sulfanilic acid, 100 ml concentrated HCl, 400 ml water; final pH −0.38) and 1 ml 0.58% N-(1-naphthyl) ethylenediamine. An...
immediate colour change to deep pink to purple indicated the production of nitrite. For all isolates, nitrite reduction was determined using a protocol comparable to the nitrate reduction protocol described above, except that KNO$_2$ was used instead of KNO$_3$ in BAM medium M108. Nitrite reduction was negative if the reaction solution produced magenta to purple coloration. If an isolate reduces nitrate to an oxidation state lower than nitrite, the nitrate reduction test will produce a false negative. Therefore, separately testing nitrite reduction and the generation of N$_2$ (Durham tubes) measures the reduction of nitrate to nitrite. All non-motile species were found to be able to reduce nitrate, with the exception of \textit{L. floridensis} sp. nov. None of the novel species reported here was able to reduce nitrite.

Haemolysis for each isolate was determined using the CAMP test as described in BAM (US Food and Drug Administration, 2013) and performed in duplicate. To test for phosphatidylinositol-specific phospholipase C (PI-PLC) activity, single colonies from BHI agar plates were subcultured to LMPM (R&F Laboratories). PI-PLC activity-positive strains colour blue on LMPM medium. None of the novel species described here showed haemolytic activity or PI-PLC activity, suggesting that these species lack the genes (i.e. \textit{hly} and \textit{plcA}) associated with virulence in \textit{L. monocytogenes} and \textit{L. ivanovii}. Virulence genes (i.e. genes found in the \textit{prfA} cluster; Schmid \textit{et al.}, 2005) or LIPI-2 (Domínguez-Bernal \textit{et al.}, 2006) were absent from the draft genomes, further supporting the conclusion that none of the novel species described here are pathogenic.

API \textit{Listeria} and API 50 CH (with API 50 CHB/E medium) kits (bioMérieux) were utilized for further phenotypic characterization, using bacterial colonies taken from BHI agar plates. API \textit{Listeria} tests were incubated for 24 h at 34°C for all isolates (within the manufacturer’s recommended temperature range of 36 ± 2°C). API 50 CH tests were incubated for 48 h at 37°C for all isolates. A mineral oil cover was not used, since all species studied here are facultative anaerobes. Results were recorded at 48 h, unless there were positive or variable results at 24 h with a corresponding negative result at 48 h, in which case 24 h results were reported (as instructed in the API 50 CHB/E manual). Results are summarized in the species descriptions and Table 1. Results for individual isolates are reported in Table S3.

To test growth characteristics, isolates were grown in BHI broth at 4, 7, 22.5, 30, 37 and 41°C. A Synergy H1 hybrid multi-mode microplate reader (BioTek Instruments) was employed to test for growth at 22.5 ± 0.5, 30 ± 0.1, 37 ± 0.1...
Table 1. Physiological characteristics of members of the genus *Listeria* based on observations made in this study and the current literature

Strains/species: 1, *L. monocytogenes* 10403S (additional data from McLauchlin & Rees, 2009; Bertsch et al., 2013); 2, *L. innocua* FSL S4-378 (additional data from McLauchlin & Rees, 2009; Bertsch et al., 2013); 3, *L. seeligeri* (data from McLauchlin & Rees, 2009; Bertsch et al., 2013); 4, *L. ivanovii* ATCC BAA-678 (additional data from McLauchlin & Rees, 2009; Bertsch et al., 2013); 5, *L. welshimeri* (data from Bille et al., 1992; McLauchlin & Rees, 2009; Bertsch et al., 2013); 6, *L. marthii* FSL S4-120; 7, *L. grayi* strains ATCC 19120T and ATCC 25401 (type strain of *L. murrayi*); 8, *L. rocourtiae* CIP 109804T; 9, *L. weissenstephanensis* DSM 24698T; 10, *L. cornellensis* sp. nov, strains TTU A1-0210T and FSL F6-0970; 11, *L. riparia* sp. nov, strains FSL S10-1204T and FSL S10-1219; 12, *L. grandensis* sp. nov. TTAU A1-0211T; 13, *L. fleischmannii* strains DSM 24987T, ATCC BAA-2414T, FSL F6-1019, FSL S10-1186, FSL S10-1203 and FSL S10-1220; 14, *L. aquatica* sp. nov. strains FSL S10-1188T and FSL S10-1181; 15, *L. floridensis* sp. nov. FSL S10-1187T. All species/strains are catalase- and methyl red-positive. All strains are positive for acid production from D-arabinose, D-adonitol, methyl b-D-sylpyranoside, raffinose, glycogen, L-fucose, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate. +, Positive; (+) weakly positive; −, negative; V, variable (between replicates and/or between strains); V!, variable between studies, possibly due to differences in incubation times and temperatures between studies; ND, not done or not reported.

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*Motility was reported for these species in the original descriptions (Lang Halter et al., 2013; Leclercq et al., 2010). We did not observe motility in the type strains of these species, nor did we find genes encoding a flagellar apparatus in the genomes of the type strains (Table S4). In agreement with our observations, Bertsch et al. (2013) did not observe motility in the type strain of *L. rocourtiae.*
for 26 h and 41 °C for 41 h in the Synergy H1 reader. The OD_{600} was measured after 10 s of orbital shaking as follows: every 2 min for the first 20 min, every 5 min for the next 40 min and every 10 min for the remainder of the experiment. Growth was quantified by finding the first time the cell number had increased by 0.6 log (c.f.u. ml⁻¹), i.e. a 4-fold increase in cell number. Measured increases in log(OD_{600}) were converted to increases in log(c.f.u. ml⁻¹) using the slope of the strain-specific linear function relating log(OD_{600}) to log(c.f.u. ml⁻¹). The linear function was created with a series of eight 2-fold serial dilutions of a 24 h culture of each strain, performed in a microtitre plate, and the resulting OD_{600} values for each dilution. The mean slope of the resulting best-fit lines was 0.92 log(OD_{600})/log(c.f.u. ml⁻¹), with a mean r² of 0.99. For the measurement of growth characteristics at temperatures below ambient temperature, BHI culture tubes were inoculated at 10³ c.f.u. ml⁻¹ (+0.3 log) and incubated at 4 °C (low, 3 °C; high, 9 °C) and 7 °C (6.8±0.2 °C) in a refrigerated incubator. Cultures were spiral-plated on BHI agar (to determine c.f.u. ml⁻¹) every 24 h for 12 days. Growth was quantified by calculating the time for the number of cells to increase by 1.0 log(c.f.u. ml⁻¹), i.e. a 10-fold increase in cell number, by linear interpolation of the measured points immediately before and after the observed 1.0 log increase. Growth characteristics are reported in the species descriptions and additional data on growth characteristics of the individual isolates can be found in Table S4.

Motility was determined in motility test medium (MTM) (prepared according to BAM medium M103; US Food and Drug Administration, 2013). For each isolate, 13 mm tubes containing MTM were inoculated, 1 cm under the surface, with a stab from a colony on BHI agar. Isolates were incubated aerobically for 7 days at 22, 30 or 37 °C or for 10 days at 4 °C. An isolate was considered motile if cloudy growth beyond the stab was observed in addition to the characteristic mushroom- or umbrella-shaped growth. Results are reported in Table 1 and the species descriptions. *L. monocytogenes* 10403S was included as a positive control, while *L. fleischmannii* DSM 24998T was included as a negative control. In addition to the motility test, draft genomes of the type strains sequenced were queried using BLAST (Altschul et al., 1990) for genes associated with motility in *L. monocytogenes* (Table S5). Consistent with observations of the motility test, no flagellar motility-associated genes were found in the novel species described here.

Light microscopic observations were performed on isolates that were cultured on BHI agar aerobically for 18 h at 30 °C. Stationary-phase bacteria were examined on agar-coated slides and viewed by phase-contrast microscopy using an Olympus BX61 microscope. Digital photographs were captured using the SlideBook version 4.0.2.2 software (Intelligent Imaging Innovations) and length/width ratios were based on the mean length and width of five bacteria. Results are reported in Table 1 and the species descriptions. Photographs of individual isolates can be found in Fig. S5.

**Description of Listeria floridensis sp. nov.**

*Listeria floridensis* (flo.ri.den’sis. N.L. fem. adj. floridensis of or belonging to Florida, the state in the United States from which the type strain was isolated).

Cells are 0.6 × 1.3–1.9 µm; length/width ratio 2.7. Gram-stain-positive, straight rods with rounded ends. No growth at 7 °C or below. Optimum growth temperature 37–41 °C. No motility at 4, 22, 30 or 37 °C. Voges–Proskauer-negative and catalase-positive. CAMP-negative. Negative for reduction of nitrate and nitrite. Other characteristics are reported in Table 1. Currently the only member of the genus *Listeria* that both lacks motility and is unable to reduce nitrate. A unique API *Listeria* numerical profile (2 710) is observed for the type strain.

The type strain, FSL S10-1187T (=DSM 26687T=LMG 28121T=BEI NR-42632T), was isolated from running water in Florida, USA. The DNA G + C content of the type strain is 41.8 mol% (determined by genome sequencing).

**Description of Listeria aquatica sp. nov.**

*Listeria aquatica* (a.qua’ti.ca. L. fem. adj. aquatica found in water, aquatic).

Cells are 0.6–0.7 × 1.5–2.4 µm; mean length/width ratio 3.2. Gram-stain-positive, straight rods with rounded ends. No growth at 7 °C or below. Optimum growth temperature 37–41 °C. No motility at 4, 22, 30 or 37 °C. Voges–Proskauer-negative for type strain; positive for strain FSL S10-1181. Catalase-positive. CAMP-negative. Nitrate reduction-positive; nitrite reduction-negative. Other characteristics are reported in Table 1. Currently, the only member of the genus *Listeria* that is unable to ferment maltose. Among non-motile species of the genus *Listeria*, it is currently unique in its ability to ferment D-tagatose. A unique API *Listeria* numerical profile (6 731) is observed for the type strain and FSL S10-1181.

The type strain, FSL S10-1188T (=DSM 26686T=LMG 28120T=BEI NR-42633T), was isolated from running water in Florida, USA. The DNA G + C content of the type strain is 40.9 mol% (determined by genome sequencing).

**Description of Listeria cornellensis sp. nov.**

*Listeria cornellensis* (cor.nel.len’sis. N.L. fem. adj. cornel-\*lens* named after Cornell, the university where most of the research was performed that led to the discovery of the species described in this study).

Cells are 0.4–0.7 × 2.4–3.8 µm; mean length/width ratio 5.3. Gram-stain-positive, straight rods with rounded ends. Optimal growth at 30–37 °C. No motility at 4, 22, 30 or 37 °C. Voges–Proskauer-negative, catalase-positive. CAMP-negative. Nitrate reduction-positive, nitrite reduction-negative. Other characteristics are indicated in Table 1. While phylogenetically and genomically distinct, this species resembles *L. grandensis* for the phenotypic characteristics.
recorded here. Among the non-motile species of the genus *Listeria*, *L. cornellensis* and *L. grandensis* are the only ones that are L-rhamnose-negative in the API 50 CH test (performed at 37 °C); however, the API *Listeria* test strip (incubated at 34 °C) shows acidification of L-rhamnose for *L. grandensis* and for one of the two *L. cornellensis* strains tested. An operon involved in rhamnose utilization could be found in the draft genome of *L. grandensis* TTU A1-0210T, but is absent from the draft genome of the type strain of *L. cornellensis*, suggesting the absence of rhamnose utilization for the type strain of *L. cornellensis* and temperature-dependent ability of *L. grandensis* to acidify L-rhamnose. *L. cornellensis* can be further distinguished from *L. grandensis* by weak acidification of lactose. API *Listeria* numerical profile 2 730 is observed for the two known isolates (TTU A1-0210T and FSL F6-0970) of this species; this numerical profile is associated with *L. ivanovii* according to the API *Listeria* manual (bioMérieux; version 04/2007).

The type strain, TTU A1-0210T (≡DSM 26689T = LMG 28123T = BEI NR-42630T), was isolated from water in Colorado, USA. The DNA G+C content of the type strain is 42.5 mol% (determined by genome sequencing).

**Description of *Listeria grandensis* sp. nov.**

*Listeria grandensis* (gran.den’sis. N.L. fem. adj. grandensis of or belonging to Grand, the county where the type strain was isolated).

Cells are 0.6–0.7 × 2.0–3.1 μm; mean length/width ratio 4.0. Gram-stain-positive, straight rods with rounded ends. Optimal growth temperature 30–37 °C. No motility at 4, 22, 30 or 37 °C. Voges–Proskauer-negative, catalase-positive. CAMP-negative. Nitrite reduction-negative and nitrate reduction-positive. Other characteristics are reported in Table 1. See the description of *L. cornellensis* for differentiation of *L. grandensis* from this species. A unique API *Listeria* numerical profile (2 730) is observed for the type strain.

The type strain, TTU A1-0212T (≡DSM 26685T = LMG 28119T = BEI NR-42634T), was isolated from running water in Florida, USA. The DNA G+C content of the type strain is 41.9 mol% (determined by genome sequencing).

**Acknowledgements**

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**Reference**


