Lactobacillus brantae sp. nov., isolated from faeces of Canada geese (*Branta canadensis*)

Dmitriy V. Volokhov,¹ Megan Amselle,² Brian J. Beck,² David L. Popham,³ Paul Whittaker,⁴ Hua Wang,⁴ Elizabeth Kerrigan² and Vladimir E. Chizhikov¹

Correspondence

Dmitriy V. Volokhov
dmitriy.volokhov@fda.hhs.gov

¹Center for Biologics Evaluation and Research, US Food and Drug Administration, 1401 Rockville Pike, HFM-470, Rockville, MD 20852, USA

²American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110, USA

³Department of Biological Sciences, Virginia Tech, Life Sciences I - MC0910, Blacksburg, VA 24061, USA

⁴Center for Food Safety and Applied Nutrition, US Food and Drug Administration, 5100 Paint Branch Parkway, College Park, MD 20740, USA

Three strains of lactic acid bacteria (LAB) were isolated from the faeces of apparently healthy wild Canada geese (*Branta canadensis*) in 2010 by cultivating faecal LAB on Rogosa SL agar under aerobic conditions. These three isolates were found to share 99.9% gene sequence similarity of their 16S rRNA, their 16S–23S intergenic transcribed spacer region (ITS), partial 23S rRNA, *rpoB*, *rpoC*, *rpoA* and *pheS* gene sequences. However, the three strains exhibited lower levels of sequence similarity of these genetic targets to all known LAB, and the phylogenetically closest species to the geese strains were *Lactobacillus casei*, *Lactobacillus paracasei*, *Lactobacillus rhamnosus* and *Lactobacillus saniviri*. In comparison to *L. casei* ATCC 393^T, *L. paracasei* ATCC 25302^T, *L. rhamnosus* ATCC 7469^T and *L. saniviri* DSM 24301^T, the novel isolates reacted uniquely in tests for cellobiose, galactose, mannitol, citric acid, aesculin and dextrin, and gave negative results in tests for L-proline arylamidase and L-pyrrolydonyl-arylamidase, and in the Voges-Proskauer test. Biochemical tests for cellobiose, aesculin, galactose, gentiobiose, mannitol, melezitose, ribose, salicin, sucrose, trehalose, raffinose, turanose, amygdalin and arbutin could be used for differentiation between *L. saniviri* and the novel strains. On the basis of phenotypic and genotypic characteristics, and phylogenetic data, the three isolates represent a novel species of the genus *Lactobacillus*, for which the name *Lactobacillus brantae* sp. nov. is proposed. The type strain is SL1108^T (=ATCC BAA-2142^T=LMG 26001^T=DSM 23927^T) and two additional strains are SL1170 and SL60106.

At the time of writing, the genus *Lactobacillus* contained more than 150 species and subspecies (<http://www.bacterio.cict.fr/l/lactobacillus.html>) (Claesson *et al.*, 2007,

Abbreviations: FAME, fatty acid methyl ester; ITS, intergenic transcribed spacer region; LAB, lactic acid bacteria.

The GenBank/EMBL/DDBJ accession numbers for the 16S-ITS-23S, *rpoB* and *rpoC* sequences of *Lactobacillus saniviri* DSM 24301^T are JN644756, JN644760, and JN644761, respectively. The GenBank/EMBL/DDBJ accession numbers for the 16S-ITS-23S, *rpoB*, *rpoA*, *rpoC*, and *pheS* sequences of strain SL1108^T are HQ022861, HQ022858, HQ917698, JN644762 and JN644763, respectively. The GenBank/EMBL/DDBJ accession number for an unknown DNA sequence of strain SL1108^T amplified with *gyrB* primers is JF313457.

Four supplementary figures and three supplementary tables are available with the online version of this paper.

2008; Felis & Dellaglio, 2007). Species of the genus *Lactobacillus* have been isolated from a wide range of hosts and environmental matter (Claesson *et al.*, 2008; London, 1976), and many of them are known to provide a beneficial effect in their natural hosts (Ohashi & Ushida, 2009; Patterson & Burkholder, 2003). During a study aimed at the isolation of lactobacilli from different environmental samples in March–August 2010, we isolated several species of lactic acid bacteria (LAB), including *Lactobacillus sakei*, *Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus fermentum* and *Lactobacillus lactis*, from the faeces of apparently healthy wild Canada geese (*Branta canadensis*). LAB microflora of geese and other *Anseriformes* remains to be elucidated with only a few publications describing LAB diversity in these avian hosts (Angelakis & Raoult, 2010;

Damaré *et al.*, 1979; Ehrmann *et al.*, 2002; Kovalenko *et al.*, 1989; Kurzak *et al.*, 1998). Isolation of species of the genus *Lactobacillus*, including *Lactobacillus coryniformis*, *L. plantarum* and *Lactobacillus acidophilus*, has been reported from geese (Damaré *et al.*, 1979; Kovalenko *et al.*, 1989), but other LAB including *Enterococcus faecium* and *Enterococcus faecalis* were found to be the most prevalent species in the digestive tract of geese and ducks (Kovalenko *et al.*, 1989; Kurzak *et al.*, 1998). Among species of the genus *Lactobacillus*, the predominant isolate from geese has been *L. plantarum*, and it was suggested that this observation can be explained by the herbivore diet of geese (Kovalenko *et al.*, 1989), and by the fact that *L. plantarum* are a frequent associate of epiphytic microflora of terrestrial plants (Endo *et al.*, 2010; Mundt & Hammer, 1968).

In this study, we describe a novel species of the genus *Lactobacillus*, which was isolated from the faeces of Canada geese and demonstrated low 16S rRNA gene sequence similarity to all previously described LAB, and had distinctive biochemical features. Ten faecal specimens were collected from Canada geese in Great Falls Park and Assateague State Park, Maryland, USA. Faecal specimens (1 g per bird) were diluted 1:10 (w/v) in sterile PBS and serially diluted in PBS. Aliquots from 10^{-1} to 10^{-5} dilutions were either inoculated 1:20 (v/v) into Rogosa SL broth (Difco; BD) or 0.5 ml of each dilution were plated on Rogosa SL agar. Broth and plates were incubated at 36 ± 1 °C under aerobic conditions with 5 % CO₂. Bacterial growth in broth and on plates was observed after 2–3 days. The broth samples where bacterial growth was visibly detected were streaked on Rogosa SL agar and incubated under the same conditions. Individual colonies from Rogosa SL agar were subcultivated on MRS agar (Difco; BD) under both aerobic and anaerobic conditions. Following triple subculturing from single colonies, the isolates were maintained in MRS broth (Difco; BD). The almost complete 16S rRNA gene sequence (Pérez Pulido *et al.*, 2005) of the randomly selected colonies suggested that most isolates were represented by the abovementioned known LAB, which were later also confirmed by partial sequencing of their housekeeping genes (*rpoB*, *rpoA* and *gyrB*). However three isolates had a 16S rRNA gene sequence different from the 16S rRNA gene sequences of all LAB available in the GenBank database. The three isolates were designated SL1108^T, SL1170 and SL60106, and were isolated in June, July and August 2010, respectively. Strain SL1108^T was selected as the type strain for the novel species because it was isolated first.

The three isolates grew readily on MRS agar and on Trypticase Soy Agar (TSA) with 5 % sheep blood. When incubated at 36 ± 1 °C, good growth appeared in 48 h under both aerobic and anaerobic conditions. An aerobic atmosphere of 5 % CO₂ slightly enhanced growth, although it was not essential for growth. Colonies on MRS agar were approximately 1 mm in diameter, circular, entire, translucent, cream, convex and smooth. Growth appeared more dense in MRS broth, producing a thick settled layer of cells at the bottom of the tube. α -haemolysis was observed on TSA

containing sheep blood after 5–7 days incubation under anaerobic conditions. In agreement with the phylogenetically closely related species *Lactobacillus casei* ATCC 393^T, *Lactobacillus paracasei* ATCC 25302^T, and *Lactobacillus rhamnosus* ATCC 7469^T, the three novel strains grew slowly on CDC Anaerobe 5 % sheep blood agar (BD) with phenylethyl alcohol (PEA) during anaerobic incubation at 36 ± 1 °C. Faint and slow growth of all the tested strains was observed in Schaedler broth (BD) during aerobic incubation at 36 ± 1 °C. Microscopically, the novel isolates appeared as chains of plump coccobacilli, with a few bacilli and cocci forms also present. Like most species of the genus *Lactobacillus*, the isolates did not produce catalase or oxidase, and cells were non-motile and Gram-stain-positive.

Genomic DNA was isolated from 1.0 ml of actively growing broth culture using the DNeasy Blood & Tissue kit (Qiagen) according to the manufacturer's protocol. PCR amplification of the entire 16S rRNA gene, the 16S–23S rRNA intergenic transcribed spacer region (ITS) and the partial 23S rRNA gene as a single PCR product (hereinafter referred to as the 16S-ITS-23S) was performed using previously published primers, 16SF and 23S-R1-MYC (Volokhov *et al.*, 2007). The partial *rpoB*, *rpoA*, and *rpoC* gene sequences of these isolates were amplified by PCR with primers *rpoBF* (GATGATATTGAYCACTTRGGTAACCG) and *rpoBR* (GCCCADACTTCCATTTCMC-CRAACG), *rpoAF* (TTAGTCTTCTTTACGCAATCCTA-AGC) and *rpoAR* (ATGATCGAATTCGAAAAGCCGAAC), *rpoCF* (CATCCCGGTACCAGCCGGAATGATCT) and *rpoCR* (TGATTGATGTCAATAAGTTTGAAAGTATGCA), respectively. These primers were designed from the GenBank sequences of the corresponding genes of *L. casei*, *L. paracasei* and *L. rhamnosus*, the species which were found to be closely related to the novel geese strains based on the phylogenetic analysis of their 16S rRNA genes. PCR amplification of the phenylalanyl-tRNA synthase alpha subunit gene (*pheS*) was performed using previously published primers, *pheS*-21F and *pheS*-952R (Naser *et al.*, 2005). The determined sequences of the 16S-ITS-23S, *rpoB*, *rpoC*, *rpoA* and *pheS* genes, and deduced amino acid sequences of RpoB, RpoC, RpoA and PheS were compared to the GenBank nucleotide and protein databases using BLASTN and BLASTP algorithms, respectively. Nucleotide and amino acid sequences were aligned using CLUSTAL_X software (<http://www.clustal.org>). Inter- and intra-species similarity scores for each gene were generated using BioEdit software (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>). Phylogenetic analysis was conducted using MEGA version 4 software (Tamura *et al.*, 2007). Phylogenetic trees were constructed and compared in MEGA 4 software using neighbour-joining, maximum-parsimony, UPGMA and minimum evolution algorithms. The robustness of the individual branches was estimated by bootstrap analysis with 1000 replicates. The analogous genetic targets of *Streptococcus equinus* were used as outgroup sequences during phylogenetic comparisons, and bootstrap analyses were performed using 1000 bootstrapped iterations. No differences were observed between

the trees created with the different phylogenetic algorithms. At the time of the isolation of strain SL1108^T, its 16S rRNA gene sequence showed low sequence similarity to known species of LAB; all similarities were 94–95 % or less, and the closest known relatives were *L. casei*, *L. paracasei* and *L. rhamnosus*, LAB species that are widely distributed in nature (Claesson *et al.*, 2008). However, the recently published species *Lactobacillus saniviri* (Oki *et al.*, 2012) demonstrated the highest 16S rRNA gene sequence similarity (97 %) to strain SL1108^T. Phylogenetically, based on the 16S rRNA, *rpoB* and *rpoA* gene sequences, strain SL1108^T was found to be a member of the *L. casei* group (Felis & Dellaglio, 2007), which includes *L. casei*, *L. paracasei*, *L. rhamnosus* and *L. saniviri* (Fig. 1 and Figs S1 and S2, available in IJSEM Online). The 16S rRNA gene sequence similarities of strain SL1108^T with *L. casei*, *L. paracasei* and *L. rhamnosus* were lower than the similarity threshold of 97 % recommended for species differentiation by using genomic DNA–DNA hybridization (Stackebrandt & Goebel, 1994). Therefore DNA–DNA hybridization between strain SL1108^T and the phylogenetically closely related LAB species was not performed, in agreement with reports for other LAB with similar 16S rRNA gene sequence similarity values (Cousin *et al.*, 2012; Kawasaki *et al.*, 2011a, b). However, because *L. saniviri* DSM 24301^T was found to share 97 % 16S rRNA gene sequence similarity to strain SL1108^T, the comparison of several genetic regions (*rpoB*, *rpoC*, *pheS* and *rpoA* genes, and the 16S-ITS-23S) and *EcoRI* ribotyping, in addition to the biochemical tests, were performed for both species. For this purpose, in addition to the sequences available for *L. saniviri* DSM 24301^T in GenBank (16S rRNA, *pheS* and *rpoA*), sequencing of the 16S-ITS-23S, *rpoB* and *rpoC* genes for this species was performed during this study, and resulting sequences were deposited in Genbank under accession numbers JN644756, JN644760, and JN644761, respectively.

Despite the 97 % 16S rRNA gene sequence similarity between strain SL1108^T and *L. saniviri* DSM 24301^T, several species-specific regions within the 16S rRNA gene and the ITS were found for both species (Figs S3 and S4). These differences clearly demonstrate that strain SL1108^T and *L. saniviri* DSM 24301^T are distinct species. During our search for other closely related sequences to the 16S rRNA gene of strain SL1108^T or *L. saniviri* DSM 24301^T, we found one 16S rRNA gene sequence (GenBank accession number HM534767) that was identical to the 16S rRNA gene of *L. saniviri* DSM 24301^T. This sequence from *Lactobacillus* sp. Akpobro1 was isolated from the stomach of a honey bee and deposited in Genbank, and was published (Vásquez *et al.*, 2012) while this manuscript was in press.

The similarities among the *rpoA*, *rpoC*, *rpoB* and *pheS* gene sequences of strain SL1108^T and the closest neighbouring species ranged from 75–85 % [Tables S1 (*rpoA*) and S2 (*rpoB*), and data not shown]. Gene sequences from this study were deposited in GenBank under accession numbers HQ022861, HQ022858, HQ917698, JF313457, JN644756 and JN644760–JN644763.

Our attempt to amplify the *gyrB* gene of strain SL1108^T and *L. saniviri* DSM 24301^T using primers designed from the *gyrB* gene sequences of *L. casei* and *L. rhamnosus*, *gyrBF* (GTGACGGACAAGAAAGAAWCGGCCGAAG) and *gyrBR* (CGCCGCGGYTCAACATGATCRCCCATC), failed for both species. However these primers did efficiently amplify an unknown DNA sequence (GenBank accession number JF313457) that was unique to strain SL1108^T and found to be partially similar to *orf106* gene (70 %) of *L. casei* BL23 (FM177140), phage hypothetical protein (74 %) of *L. casei* bacteriophage A2 (AJ251789), and phage hypothetical protein (70 %) of *Macrococcus caseolyticus* JCSC5402 (AP009484). Using this unique DNA sequence of strain SL1108^T, species-specific primers were designed (*L.brantaef* GTTGCGTCTGGTTGCGAGTTGTG and *L.brantaer* TGA-TAGCGGGCGTGACGTTGATG) which subsequently amplified a 776 bp DNA fragment from strains SL1108^T, SL1170 and SL60106, but not from the phylogenetically closely related species *L. casei* ATCC 393^T, *L. paracasei* ATCC 25302^T, *L. rhamnosus* ATCC 7469^T, and *L. saniviri* DSM 24301^T (data not shown).

On the basis of phylogenetic analysis of the 16S rRNA, *rpoA* and *rpoB* gene sequences as well as sequence analysis of additional genetic targets (*rpoC* and *pheS* genes, and the ITS), the novel strain SL1108^T did not belong to any recognised species of the genus *Lactobacillus*. The two additional strains SL1170 and SL60106 shared the same growth characteristics as strain SL1108^T, and had 99.9 % nucleotide similarity in 16S rRNA, *rpoA*, *rpoB*, *rpoC* and *pheS* gene sequences to strain SL1108^T.

Phylogenetic relatedness of the strains SL1108^T, SL1170 and SL60106 to *L. casei*, *L. paracasei*, *L. rhamnosus* and *L. saniviri* directed us to perform ribotyping of representative strains as an additional discriminating test. Automated ribotyping was performed on a Riboprinter system (DuPont Qualicon) using *EcoRI* as the restriction enzyme as described previously (Brisse *et al.*, 2002; Massi *et al.*, 2004; Švec *et al.*, 2010). The ribopatterns of strains SL1108^T, SL1170 and SL60106 were very similar (Fig. 2). However they did not match (≥ 0.7 – 0.8 similarity index value indicates high level similarity) any pattern in the database which included more than 70 unique species of the genus *Lactobacillus* (data not shown). Comparison of the ribotype pattern of SL1108^T with *L. casei* ATCC 393^T, *L. paracasei* ATCC 25302^T, *L. rhamnosus* ATCC 7469^T, *L. saniviri* DSM 24301^T and strains of other species of the genus *Lactobacillus* showed distinct differences among the species (Fig. 3) with similarity indexes ranging from 0.03 to 0.51, indicating a low degree of similarity (Brisse *et al.*, 2002).

The biochemical characteristics of strain SL1108^T were compared to *L. casei* ATCC 393^T, *L. paracasei* ATCC 25302^T, *L. rhamnosus* ATCC 7469^T, and *L. saniviri* DSM 24301^T (Table 1). For strain SL1108^T, *L. casei* ATCC 393^T, *L. paracasei* ATCC 25302^T and *L. rhamnosus* ATCC 7469^T biochemical characteristics were determined using standard laboratory methods (conventional tube tests) and rapid

Fig. 1. Dendrogram showing phylogenetic relationships of *Lactobacillus brantae* sp. nov. with other lactic acid bacteria based on 16S rRNA gene sequences. The trees were constructed based on the gene sequences of 151 different *Lactobacilli* using the minimum evolution method in the MEGA 4 software package. Bootstrap values presented at corresponding branches were evaluated from 1000 replications. GenBank accession numbers are indicated in parentheses. Major phylogenetic groups of *Lactobacilli* are indicated. Bar, 0.01 substitutions per nucleotide position.

commercial systems, including API 50CH strips, Vitek Compact 2 GP and ANC cards (bioMérieux), and Gen III plates (Biolog), all commonly used for biochemical characterization of *Lactobacilli* (Charteris *et al.*, 2001; Di Cagno *et al.*, 2010; Johansson *et al.*, 1995). All commercial biochemical tests were performed according to manufacturers' procedures. The biochemical test results for *L. saniviri* DSM 24301^T were taken from a recent publication by Oki *et al.* (2012). In addition, several tests with *L. saniviri* DSM 24301^T (arabinose, fructose, glucose, glycerol, lactose, maltose, mannitol, mannose, rhamnose, ribose, sucrose and xylose) were evaluated in this study using conventional tube tests and agreed with the previously published data of Oki *et al.* (2012) (Table 1). The two additional novel strains, SL1170 and SL60106, were also analysed for their biochemical characteristics using conventional tube tests and Vitek Compact 2 GP and ANC cards. The Vitek Compact 2 GP and ANC cards and the API 50CH strip

profiles failed to produce a clear identification of these novel strains to the species level, but were useful to determine the biochemical capability of the strains. Use of the Biolog Gen III system indicated *Lactobacillus sake* and *Lactobacillus brevis* to be the closest relatives of strain SL1108^T, although the similarity scores were very low (0.12 and 0.07, respectively). All four species of the genus *Lactobacillus* tested (*L. casei* ATCC 393^T, *L. paracasei* ATCC 25302^T, *L. rhamnosus* ATCC 7469^T and strain SL1108^T) were able to utilize glucose, fructose, mannose, *N*-acetyl-D-glucosamine (NAG) and butyrate, but not arabinose, fucose, glycogen, raffinose, xylose, adonitol, arabitol, dulcitol, inulin, inosine, xylitol, acetic acid, formic acid, fusidic acid, glucuronic acid, mucic acid, propionic acid or quinic acid. All four species were negative in tests for urease, indole and gelatinase; were unable to grow in increased concentrations of NaCl (6.5 %, w/v); did not utilise any of the amino acids tested (alanine, arginine,

Fig. 2. *Eco*RI riboprint patterns for the three strains of *L. brantae* sp. nov. Similarity Index (SIM) is relative to strain SL1108^T.

aspartic acid, glutamic acid, histidine, proline and serine); and were unable to grow in the presence of guanidine, lincomycin, minocycline, nalidixic acid, rifamycin, troleandomycin or vancomycin. However many of the biochemical tests did provide differential reactions for these four species (Table 1); strain SL1108^T reacted uniquely with cellobiose, galactose, mannitol, citric acid and aesculin (all negative), and dextrin and melibiose (both positive). Strain SL1108^T was also negative in tests for L-proline arylamidase, L-pyrrolydonyl-arylamidase, and in the Voges-Proskauer test. Furthermore, comparison of data for strain SL1108^T and *L. saniviri* DSM 24301^T demonstrated that the biochemical tests for cellobiose, aesculin, galactose, gentiobiose, mannitol, melezitose, ribose, salicin, sucrose, trehalose, raffinose, turanose, amygdalin and arbutin could be used for differentiation between *L. saniviri* DSM 24301^T and the novel strains, SL1108^T, SL1170 and SL60106.

Although SL1108^T, SL1170 and SL60106 demonstrated complete concordance with their biochemical profiles obtained with the Vitek Compact 2 GP and ANC cards, there were conflicting results for strain SL1108^T, *L. paracasei* ATCC 25302^T and *L. rhamnosus* ATCC 7469^T in a few tests (D-galactose, lactose, maltose, salicin and sorbitol) obtained with these cards compared to the Biolog

Gen III system. All discrepant tests were investigated using the sugars in conventional test tubes and were determined to match the results from the Biolog Gen III system (data not shown).

Peptidoglycan type analysis was performed for strain SL1108^T, *L. casei* ATCC 393^T, *L. paracasei* ATCC 25302^T and *L. rhamnosus* ATCC 7469^T. The strains were grown in MRS broth (Sigma-Aldrich Inc.) and harvested at the time when the OD₆₀₀ of the cultures were 1.0 as measured by using an Ultrospec 3100 pro spectrophotometer (Amersham Biosciences). Peptidoglycan from the harvested cells was purified as described by McPherson & Popham (2003) and amino acid analysis was performed as described by González-Castro *et al.* (1997). Strain SL1108^T had the peptidoglycan type L-Lys-D-Asp, which was identical to the previously published peptidoglycan type for *L. casei*, *L. paracasei* and *L. rhamnosus* (Felis & Dellaglio, 2007), and confirmed in this study. It was also identical to the peptidoglycan type for the recently published *L. saniviri* (Oki *et al.*, 2012). In all the tested strains there was no diaminopimelic acid detected in the peptidoglycan, and the peptidoglycan side chains appeared to be Ala-Glu-Lys-Ala (with approximate ratios 2 Ala : 1 Glu : 1 Asp : 1 Lys) with Asp crossbridges in the cross-links, in agreement with that previously described for *L. casei* (Billot-Klein *et al.*, 1997). Thus, the primary structure of the peptidoglycan of all the tested species belonged to subgroup A4 (Billot-Klein *et al.*, 1997; Schleifer & Kandler, 1972).

Fatty acid profile analysis was performed for strain SL1108^T, *L. casei* ATCC 393^T, *L. paracasei* ATCC 25302^T and *L. rhamnosus* ATCC 7469^T. The strains were grown in MRS broth at 37.0 ± 1.0 °C for 48 h. For gas chromatography with flame-ionization detection (GC-FID) analysis, bacterial cells were harvested and whole-cell fatty acid methyl esters (FAMES) from triplicate samples were prepared by saponification, methylation and extraction into hexane/methyl *tert*-butyl ether, as previously described

Fig. 3. *Eco*RI riboprint patterns for various species of the genus *Lactobacillus* in comparison with strain SL1108^T. Similarity Index (SIM) is relative to strain SL1108^T.

Table 1. Biochemical characteristics of strain SL1108^T and phylogenetically closely related species of the genus *Lactobacillus*

Strains: 1, SL1108^T; 2, *L. casei* ATCC 393^T; 3, *L. paracasei* ATCC 25302^T; 4, *L. rhamnosus* ATCC 7469^T; 5, *L. saniviri* DSM 24301^T. Data are from this study except for *L. saniviri* DSM 24301^T which is taken from Oki *et al.*, 2012. +, Positive; –, negative; ND, no data available.

Tests*	1	2	3	4	5
Aesculin ^{a,b}	–	+	+	+	+
Amylose ^c	–	+	–	+	ND
Cellobiose ^c	–	+	+	+	+
Citric acid ^c	–	+	+	+	ND
Dextrin ^c	+	–	–	–	ND
Galactose ^c	–	+	+	+	+
D-Galactose ^d	–	+	–	+	+
D-Galactose ^b	–	+	+	+	+
Gentiobiose ^c	–	+	–	+	+
Gluconic acid ^c	+	+	+	–	ND
Glycerol ^c	+	+	–	+	+
Inositol ^c	–	–	–	+	–
1 % Sodium lactate ^c	–	–	+	–	ND
Lactic acid ^c	+	–	–	+	ND
Lactose ^c	–	+	+	+	–
Lactose ^d	–	+	–	+	–
Maltose ^c	+	–	+	+	+
Maltose ^b	–	–	+	–	+
Mannitol ^c	–	+	+	+	+
D-Mannitol ^d	–	+	+	+	+
Melezitose ^a	–	+	–	+	+
Melibiose ^c	+	–	–	–	+
Pectin ^c	+	–	–	+	ND
Pyruvate ^c	–	–	+	+	ND
Rhamnose ^c	–	–	–	+	–
Ribose ^c	–	–	+	+	+
D-Ribose ^b	–	–	+	+	+
Salicin ^c	–	+	+	+	+
Salicin ^d	–	+	–	+	+
Sorbitol ^c	–	–	–	+	–
D-Sorbitol ^d	–	–	+	–	–
Sucrose ^{b,c}	–	–	+	+	+
Tellurite ^c	–	+	–	+	ND
Trehalose ^{c,d}	–	+	+	+	+
Raffinose ^c	–	–	–	–	+
Turanose ^c	+	–	–	+	–
Tween 40 ^c	+	–	–	–	ND
Voges–Proskauer ^a	–	+	+	+	ND
D-Amygdalin ^d	–	+	–	+	+
β-Galactosidase ^d	–	+	–	–	ND
Ala-Phe-Pro arylamidase ^d	+	–	+	–	ND
Ala-Phe-Pro arylamidase ^b	+	–	–	–	ND
β-Galactopyranosidase ^d	–	+	–	–	ND
L-Proline arylamidase ^d	–	+	+	+	ND
L-Pyrrolydonyl-arylamidase ^d	–	+	+	+	ND
Methyl-β-D-glucopyranoside ^d	–	+	–	+	ND
Arbutin ^b	–	+	–	+	+
5-Bromo-4-chloro-3-indoxyl-β-glucoside ^b	–	+	–	+	ND
α-Arabinosidase ^b	–	+	–	–	ND
Growth on CDC Anaerobe 5 % SBA with PEA ^e	Slow	Slow	Slow	Slow	ND
Growth in Schaedler broth ^e	Slow	Slow	Slow	Slow	ND

*Results for columns 1–4 obtained with: *a*, API 50CH strips; *b*, VITEK 2 ANC card; *c*, Biolog Gen III plates; *d*, VITEK 2 GP card; *e*, conventional tube.

(Whittaker *et al.*, 2007). The GC was an Agilent 6890 with a flame-ionization detector and an auto sampler and injector (7683; Agilent). A 25 cm (length) x 0.2 mm ID x 0.33 µm film thickness, cross-linked 5% phenylmethyl silicone fused silica capillary column (19091B-102; Agilent) was used to separate the fatty acids. The FAMES were analysed by GC using the rapid Microbial Identification System (MIS, MIDI Inc.) software (RTSB50) to identify the relative amounts of fatty acids in the bacteria (Sasser, 1990). Results were expressed as percentage of total fatty acids (Table 2). The major fatty acids of the four species of the genus *Lactobacillus* were C_{14:0}, C_{16:0}, C_{18:1ω9c}, C_{18:1ω7c}, and summed feature C_{16:1ω7c}/C_{16:1ω6c}. The percentages of all the major fatty acids were significantly different for strain SL1108^T compared to the other three species (Table 2). Therefore FAME analysis can be used to differentiate strain SL1108^T from the phylogenetically closely related species of the genus *Lactobacillus*.

The isomers of lactic acid formed from glucose during the growth of strain SL1108^T in MRS broth were determined with the TC D-/L-Lactic Acid test kit (Boehringer Mannheim), and strain SL1108^T was found to produce L-lactic acid isomer (data not shown).

Assessment and prediction of genomic G+C content for strain SL1108^T in comparison with *L. casei* ATCC 334, *L. rhamnosus* GG, *L. saniviri* DSM 24301^T and *Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC 11842 was performed by the analysis of G+C content for the 16S-ITS-23S, *rpoB*, *rpoC*, *pheS* and *rpoA* gene sequences (Table S3). This approach of assessing and predicting genomic G+C content based on the comparison of G+C content of individual housekeeping genes compared to the results of DNA G+C content determined experimentally by the HPLC (high performance liquid chromatography) technique, has been successfully

demonstrated and published for multiple bacterial species (Fournier *et al.*, 2006; Kuhnert & Korczak, 2006; Lorén *et al.*, 2010; Zavala *et al.*, 2005). Previously published data demonstrated minimal differences (± 3 –5%) between genomic G+C content predicted from analysis of individual housekeeping genes and the results of G+C content estimated using HPLC. The predicted genomic G+C contents based on the analysis of individual housekeeping genes for *L. casei* ATCC 334, *L. rhamnosus* GG, *L. saniviri* DSM 24301^T and *L. delbrueckii* subsp. *bulgaricus* ATCC 11842 (Table S3) were very similar to previously published data (Felis & Dellaglio, 2007; Oki *et al.*, 2012) for these four reference species, and/or the data available in GenBank from their whole genome sequencing projects (Genbank accession number CP000423, FM179322, and CR954253 for *L. casei*, *L. rhamnosus*, and *L. delbrueckii* subsp. *bulgaricus*, respectively). The predicted genomic G+C content for strain SL1108^T was 48.2 ± 2.6 mol%.

On the basis of phenotypic, chemotaxonomic characteristics and phylogenetic data, the three isolates, SL1108^T, SL1170 and SL60106, represent a novel species of the genus *Lactobacillus*, for which the name *Lactobacillus brantae* sp. nov. is proposed.

Description of *Lactobacillus brantae* sp. nov.

Lactobacillus brantae (bran'ta.e. L. gen. n. *brantae* of *Branta*, isolated from *Branta canadensis*).

Cells are Gram-stain-positive, catalase-negative, non-spore-forming, non-motile rods, approximately 0.5×4.0 µm, appearing as chains of plump coccobacilli, with a few bacilli and cocci forms also present. Colonies are formed on MRS agar, Rogosa SL agar and sheep blood agar under aerobic and anaerobic conditions. When incubated at 37 °C, good

Table 2. Cellular fatty acid compositions of strain SL1108^T and phylogenetically closely related species of the genus *Lactobacillus*

Strains: 1, SL1108^T; 2, *L. casei* ATCC 393^T; 3, *L. rhamnosus* ATCC 7469^T; 4, *L. paracasei* ATCC 25302^T. All data are from this study. Values are percentages of total fatty acids and are the mean of triplicate analysis $\pm$ standard deviation. Means for a variable not sharing a common superscript letter are significantly different ($P < 0.05$) as determined by the Duncan multiple comparison method, which was applied only if significant differences were determined to exist by ANOVA.

Cellular fatty acid	1	2	3	4	P-value
C _{14:0}	9.72 $\pm$ 0.30 ^c	14.01 $\pm$ 0.81 ^d	3.78 $\pm$ 0.30 ^a	8.19 $\pm$ 0.44 ^b	<0.001
C _{15:0}	1.01 $\pm$ 0.02 ^c	0.69 $\pm$ 0.06 ^b	0.00 $\pm$ 0.00 ^a	0.70 $\pm$ 0.09 ^b	<0.001
C _{16:1ω5c}	1.32 $\pm$ 0.03 ^b	1.48 $\pm$ 0.06 ^c	0.38 $\pm$ 0.05 ^a	1.77 $\pm$ 0.08 ^d	<0.001
C _{16:0}	27.57 $\pm$ 0.64 ^a	42.88 $\pm$ 1.44 ^c	37.23 $\pm$ 1.23 ^b	41.06 $\pm$ 0.59 ^c	<0.001
C _{17:0 ante}	2.18 $\pm$ 0.08 ^c	1.13 $\pm$ 0.33 ^b	0.08 $\pm$ 0.13 ^a	1.26 $\pm$ 0.03 ^b	<0.001
C _{17:1ω8c}	2.10 $\pm$ 0.03 ^d	0.35 $\pm$ 0.30 ^b	0.00 $\pm$ 0.00 ^a	0.90 $\pm$ 0.03 ^c	<0.001
C _{18:1ω9c}	26.27 $\pm$ 1.63 ^d	11.95 $\pm$ 0.25 ^b	3.99 $\pm$ 0.13 ^a	20.21 $\pm$ 0.43 ^c	<0.001
C _{18:1ω7c}	6.46 $\pm$ 0.35 ^a	14.01 $\pm$ 0.29 ^c	17.93 $\pm$ 1.13 ^d	12.40 $\pm$ 0.10 ^b	<0.001
C _{18:0}	0.73 $\pm$ 1.26 ^a	1.21 $\pm$ 1.06 ^a	3.71 $\pm$ 0.82 ^b	2.19 $\pm$ 0.20 ^{a,b}	0.019
C _{19:0 cyclo ω8c}	0.00 $\pm$ 0.00 ^a	0.00 $\pm$ 0.00 ^a	23.33 $\pm$ 1.31 ^b	0.00 $\pm$ 0.00 ^a	<0.001
C _{20:0}	2.60 $\pm$ 0.76 ^d	0.13 $\pm$ 0.11 ^a	1.59 $\pm$ 0.12 ^c	0.83 $\pm$ 0.42 ^b	0.001
Summed feature C _{16:1 ω7c} / C _{16:1 ω6c}	14.44 $\pm$ 0.22 ^d	8.98 $\pm$ 0.44 ^c	5.48 $\pm$ 0.78 ^a	7.79 $\pm$ 0.25 ^b	<0.001

growth appears in 48 h. Growth occurs at 25–40 °C, but not at 45 °C. At 37 °C, growth occurs in the presence of 2.0–4.0 % (w/v) NaCl but not in the presence of 6.5 % (w/v) NaCl. Colonies grow slowly on CDC Anaerobe 5 % sheep blood agar with phenylethyl alcohol during anaerobic incubation. Colonies on MRS plates are 1 mm in diameter, circular, entire, translucent, cream, convex and smooth. Growth appeared more dense in MRS broth, producing a thick settled layer of cells at the bottom of the tube. Cells are facultatively heterofermentative and do not produce gas from glucose. Negative result in tests for urease, indole and gelatinase. The type strain SL1108^T utilizes glucose, fructose, mannose, NAG and butyrate, but not arabinose, fucose, glycogen, raffinose, xylose, adonitol, arabitol, dulcitol, inulin, inosine, xylitol, acetic acid, formic acid, fusidic acid, glucuronic acid, mucic acid, propionic acid or quinic acid. The type strain SL1108^T did not use alanine, arginine, aspartic acid, glutamic acid, histidine, proline or serine, and did not grow in the presence of guanidine, lincomycin, minocycline, nalidixic acid, rifamycin, troleandomycin or vancomycin. In comparison to the phylogenetically closely related species *L. casei* ATCC 393^T, *L. paracasei* ATCC 25302^T, *L. rhamnosus* ATCC 7469^T and *L. saniviri* DSM 24301^T, strain SL1108^T reacted uniquely with cellobiose, galactose, mannitol, citric acid and aesculin (all negative), and dextrin (positive), and was negative in tests for L-proline arylamidase, L-pyrrolydonyl-arylamidase, and in the Voges-Proskauer test. Biochemical tests for cellobiose, aesculin, galactose, gentiobiose, mannitol, melezitose, ribose, salicin, sucrose, trehalose, raffinose, turanose, amygdalin and arbutin can be used for differentiation between *Lactobacillus saniviri* and the novel strains. The type strain SL1108^T has peptidoglycan type L-Lys-D-Asp, and major fatty acids are C_{14:0}, C_{16:0}, C_{18:1ω9c}, C_{18:1ω7c} and summed feature C_{16:1ω7c}/C_{16:1ω6c}.

The type strain is SL1108^T (=ATCC BAA-2142^T=LMG 26001^T=DSM 23927^T) and was isolated from the faeces of apparently healthy wild Canada geese (*Branta canadensis*) collected in Great Falls Park, Maryland, USA in June 2010. Two additional strains are SL1170 and SL60106.

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