**Lactobacillus composti** sp. nov., a lactic acid bacterium isolated from a compost of distilled shochu residue

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Two strains of lactic acid bacteria, strains NRIC 0689\(^T\) and NRIC 0690, were isolated from a compost of distilled shochu residue in Japan. The isolates showed quite low sequence similarity to known species of lactic acid bacteria on the basis of 16S rRNA gene sequence; the highest sequence similarities to NRIC 0689\(^T\) were shown by the type strains of *Lactobacillus satsumensis*, *L. plantarum*, *L. hilgardii*, *L. buchneri* and *L. parabuchneri* (92.9, 92.9, 92.8, 92.6 and 92.5 %, respectively). The isolates formed a distinct subcluster in the *Lactobacillus casei*–*Pediococcus* phylogenetic cluster. Levels of DNA–DNA relatedness revealed that the isolates belonged to the same taxon. Therefore, the isolates represent a novel species, for which the name *Lactobacillus composti* sp. nov. is proposed. The type strain is NRIC 0689\(^T\) (=JCM 14202\(^T\)=DSM 18527\(^T\)).

The closest known relatives of the isolates were determined by performing DataBase searches, and the sequences of closely related species were retrieved from the DDBJ database. Multiple alignments of the sequences were carried out with the program CLUSTAL_X, version 1.18 (Thompson et al., 1997). Distance matrices for the aligned sequences were calculated by using the two-parameter method of Kimura (1980). The neighbour-joining method (Saitou & Nei, 1987) was used to construct a phylogenetic tree. The robustness of individual branches was estimated by bootstrapping with 1000 replicates (Felsenstein, 1985). Phylogenetic trees were also constructed by using the maximum-likelihood (Cavalli-Sforza & Edwards, 1967) and maximum-parsimony (Klug & Farris, 1969) methods with PHYLIP version 3.65 (Felsenstein, 2005). The 16S rRNA gene sequences of the isolates were compared with each other, and the sequence of NRIC 0689\(^T\) was used to search for sequence similarity with DataBase. Approximately 1350 bp of the 16S rRNA gene sequences of the isolates and related species were used to construct phylogenetic trees. The sequences of NRIC 0689\(^T\) and NRIC 0690 were identical, and they showed quite low sequence similarity to known species of LAB. The 16S rRNA gene sequence similarity of NRIC 0689\(^T\) to known LAB species was less than 93 %, and the highest sequence similarity to NRIC 0689\(^T\) was shown by the type strains of *Lactobacillus satsumensis*, *L. plantarum*, *L. hilgardii*, *L. buchneri* and *L. parabuchneri* (92.9, 92.9, 92.8, 92.6 and 92.5 %, respectively). The genus *Lactobacillus* consists of several phylogenetic groups, originally including the *Lactobacillus acidophilus*, *Lactobacillus buchneri*, *Lactobacillus plantarum*, *Lactobacillus reuteri* and *Lactobacillus salivarius* phylogenetic groups (Schleifer & Ludwig, 1995); later, the *Lactobacillus casei* and *Lactobacillus sakei*
phylogenetic groups were proposed (Hammes & Hertel, 2006). However, the novel isolates did not belong to any of the phylogenetic groups, and formed a distinct subcluster in the L. casei–Pediococcus phylogenetic cluster by using the neighbour-joining analysis (Fig. 1) (Collins et al., 1991). Similar tree topologies were obtained by using maximum-likelihood and maximum-parsimony methods (see Supplementary Figs S1 and S2 in IJSEM Online). A few species, such as Lactobacillus bifereaments and Lactobacillus coryniformis, are known to be outmembers of the phylogenetic groups (Hammes & Hertel, 2006). The phylogenetic positions of the isolates indicated that the isolates were also outmembers. Recently, the genus Paralactobacillus, related to the L. casei–Pediococcus phylogenetic cluster, was described (Leisner et al., 2000). The sequence similarity between the genus Paralactobacillus and species in the L. casei–Pediococcus phylogenetic cluster was less than 91.7 % (Leisner et al., 2000), and this value was close to that mentioned above for NRIC 0689T.

Levels of DNA–DNA relatedness among the isolates and DNA base compositions (G + C content) were determined by methods described previously (Endo & Okada, 2005). The extraction and isolation of bacterial DNAs were performed by the method of Marmur (1961) as modified by Ezaki et al. (1983). The isolates showed a high level of DNA–DNA relatedness (93 %) to each other. Therefore, we concluded that the isolates belonged to the same taxon. The sequence similarities of the 16S rRNA genes of the isolates to known LAB species were less than 93 %, as mentioned above, and this value is considerably lower than the recommended value for species differentiation (97 %; Stackebrandt & Goebel, 1994). Therefore, DNA–DNA hybridization between the isolates and known LAB species was not carried out. Strains NRIC 0689T and NRIC 0690 both had a G + C content of 47 mol%.

For differentiation of the isolates, randomly amplified polymorphic DNA (RAPD) fingerprinting was performed by a method described previously (Endo & Okada, 2006). Primers A (5′-CCGCAGCCCA) and E (5′-GGCTCGGTT) were used in this study. The isolates showed slightly different fingerprints (Fig. 2), and the difference was reproducible. DNA–DNA relatedness revealed that the isolates belonged to the same species, and RAPD fingerprinting indicated that they were separated within the species at the strain level.

Morphological, physiological and biochemical characteristics of the isolates were determined by methods described previously (Endo & Okada, 2005) and MRS both was used as a basal medium. The detailed characteristics of the isolates are described in the species description. The characteristics of the isolates were not compared with any other species in the genus Lactobacillus because the sequence similarity was so low. The isolates were facultatively heterofermentative LAB and produced DL-lactic acid from D-glucose. They grew well at 15 °C and 37 °C and grew slowly at 10 °C, but not at 45 °C. The strains produced acid from various pentoses, hexoses and oligosaccharides. These characteristics are common amongst the facultatively heterofermentative

Fig. 1. Phylogenetic relationships of strains NRIC 0689T and NRIC 0690 to related species based on 16S rRNA gene sequences. The tree was constructed by the neighbour-joining method. Leuconostoc mesenteroides NRIC 1541T was used as an outgroup. Bootstrap values above 70 % are given at branching points. Maximum-likelihood- and maximum-parsimony-based trees are available as supplementary material in IJSEM Online.

Fig. 2. RAPD-PCR fingerprinting of isolates NRIC 0689T (lanes 1) and NRIC 0690 (lanes 2). Primers A and E were used. Lane M, size marker (500 bp DNA ladder; Takara-bio).
lactobacilli, meaning that identification of this species based on phenotypic characteristics is difficult. DNA–DNA hybridization is therefore appropriate for identification of this species.

Based on the data provided, the phenotypic characteristics of the isolates were similar to those of members of the facultatively heterofermentative lactobacilli. However, they showed low 16S rRNA gene sequence similarity to known species of LAB, including facultatively heterofermentative lactobacilli, and formed a distinct subcluster in the L. casei–Pediococcus group by phylogenetic analysis. Thus, the isolates represent a novel species, for which the name Lactobacillus composti sp. nov. is proposed.

**Description of Lactobacillus composti sp. nov.**

*Lactobacillus composti* (com.pos’ti. N.L. gen. n. composti of compost, from which the type strain was isolated).

Cells are Gram-positive, non-motile rods, 0.8 × 2–6 μm. Cells occur singly or in pairs and chains. They are facultatively anaerobic and catalase-negative. Colonies on MRS agar are beige, smooth and approximately 1 mm in diameter after incubation for 2 days at 30 °C. Facultatively heterofermentative. No gas is produced from D-glucose. DL-Lactic acid is a major end-product from D-glucose; D- and L-lactic acid are produced in the ratio 1 : 1. Nitrate is not reduced. Acid is produced from D-glucose, D-fructose, D-galactose, D-mannose, L-arabinose, D-xylene, maltose, melibiose, sucrose, D-trehalose, D-melezitose, D-mannitol and D-sorbitol and produced weakly from D-glucanate, L-rhamnose and salicin, but not from D-ribose, cellobiose, lactose, raffinose or starch. Dextran is not formed from sucrose. Cells grow at 15–37 °C and grow slowly at 10 °C, but not at 45 °C. Both known strains grow at pH 4.0–8.5 and grow weakly at pH 3.5 and 9.0. No growth is determined in MRS broth containing 5 % (w/v) NaCl. Cells do not contain meso-diaminopimelic acid in their peptidoglycan. The DNA G + C content is 47 mol%.

The type strain is NRIC 0689T (=JCM 14202T=DSM 18527T). Strain NRIC 0690 is a reference strain. Both known strains were isolated from a compost of distilled shochu residue collected at a shochu distillery in Miyazaki prefecture, southern Kyushu area, Japan, in 2003.

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**References**


