***Lactobacillus paralimentarius* sp. nov., isolated from sourdough**

Yimin Cai,¹ Hisashi Okada,² Haruhiko Mori,² Yoshimi Benno¹
and Takashi Nakase¹

Author for correspondence: Yimin Cai. Tel: +81 48 467 9562. Fax: +81 48 462 4619.
e-mail: cai@ulmus.riken.go.jp

¹ Japan Collection of Microorganisms, The Institute of Physical and Chemical Research, Wako, Saitama 351-0198, Japan

² Laboratory of Microorganism, Takaki Bakery Company Ltd, Hiroshima-shi, Hiroshima 739-0323, Japan

Six strains of lactic acid bacteria isolated from sourdough were characterized taxonomically. They were Gram-positive, catalase-negative, facultatively anaerobic rods that did not produce gas from glucose. Morphological and physiological data indicated that the strains belong to the genus *Lactobacillus* and they were similar to *Lactobacillus alimentarius* in phenotypic characteristics. These strains shared the same phenotypic characteristics and exhibited intragroup DNA homology values of over 89.8 %, indicating that they comprised a single species. The G+C content of the DNA for the strains was 37.2–38.0 mol%. The 16S rRNA sequence of representative strain TB 1^T was determined and aligned with that of other *Lactobacillus* species. This strain was placed in the genus *Lactobacillus* on the basis of phylogenetic analysis. *L. alimentarius* was the most closely related species in the phylogenetic tree and this species also showed the highest sequence homology value (96 %) with strain TB 1^T. DNA–DNA hybridization indicated that strain TB 1^T did not belong to *L. alimentarius*. It is proposed that these strains are placed in the genus *Lactobacillus* as a new species, *Lactobacillus paralimentarius* sp. nov. The type strain of *L. paralimentarius* is TB 1^T, which has been deposited in the Japan Collection of Microorganisms (JCM) as strain JCM 10415^T.

Keywords: *Lactobacillus paralimentarius* sp. nov., 16S rRNA, DNA–DNA hybridization

INTRODUCTION

The genus *Lactobacillus* encompasses an assemblage of Gram-positive, catalase-negative, non-sporing, rod-shaped organisms and is presently composed of over 50 recognized species (Collins *et al.*, 1991). This classification is based on phenotypic and genetic characteristics. The genetic interrelationships of the genus *Lactobacillus* and related lactic acid bacteria have been studied extensively by using 16S rRNA sequence analysis and DNA–DNA hybridization experiments, and new genera and new species have been added (Cai & Collins, 1994; Collins *et al.*, 1989, 1993, 1991, 1990). Recent results clearly indicate that the genus *Lactobacillus*, as presently constituted, is very heterogeneous phylogenetically, and that some *Pediococcus* and *Lactobacillus* species exhibit a high degree of 16S rRNA sequence similarity with each other and

form a phylogenetically coherent group that is quite separate from other lactic acid bacteria (Collins *et al.*, 1989, 1993, 1991, 1990). Collins *et al.* (1991) reported that comparative analysis of 16S rRNA sequence data revealed the presence of three phylogenetically distinct clusters. Cluster 1 (designated the *Lactobacillus delbrueckii* group) includes *L. delbrueckii*, the type species of the genus, and 11 other obligately homofermentative species. Cluster 2 (designated the *Lactobacillus casei*/*Pediococcus* group) comprises 32 *Lactobacillus* species (including *Lactobacillus alimentarius*) and five *Pediococcus* species. Cluster 3 (designated the *Leuconostoc paramesenteroides* group) includes the heterofermentative *Weissella*. The 16S rRNA groups of the genus *Lactobacillus* do not support the classical division into the subgenera *Thermobacterium*, *Streptobacterium* and *Betabacterium* (Collins *et al.*, 1991) or the three physiological groups (obligately homofermentative, facultatively heterofermentative and obligately heterofermentative) outlined in *Bergey's Manual of Systematic Bacteriology* (Kandler & Weiss, 1986).

The DDBJ accession number for the 16S rRNA sequence of TB 1^T is AB018528.

Lactobacillus species are widely distributed in various fermented foods, dairy products, and plant and animal material. There have been several reports (Okada *et al.*, 1992; Oura *et al.*, 1982; Spicher, 1984; Spicher & Schroder, 1978, 1980) of lactobacilli occurring among the dominant microbial population on sourdough, where they may contribute to dough fermentation. Some isolates from sourdough have been identified with an early classification scheme as belonging to the previously described *Lactobacillus* species (Okada *et al.*, 1992; Spicher, 1984; Spicher & Schroder, 1978). However, in our experience many isolates cannot be identified as any previously described species.

In the present study, *Lactobacillus* strains isolated from five samples of sourdough were examined. To determine their taxonomic status, the isolates were also studied by 16S rRNA sequence analysis and DNA hybridization experiments, which revealed one new species, for which the name *Lactobacillus paralimentarius* was proposed.

METHODS

Strains studied. Six strains were isolated from two samples of sourdough (Takaki Bakery Company) on LBS agar (Becton Dickinson) and Lactobacilli MRS broth (Difco) containing 1.5% agar incubated at 30 °C for 2 d under anaerobic conditions. The sourdough, without yeast or bacteria addition, was fermented at 27 °C for 12 h. Each strain was purified twice by streaking on MRS agar and the pure cultures were grown in the same agar at 30 °C for 24 h, suspended with nutrient broth (Difco) and dimethyl sulfoxide in the ratio of 9:1 and stored as stock cultures at -80 °C for further examination. The *Lactobacillus* type strains were obtained from the Japan Collection of Micro-organisms (JCM), The Institute of Physical and Chemical Research, Wako, Saitama, Japan.

Morphological, physiological and biochemical tests. Gram stain and morphology were examined after 24 h incubation on MRS agar. Catalase activity, nitrite reduction and gas production from glucose were determined as described by Kozaki *et al.* (1992). Tests for growth at pH 4.5 and 7.0, in 4.5 and 7.0% NaCl, and at 10, 15, 45 and 50 °C were performed in duplicate in MRS broth. Results were recorded after 48 h. Carbohydrate fermentation tests were carried out with the API 50 CH system for Lactobacilli (bioMérieux) according to the manufacturer's instructions; reactions were determined after incubation at 30 °C for 48 h.

16S rRNA sequencing. The 16S rRNA sequence coding region of strain TB 1^T was amplified by PCR performed in a PCR ThermalCycler (Takara Shuzo) as described by Suzuki *et al.* (1996). The sequences of the PCR products were determined directly by using an ALFexpress AutoCycle Sequence kit (Pharmacia Biotech) with the primers as described by Suzuki *et al.* (1996). Analyses of DNA sequence reactions were performed with an ALFexpress DNA sequencer (Pharmacia Biotech). The 16S rDNA sequence of strain TB 1^T was deposited in the DNA Database of Japan (DDBJ). Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura & Ohta, 1972) and a phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987). The topology of trees was evaluated by bootstrap analysis of the sequence data with CLUSTAL W

software (Thompson *et al.*, 1994) based on 100 random resamplings. This sequence was aligned with the following published sequences from DDBJ, GenBank and EMBL: *Enterococcus faecalis* JCM 5803^T (AB012212), *Enterococcus faecium* JCM 5804^T (AB012213), *Lactobacillus acetotolerans* DSM 20749^T (M58801), *Lactobacillus acidophilus* ATCC 4365^T (M58802), *Lactobacillus agilis* DSM 20509^T (M58803), *L. alimentarius* DSM 20249^T (58804), *Lactobacillus amylophilus* DSM 20533^T (M58806), *Lactobacillus amylovorus* DSM 20531^T (M58805), *Lactobacillus animalis* NCDO 2425^T (X61133), *Lactobacillus aviarius* DSM 20655^T (M58808), *Lactobacillus bifementans* DSM 20003^T (M58809), *Lactobacillus brevis* NCDO 1749^T (X61134), *Lactobacillus buchneri* DSM 20057^T (M58811), *Lactobacillus carnis* DSM 20722^T (M58812), *L. casei* ATCC 334^T (D86517), *Lactobacillus catenaformis* ATCC 25536^T (M23729), *Lactobacillus collinoides* JCM 1123^T (AB005893), *Lactobacillus coryniformis* DSM 20001^T (M58813), *L. delbrueckii* JCM 1002^T (AB007908), *Lactobacillus fermentum* NCDO 1750^T (X61142), *Lactobacillus farciminis* ATCC 29644^T (M58817), *Lactobacillus fructivorans* DSM 20203^T (X76330), *Lactobacillus fructosus* NCDO 2345^T (X61140), *Lactobacillus gasseri* NCDO 2233^T (X61137), *Lactobacillus helveticus* NCDO 2712^T (X61141), *Lactobacillus hilgardii* DSM 20176^T (M58821), *Lactobacillus johnsonii* ATCC 33200^T (AJ-002515), *Lactobacillus lactis* DSM 20072^T (M58823), *Lactobacillus lindneri* DSM 20690^T (X95421), *Lactobacillus mali* DSM 20444^T (M58824), *Lactobacillus maltaromicus* JCM 1154^T (X54420), *Lactobacillus minutus* (S44204), *Lactobacillus murinus* DSM 20452^T (M58826), *Lactobacillus oris* DSM 4864^T (X94229), *Lactobacillus panis* DSM 6035^T (X94230), *Lactobacillus pentosus* JCM 1558^T (D79211), *Lactobacillus plantarum* JCM 1149^T (D79210), *Lactobacillus pontis* LTH 2587 (X76329), *Lactobacillus rhamnosus* JCM 1136^T (D16552), *Lactobacillus reuteri* DSM 20016^T (L23-507), *Lactobacillus ruminis* DSM 20403^T (M58828), *Lactobacillus sakei* DSM 20017^T (M58829), *Lactobacillus salivarius* DSM 20554^T (M59054), *Lactobacillus sanfranciscensis* JCM 5668^T (X61132), *Lactobacillus sharpeae* DSM 20505^T (M58831), *Lactobacillus* sp. ATCC 8317 (M58832), *Lactobacillus vaginalis* NCTC 12197^T (X61136), *Lactobacillus* sp. ATCC 13133 (M59295), *Lactobacillus zeae* ATCC 15820^T (D86516), *Lactococcus lactis* NCDO 2118^T (X54260), *Pediococcus acidilactici* NCDO 2767^T (X95976), *Pediococcus dextrinicus* JCM 5887^T (D87679), *Pediococcus parvulus* JCM 5889^T (D88528), *Pediococcus pentosaceus* DSM 20336^T (M58834), *Weissella confusa* NCDO 1586^T (X52567), *Weissella halotolerans* DSM 20190^T (M23037), *Weissella hellenica* NCFB 2973^T (X95981), *Weissella kandleri* NCDO 2753^T (X52570), *Weissella minor* NCDO 1973^T (X52569) and *Weissella viridescens* NCDO 1655^T (X52568). *Bacillus subtilis* NCDO 1769^T (X60646) was used as an outgroup organism.

DNA base composition and DNA-DNA hybridization. DNA was extracted from cells harvested from MRS broth culture which had been incubated for 8 h at 30 °C. It was purified by the procedure of Saitou & Miura (1963). DNA base composition was determined by the method of Tamaoka & Komagata (1984) by using HPLC following enzymic digestion of DNA to deoxyribonucleosides. The equimolar mixture of four deoxyribonucleotides in a GC kit (Yamasa Shoyu) was used as the quantitative standard. DNA-DNA relatedness was determined by the method of Ezaki *et al.* (1989) by using photobiotin and microplates.

Table 1. Carbohydrate fermentation patterns of *Lactobacillus* species

All strains were Gram-positive, catalase-negative rods that produce acid from D-glucose, D-fructose, D-mannose and N-acetylglucosamine, but failed to produce acid from erythritol, D-arabinose, L-xylose, adonitol, methyl β -xyloside, dulcitol, inositol, inulin, xylitol, D-lyxose, D- and L-fucose, D- and L-arabitol, and 2- and 5-ketogluconate. +, Positive; -, negative; + -, 3/5 strains positive; w, weakly positive. Strains: 1, *L. paralimentarius* TB 1^T; 2, other *L. paralimentarius* isolates ($n = 5$); 3, *L. alimentarius* JCM 1095^T; 4, *L. acidophilus* JCM 1132^T; 5, *L. amylophilus* JCM 1125^T; 6, *L. amylovorus* JCM 1127^T; 7, *L. helveticus* JCM 1120^T; 8, *L. pentosus* JCM 1558^T; 9, *L. plantarum* JCM 1149^T; 10, *L. rhamnosus* JCM 1136^T.

Carbohydrate	1	2	3	4	5	6	7	8	9	10
Glycerol	-	-	-	-	-	-	-	+	-	-
L-Arabinose	-	-	-	-	-	-	-	+	+	-
Ribose	+	+	+	-	-	-	-	+	+	+
D-Xylose	-	-	+	-	-	-	-	+	-	-
Galactose	-	-	+	-	+	+	+	+	+	+
L-Sorbitol	-	-	-	-	-	-	-	-	-	+
Rhamnose	-	-	-	-	-	-	-	w	+	+
Mannitol	-	-	-	-	-	+	-	+	+	+
Sorbitol	-	-	-	-	-	-	-	-	+	+
Methyl α -D-mannoside	-	-	-	-	-	-	-	-	+	-
Methyl α -D-glucoside	-	-	-	-	-	-	-	-	+	+
Amygdalin	+	+	+	w	-	+	-	+	+	+
Arbutin	+	+	+	-	-	+	-	+	+	+
Aesculin	+	+	+	+	-	+	-	+	-	+
Salicin	+	+	+	+	-	w	-	+	+	+
Cellobiose	+	+	+	+	-	+	-	+	+	+
Maltose	+	+	+	w	+	+	+	+	+	+
Lactose	-	-	-	+	-	-	+	+	+	+
Melibiose	-	-	-	-	-	-	-	+	+	-
Saccharose	+	+	+	+	-	+	-	+	+	-
Trehalose	+	+	+	+	-	+	+	+	+	+
Melezitose	-	-	-	-	-	-	-	-	+	+
D-Raffinose	-	-	-	-	-	-	-	-	w	-
Starch	-	-	-	-	+	+	-	-	-	-
Glycogen	-	-	-	-	+	+	-	-	-	-
β -Gentiobiose	+	+	-	+	-	+	-	w	+	+
D-Turanose	-	-	-	-	-	-	-	-	+	+
D-Tagatose	-	-	-	-	-	-	-	-	-	+
Gluconate	-	-	-	-	-	-	-	w	w	-

RESULTS AND DISCUSSION

Morphology and biochemical properties

All isolates were Gram-positive, catalase-negative, facultatively anaerobic rods that did not produce gas from glucose. These isolates were able to grow at pH 4.5 and in 6.5% NaCl, but not above 45 °C. As shown in Table 1, strain TB 1^T and the other five isolates showed the same phenotypic characteristics, with the exception of maltose fermentation patterns. These characteristics were similar to those of *L. alimentarius*, although D-xylose, galactose and β -gentiobiose fermentation patterns differed in *L. alimentarius* and the isolates. These isolates were quite different from other *Lactobacillus* type strains in some biochemical characteristics and sugar fermentation patterns.

Lactobacillus species are a major component of the microbial flora which are usually present in sourdough sponges (Okada *et al.*, 1992; Spicher, 1984; Spicher & Schroder, 1978, 1980). Some sourdough-associated lactobacilli have been characterized by phenotypic features and they have been identified as *L. acidophilus*, *L. alimentarius*, *L. brevis*, *L. buchneri*, *L. casei*, *Lactobacillus curvatus*, *L. farciminis*, *L. fermentum*, *L. fructivorans*, *L. hilgardii*, *L. reuteri* and *L. sanfranciscensis* (Okada *et al.*, 1992; Oura *et al.*, 1992; Spicher, 1984; Spicher & Schroder, 1978, 1980). In the present study, all strains were isolated from sourdough and, from their morphology and biochemical properties, these isolates were considered to be typical lactobacilli. As shown in Table 1, the strains were phenotypically similar to *L. alimentarius* JCM 1095^T,

Fig. 1. Phylogenetic tree derived from 16S rDNA sequence of the genus *Lactobacillus* and related lactic acid bacteria. The tree was created using the neighbour-joining method and K_{nuc} values. Numbers indicate bootstrap values for branch points.

but they differed from *L. alimentarius* JCM 1095^T in the fermentation patterns of three sugars. These strains can be distinguished from other *Lactobacillus* species by physiological and biochemical characteristics. Results of the present study have shown that the isolates were closely related to *L. alimentarius* and that they could not be identified down to a species level on the basis of phenotypic characteristics.

16S rRNA sequence and DNA-DNA hybridization

The 16S rRNA sequence of TB 1^T consisted of 1476 bases. The phylogenetic tree shown in Fig. 1 was reconstructed from evolutionary distances by the neighbour-joining method. Phylogenetic analysis placed representative strain TB 1^T in the cluster comprising *L. plantarum* JCM 1149^T, *L. pentosus* JCM 1558^T, *L. collinoides* JCM 1123^T and *L. alimentarius* JCM 1095^T. This cluster was recovered in 71% of the bootstrap trees. *L. alimentarius* JCM 1095^T was the most closely related species in the phylogenetic tree and showed the highest sequence homology value (96%) with strain TB 1^T.

DNA base composition and DNA-DNA hybridization data are shown in Table 2. Strains TB 1^T, TB 16, TB 30, TB 56, TB 60 and TB 110 had a G + C content of 37.2–38.0 mol%. The data are within the range for the genus *Lactobacillus* (32–53 mol%). These strains had DNA homology values of 89.8–99.7% with each other, showing that they belonged to a single species, and they had DNA homology values of less than 20.5% with *L. alimentarius* JCM 1095^T, and values of less than 12.5% with *L. collinoides* JCM 1123^T, *L. plantarum* JCM 1149^T and *L. pentosus* JCM 1558^T.

Following 16S rRNA sequence analysis, *L. alimentarius* formed a relatively long branch indicative of no

Table 2. DNA base compositions and DNA relatedness among *Lactobacillus paralimentarius* and phylogenetically closely related *Lactobacillus* species

JCM, Japan Collection of Microorganisms. ^T, type strain.

Strain	G + C content (mol %)	DNA-DNA reassociation (%) with:				
		TB 1 ^T	TB 16	JCM 1095 ^T	JCM 1149 ^T	JCM 1558 ^T
<i>L. paralimentarius</i> TB 1 ^T	37.4	100	98.5	14.9	8.2	3.5
<i>L. paralimentarius</i> TB 16	37.8	98.4	100	18.2	3.4	4.2
<i>L. paralimentarius</i> TB 30	38.0	92.3	95.5	20.3	5.6	5.8
<i>L. paralimentarius</i> TB 56	37.2	95.6	99.7	18.7	10.3	6.4
<i>L. paralimentarius</i> TB 60	37.6	98.7	93.6	15.6	8.7	7.6
<i>L. paralimentarius</i> TB 110	37.8	89.8	91.8	13.3	6.4	5.0
<i>L. alimentarius</i> JCM 1095 ^T	37.5	20.5	15.6	100	3.9	4.8
<i>L. collinoides</i> JCM 1123 ^T	45.6	7.3	12.5	7.6	15.4	13.8
<i>L. plantarum</i> JCM 1149 ^T	45.2	6.5	11.3	8.4	100	18.7
<i>L. pentosus</i> JCM 1558 ^T	44.0	8.8	8.9	6.0	18.6	100

specific relationship to other species within the *L. casei*/*Pediococcus* group (Collins *et al.*, 1991). However, in the present study, the tree topology indicated that strain TB 1^T formed a cluster only with *L. alimentarius*, and that *L. plantarum*, *L. pentosus* and *L. collinoides* appear only loosely associated with this pair. As shown in Table 2, the DNA–DNA hybridization results demonstrated that strain TB 1^T had low levels of DNA relatedness (<20.5%) with *L. alimentarius* JCM 1095^T and other type strains of previously described species, showing that they were different species.

On the basis of phenotypic and genotypic characteristics, it is concluded that the strains isolated from sourdough represent a new species within the genus *Lactobacillus*, for which we propose the name *Lactobacillus paralimentarius* sp. nov.

Description of *Lactobacillus paralimentarius* sp. nov.

Lactobacillus paralimentarius (pa.ra.li.men.ta'ri.us. Gr. pref. *para* beside; L. adj. *alimentarius* pertaining to food).

Grows well anaerobically on MRS agar at 37 °C and cells are facultatively anaerobic and heterofermentative, Gram-positive, catalase-negative, non-sporing, non-motile rods with a length of about 4.0–7.0 µm and a width of 0.7–1.0 µm. Growth occurs at pH 4.5, 40 °C and 6.5% NaCl, but not above 45 °C. Acid is produced from ribose, D-glucose, D-fructose, D-mannose, N-acetylglucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, saccharose, trehalose and β-gentiobiose. Acid is not produced from glycerol, erythritol, D- and L-arabinose, D- and L-xylose, adonitol, methyl β-xyloside, galactose, L-sorbitol, rhamnose, dulcitol, inositol, mannitol, sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, lactose, melibiose, inulin, melizitose, D-raffinose, starch, glycogen, xylitol, D-turanose, D-lyxose, D-tagatose, D- and L-fucose, D- and L-arabitol, gluconate or 2- and 5-ketogluconate. Three of the six strains were positive for maltose. DNA G+C content is 37.2–38.0 mol%, as determined by HPLC. Type strain is TB 1^T (= JCM 10415^T).

REFERENCES

- Cai, J. & Collins, M. D. (1994). Evidence for a close phylogenetic relationship between *Melissococcus pluton*, the causative agent of European foulbrood disease, and the genus *Enterococcus*. *Int J Syst Bacteriol* **44**, 365–367.
- Collins, M. D., Ash, C., Farrow, J. A., Wallbanks, S. & Williams, A. M. (1989). 16S ribosomal ribonucleic acid sequence analyses of lactobacilli and related taxa. Description of *Vagococcus fluvialis* gen. nov., sp. nov. *J Appl Bacteriol* **67**, 453–460.
- Collins, M. D., Williams, A. M. & Wallbanks, S. (1990). The phylogeny of *Aerococcus* and *Pediococcus* as determined by 16S rRNA sequence analysis: description of *Tetragenococcus* gen. nov. *FEMS Microbiol Lett* **70**, 255–262.
- Collins, M. D., Rodrigues, U., Ash, C., Aguirre, M., Farrow, J. A. E., Martinez-Murcia, A., Phillips, B. A., Williams, A. M. & Wallbanks, S. (1991). Phylogenetic analysis of the genus *Lactobacillus* and related lactic acid bacteria as determined by reverse transcriptase sequencing of 16S rRNA. *FEMS Microbiol Lett* **77**, 5–12.
- Collins, M. D., Metaxopoulos, J. & Wallbanks, S. (1993). Taxonomic study on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. *J Appl Bacteriol* **75**, 595–603.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid–deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Kandler, O. & Weiss, N. (1986). Genus *Lactobacillus*. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 1209–1234. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.
- Kimura, M. & Ohta, T. (1972). On the stochastic model for estimation of mutation distance between homologous proteins. *J Mol Evol* **2**, 87–90.
- Kozaki, M., Uchimura, T. & Okada, S. (1992). *Experimental Manual for Lactic Acid Bacteria*, pp. 29–72. Tokyo: Asakura-syouten.
- Okada, S., Ishikawa, M., Yoshida, I., Uchimura, T., Ohara, N. & Kozaki, M. (1992). Identification and characteristics of lactic acid bacteria isolated from sourdough sponges. *Biosci Biotechnol Biochem* **56**, 572–575.
- Oura, H., Suomalainen, H. & Viskari, R. (1982). Breadmaking. In *Economic Microbiology*, vol. 7, *Fermented Food*, pp. 88–146. Edited by A. H. Rose. London: Academic Press.
- Saitou, H. & Miura, K. (1963). Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim Biophys Acta* **72**, 619–629.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Spicher, G. (1984). The microflora of sourdough. XVII. Communication: bacterial composition of commercial sourdough starters. *Z Lebensm Unters Forsch* **178**, 106–109.
- Spicher, G. & Schroder, R. (1978). The microflora of sourdough. IV. Communication: bacterial composition of sourdough starters (Genus *Lactobacillus* Beijerinck). *Z Lebensm Unters Forsch* **167**, 342–354.
- Spicher, G. & Schroder, R. (1980). The microflora of sourdough. IX. Communication: comparative investigations over the performance of lactic acid bacteria (Genus *Lactobacillus* Beijerinck) occurring in starter cultures ('Reinzuchtsauer'). *Z Lebensm Unters Forsch* **170**, 262–266.
- Suzuki, K., Sasaki, J., Uramoto, M., Nakase, T. & Komagata, K. (1996). *Agromyces mediolanus* sp. nov., nom. rev., comb. nov., a species for '*Corynebacterium mediolanum*' Mamoli 1939 and for some aniline-assimilating bacteria which contain 2,4-diaminobutyric acid in the cell wall peptidoglycan. *Int J Syst Bacteriol* **46**, 88–93.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL w: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.