Lactobacillus acidifarinae sp. nov. and *Lactobacillus zymae* sp. nov., from wheat sourdoughs

M. Vancanneyt,¹ P. Neysens,² M. De Wachter,¹ K. Engelbeen,¹ C. Snauwaert,¹ I. Cleenwerck,¹ R. Van der Meulen,² B. Hoste,¹ E. Tsakalidou,³ L. De Vuyst² and J. Swings^{1,4}

Correspondence

M. Vancanneyt

Marc.Vancanneyt@UGent.be

^{1,4}BCCM/LMG Bacteria Collection¹ and Laboratory of Microbiology, Faculty of Sciences⁴, Ghent University, K. L. Ledeganckstraat 35, B-9000 Ghent, Belgium

²Research Group of Industrial Microbiology, Fermentation Technology and Downstream Processing (IMDO), Department of Applied Biological Sciences, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium

³Laboratory of Dairy Research, Agricultural University of Athens (AUA), GR-11855 Athens, Greece

Three heterofermentative lactic acid bacteria, obtained from Greek and Belgian artisanal wheat sourdoughs, were preliminarily identified as *Lactobacillus brevis*-like after screening using whole-cell protein fingerprinting and 16S rRNA gene sequence analysis. The three sourdough isolates showed nearly identical sequences (>99.7% sequence similarity), and highest similarities of 98.2 and 97.6% were obtained to the species *Lactobacillus spicheri* and *Lactobacillus brevis*, respectively. Growth characteristics, biochemical features, amplified fragment length polymorphism fingerprinting, DNA–DNA hybridizations and DNA G + C contents demonstrated that the isolates represent two novel *Lactobacillus* species. The names *Lactobacillus acidifarinae* sp. nov. and *Lactobacillus zymae* sp. nov. are proposed and the type strains are LMG 22200^T (= R-19065^T = CCM 7240^T) and LMG 22198^T (= R-18615^T = CCM 7241^T), respectively.

Sourdough is a mixture of flour and water that is fermented with lactic acid bacteria (LAB) (Gänzle *et al.*, 1998; Vogel *et al.*, 1999). Sourdough fermentations improve dough properties, bread texture and flavour, retard the staling process and protect bread from mould and bacterial spoilage (Corsetti *et al.*, 1998; Hammes & Gänzle, 1998; Rosenquist & Hansen, 1998). Because of their artisanal and region-dependent handling, sourdoughs are a huge source of diverse LAB species and strains.

Lactobacillus brevis is one of the common taxa found in several sourdoughs such as artisanal Greek wheat sourdoughs (De Vuyst *et al.*, 2002) and Belgian sourdoughs (our ongoing study). In the latter two surveys, most strains

identified as *L. brevis* showed the characteristic taxonomic properties of the species. Three isolates were aberrant in their phenotypic and genotypic properties and were assigned as *L. brevis*-like. In the present study, their taxonomic position was investigated.

The three strains studied were isolated on different occasions from three different artisanal wheat sourdoughs. One strain, ACA-DC 3411 t1 (= LMG 22199), was isolated in 1997 from Greek sourdough (De Vuyst *et al.*, 2002). The strain was purified after suspension (1:10, w/v) and serial dilutions in saline (0.9% NaCl, w/v), plating on MRS agar supplemented with 2% (w/v) maltose and incubation at 30 °C for 48 h. Two further strains, LMG 22198^T and LMG 22200^T, were isolated in 2003 from Belgian sourdoughs. They were purified after suspension (1:10, w/v) and serial dilution in peptone/water (0.1%, w/v), plating on MRS agar (pH 5.4) supplemented with 1% (w/v) maltose and fructose and 0.1% (w/v) cycloheximide and incubation at 37 °C for 48 h. Bacteriological purity of all isolates was checked by plating and examining living and Gram-stained cells. Cultivation conditions for further experiments and

Published online ahead of print on 4 October 2004 as DOI 10.1099/ijs.0.63274-0.

Abbreviations: AFLP, amplified fragment length polymorphism; LAB, lactic acid bacteria.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains LMG 22198^T, LMG 22199 and LMG 22200^T are AJ632157, AJ422039 and AJ632158, respectively.

Table 1. Strains of *Lactobacillus* species studied

ACA-DC, Laboratory of Dairy Research, Agricultural University of Athens, Athens, Greece; CCM, Czechoslovak Collection of Microorganisms, Brno, Czech Republic; DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany; LMG, BCCM/LMG Bacteria Collection Laboratorium voor Microbiologie, Ghent University, Ghent, Belgium; LTH, Institute of Food Technology, University of Hohenheim, Stuttgart, Germany; NCFB, National Collection of Food Bacteria (now incorporated within NCIMB); NCIMB, National Collections of Industrial and Marine Bacteria, Aberdeen, UK; R, Research Collection Laboratorium voor Microbiologie, Ghent University, Ghent, Belgium.

Strain	Source, place and year of isolation
<i>L. acidifarinae</i> sp. nov. LMG 22200 ^T (=R-19065 ^T =CCM 7240 ^T)	Wheat sourdough, Belgium, 2003
<i>L. zymae</i> sp. nov. LMG 22198 ^T (=R-18615 ^T =CCM 7241 ^T) LMG 22199 (=ACA-DC 3411 t1=R-13317)	Wheat sourdough, Belgium, 2003 Wheat sourdough, Greece, 1997
<i>L. brevis</i> ACA-DC 4003 LMG 6906 ^T (=NCIMB 11973 ^T) LMG 8416 LMG 11435 (=NCFB 473) LMG 11438 (=NCFB 478) LMG 11495 (=NCFB 391) LMG 11989 (=DSM 2647) LMG 11993 (=NCFB 1053) LMG 14527 (=DSM 6265) LMG 16322 (=DSM 6235) LMG 18022 (=R-2085) R-18707	Wheat sourdough, Greece, 1997 Human faeces Unknown Silage, 1941 Tomato pulp Unknown Silage English hard cheese Spoiled beer, Germany Spoiled beer Zabady, Burundi, 1994 Sourdough, Belgium, 1997
<i>L. spicheri</i> LMG 21871 ^T (=LTH 5753 ^T)	Industrial processed rice sourdough, Germany

for maintenance of the sourdough isolates and reference strains listed in Table 1 were MRS agar (pH 5.4) and incubation at 30 °C for 24–48 h, unless indicated otherwise.

The three strains LMG 22198^T, LMG 22199 and LMG 22200^T were initially screened using PAGE of whole-cell proteins. Whole-cell protein extracts were prepared and SDS-PAGE was performed as described by Pot *et al.* (1994). Densitometric analysis, normalization and interpolation of protein profiles, and a numerical analysis were performed using the GELCOMP software package, versions 3.1 and 4.0, respectively (Applied Maths). After comparison with an in-house database, comprising profiles of nearly all recognized LAB species, the three isolates were identified as related to the species *L. brevis*. Among isolates of *L. brevis*, strain-specific differences were observed in the varying position of dominant protein bands in the molecular mass range 30–65 kDa and may indicate the presence of an S-layer on the outside of the bacterial cell (Boot *et al.*, 1996). The latter dense bands largely influenced the numerical analysis; after omitting this variable region from the cluster analysis, the three new isolates LMG 22198^T, LMG 22199 and LMG 22200^T occupied a distinct subgroup in a dendrogram, as shown in Fig. 1. Twelve recognized *L. brevis* strains,

including the type strain LMG 6906^T, constituted a homogeneous and separate cluster. *Lactobacillus spicheri* LMG 21871^T, the closest phylogenetic neighbour of *L. brevis* (Meroth *et al.*, 2004), occupied a unique and distinct position. Among the three new sourdough isolates, a close relationship was observed between LMG 22198^T and LMG 22199, whereas strain LMG 22200^T showed a more distant relationship.

The phylogenetic position of the three *L. brevis*-like strains, LMG 22198^T, LMG 22199 and LMG 22200^T, was determined by complete 16S rRNA gene sequence analysis. Genomic DNA was prepared according to the protocol of Niemann *et al.* (1997). 16S rRNA gene amplification, purification and sequencing were performed as described by Vancanneyt *et al.* (2004) with the following modifications. PCR-amplified 16S rRNA gene sequences were purified using a NucleoFast 96 PCR clean-up kit (Macherey-Nagel). Sequencing reactions were performed using a BigDye Terminator cycle sequencing kit (Applied Biosystems) and purified using a Montage SEQ₉₆ sequencing reaction clean-up kit (Millipore). Electrophoresis of sequence reaction products was performed using an ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Sequence assembly was

Fig. 1. Protein profiles and corresponding dendrogram, derived from UPGMA linkage of correlation coefficients (expressed as a percentage for convenience) of *Lactobacillus acidifarinae* sp. nov. and *Lactobacillus zymae* sp. nov. strains and related reference strains.

performed using the program AUTOASSEMBLER (Applied Biosystems). The 16S rRNA gene sequences (continuous stretches of 1518 bp) and sequences of strains retrieved from EMBL were aligned and a phylogenetic tree was constructed by the neighbour-joining method using the BIONUMERICS software package, version 3.50 (Applied Maths). Unknown bases were discarded for the analyses. Bootstrapping analysis was undertaken to test the statistical reliability of the topology of the neighbour-joining tree using 500 bootstrap resamplings of the data (Fig. 2). Comparison of the newly determined complete sequences revealed sequence similarities higher than 99.7%. Comparison with deposited sequences available in the EMBL database classified the strains in the *Lactobacillus buchneri* group (Schleifer & Ludwig, 1995) with nearest neighbours *L. spicheri* and *L. brevis* (sequence similarities of 98.2–98.0% and 97.6–97.5%, respectively).

In a genotypic screening approach, amplified fragment length polymorphism (AFLP) fingerprinting of whole genomes, the three *L. brevis*-like sourdough isolates and a representative set of reference strains were studied (Table 1). AFLP fingerprinting was performed as described by Thompson *et al.* (2001) with the following modifications: *EcoRI*/*TaqI* was used as the restriction enzyme combination and primer combination E01/T01 (both having an adenosine extension at the 3'-end) was applied for selective PCR. The resulting electrophoretic patterns were analysed with the GELCOMP software package, version 4.2 (Applied Maths) using a Dice coefficient and the UPGMA linkage cluster analysis. Fig. 3 shows a dendrogram and confirmed the grouping of strains obtained after protein analysis (Fig. 1). Twelve recognized *L. brevis* strains grouped in a single separate cluster. The type strain of *L. spicheri* had a unique position and the three *L. brevis*-like

Fig. 2. Distance matrix tree showing the phylogenetic relationships of *L. acidifarinae* sp. nov. and *L. zymae* sp. nov. and other reference species belonging to the *L. buchneri* group, based on 16S rRNA gene sequence comparisons. *Lactobacillus sanfranciscensis* was used as the outgroup and bootstrap probabilities (percentages of 500 tree replications) are indicated at branch points.

Fig. 3. AFLP patterns and corresponding dendrogram, derived from the UPGMA linkage of Dice coefficients (expressed as a percentage for convenience) of *L. acidifarinae* sp. nov. and *L. zymae* sp. nov. strains and related reference strains.

isolates also grouped together. In this last cluster, the Greek isolate LMG 22199 and one Belgian isolate LMG 22198^T showed highest sequence similarity, linked to the second Belgian isolate LMG 22200^T.

DNA G + C contents were determined for the three *L. brevis*-like sourdough isolates together with the reference strains *L. brevis* LMG 6906^T and *L. spicheri* LMG 21871^T. DNA was extracted from 0.75–1.25 g (wet weight) using the protocol described by Gevers *et al.* (2001), using a combination of glass beads and enzymes, but with the following modifications. Volumes were increased tenfold for application on large scale. Vortexing with beads of the SDS-treated cells was done for 30 s. After addition of 16.5 ml buffer (10 mM Tris/HCl, 100 mM EDTA, pH 8.0) and 5 ml 5 M NaCl and gentle shaking, the suspension was incubated at 65 °C for 10 min. Subsequent chloroform/isoamyl alcohol extraction, precipitation, spooling of DNA on a glass rod, washing with ethanol and RNase treatment was performed as described by Marmur (1961). For determination of the DNA G + C content, DNA was enzymically degraded into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture was then separated by HPLC using a Waters SymmetryShield C8 column maintained at 37 °C. The solvent was 0.02 M (NH₄)H₂PO₄ (pH 4.0) with 1.5 % acetonitrile. Non-methylated λ -phage DNA (Sigma) was used as the calibration reference. DNA G + C contents of strains LMG 22198^T, LMG 22199 and LMG 22200^T were 54, 53 and 51 mol%, respectively. These values were close to the value of 55 mol% determined for the type strain of *L. spicheri*, and significantly higher than the value of 46 mol% for the type strain of *L. brevis*. The values of the latter two reference species confirmed those described in the literature (Meroth *et al.*, 2004).

DNA–DNA hybridizations were performed between the

L. brevis-like strains LMG 22198^T, LMG 22199 and LMG 22200^T and the type strains of *L. brevis* and *L. spicheri* (DNA was prepared as described above). The microplate method was used as described by Ezaki *et al.* (1989) and Goris *et al.* (1998), using an HTS7000 Bio Assay Reader (Perkin Elmer) for fluorescence measurements. Biotinylated DNA was hybridized with unlabelled ssDNA, which was bound non-covalently to microplate wells. Hybridizations were performed at 44 °C in hybridization mixture (2 × SSC, 5 × Denhardt solution, 2.5 % dextran sulphate, 50 % formamide, 100 µg denatured salmon sperm DNA ml⁻¹, 1.25 µg biotinylated probe DNA ml⁻¹). The three sourdough isolates had hybridization levels of 15–18 % with the type strain of *L. spicheri* and 6–8 % with the type strain of *L. brevis*. The hybridization level between LMG 22198^T and LMG 22199 was 75 %; this indicates that the two strains comprise a single species. LMG 22199 and LMG 22200^T had a hybridization value of only 47 %, indicating separate species status for the latter strain.

Growth characteristics and colony morphology were investigated on MRS agar (pH 5.4) after 24 h of incubation at 37 °C under aerobic conditions and are given below in the species description.

Conventional biochemical tests were performed on the three *L. brevis*-like strains and the type strains of *L. brevis* and *L. spicheri*. Growth characteristics were determined in MRS broth (pH 5.4; Oxoid). Except for *L. brevis* LMG 6906^T, all strains grew at 15 °C and in the presence of 5 % NaCl, but not at 45 °C. Four strains, excluding LMG 22200^T, grew in 6 % NaCl, and only LMG 22198^T and LMG 22199 grew in the presence of 7 % NaCl. All strains were facultatively anaerobic and produced gas from 2 % glucose and 2 % gluconate in MRS broth (pH 5.4, without addition of triammonium citrate). Gas production from glucose for

L. spicheri was not confirmed by Meroth *et al.* (2004). Arginine hydrolysis was tested in a medium containing 0.5 % tryptone, 0.5 % yeast extract, 0.3 % arginine, 0.05 % glucose and 0.2 % K₂HPO₄ (pH 7.0), with phenol red as indicator; a positive reaction was recorded for all five strains tested. Metabolites from glucose were lactate, acetate and ethanol, as determined by HPLC (Waters). The proportions of D- and L-lactate were determined enzymically (R-Biopharm) and the isomers were in a ratio of 3:7 for the *L. brevis*-like strains LMG 22198^T, LMG 22199 and LMG 22200^T, 1:1 for *L. spicheri* LMG 21871^T and 1:4 for *L. brevis* LMG 6906^T.

Carbohydrate fermentation tests were carried out using API 50 CHL galleries following the manufacturer's instructions (bioMérieux). Strains were cultivated at 37 °C for 24 h under aerobic conditions on MRS agar (pH 5.4). Table 2 shows that only a single characteristic, acid formation from D-arabitol, allowed differentiation between the three *L. brevis*-like strains and recognized *L. brevis* strains and *L. spicheri*. All other features for all taxa are consistently positive or negative or are strain-dependent. None of these tests distinguished LMG 22200^T from LMG 22198^T and LMG 22199.

The overall results of the present study allowed us to assign strains LMG 22198^T and LMG 22199 to a novel species, for which we propose the name *Lactobacillus zymae* sp. nov. Strain LMG 22200^T is included in a separate novel species, for which the name *Lactobacillus acidifarinae* sp. nov. is proposed.

Description of *Lactobacillus acidifarinae* sp. nov.

Lactobacillus acidifarinae (a.ci.di.fa'ri.nae. L. adj. *acidus* sour; L. n. *farina* flour; N.L. gen. n. *acidifarinae* pertaining to sour flour).

Cells are rod-shaped, occur singly or in pairs and in chains, 2–20 µm in length and 1.0 µm wide, Gram-positive, catalase-negative, non-spore-forming and non-motile. After 24 h, colonies are beige in colour, circular with a rough and wrinkled surface and approximately 1 mm in diameter. Growth occurs at 15 °C but not at 45 °C. Growth occurs at 5 % NaCl. Facultatively anaerobic and produces DL-lactic acid heterofermentatively with acetic acid and ethanol as other metabolites from glucose. Gas is produced from glucose and gluconate. Arginine is deaminated. Acid is produced from L-arabinose, D-arabitol, galactose, gluconate, N-acetylglucosamine, D-glucose, D-fructose, maltose, mannitol, ribose and D-xylose. Negative for acid production from D-arabinose, L-arabitol, adonitol, amygdalin, arbutin, cellobiose, dulcitol, aesculin, erythritol, D-fucose, L-fucose, β-gentiobiose, 2- and 5-ketogluconate, methyl α-D-glucoside, glycerol, glycogen, inositol, inulin, lactose, D-lyxose, D-mannose, methyl α-D-mannoside, melezitose, melibiose, D-raffinose, rhamnose, sucrose, salicin, starch, sorbitol, L-sorbose, D-tagatose, trehalose, D-turanose, xylitol, L-xylose and methyl β-xyloside. The DNA G+C content is 51 mol%.

The type strain, LMG 22200^T (=R-19065^T=CCM 7240^T), was isolated from a Belgian artisanal wheat sourdough.

Description of *Lactobacillus zymae* sp. nov.

Lactobacillus zymae (zy'mae. N.L. n. *zyma* from Gr. n. *zume* leaven, sourdough; N.L. gen. n. *zymae* of sourdough).

Cells are rod-shaped, occur singly or in pairs and in chains, 2–20 µm in length and 1.0 µm wide, Gram-positive, catalase-negative, non-spore-forming and non-motile. After 24 h, colonies are beige in colour, circular with a rough and wrinkled surface and approximately 1 mm in

Table 2. Differentiating phenotypic features between and within the species *L. acidifarinae* sp. nov., *L. zymae* sp. nov., *L. brevis* and *L. spicheri*

Strains: 1, *L. brevis* LMG 6906^T; 2, *L. brevis* LMG 11435; 3, *L. brevis* LMG 11438; 4, *L. brevis* LMG 11495; 5, *L. brevis* LMG 11989; 6, *L. brevis* LMG 14527; 7, *L. brevis* LMG 16322; 8, *L. brevis* LMG 18022; 9, *L. brevis* ACA-DC 4003; 10, *L. brevis* R-18707; 11, *L. spicheri* LMG 21871^T; 13, *L. zymae* LMG 22198^T; 12, *L. zymae* LMG 22199; 14, *L. acidifarinae* LMG 22200^T. +, Positive after 48 h incubation; (+) positive after 5 days incubation; –, negative.

Production of acid from	1	2	3	4	5	6	7	8	9	10	11	12	13	14
D-Arabitol	–	–	–	–	–	–	–	–	–	–	–	+	+	+
Galactose	+	+	+	+	+	–	(+)	+	+	+	+	+	–	+
2-Ketogluconate	–	(+)	(+)	–	(+)	–	–	(+)	+	(+)	–	–	–	–
5-Ketogluconate	+	+	+	(+)	+	–	–	+	+	+	+	–	–	–
Lactose	(+)	–	(+)	–	–	–	–	–	–	–	–	+	–	–
Mannitol	–	(+)	(+)	(+)	(+)	–	–	(+)	(+)	(+)	(+)	+	–	+
Melibiose	(+)	+	+	–	+	–	+	+	+	+	+	(+)	–	–
Methyl α-D-glucoside	(+)	+	+	–	+	–	(+)	+	+	+	(+)	–	–	–
Ribose	+	+	+	+	+	–	+	+	+	+	+	+	+	+
D-Xylose	+	+	+	+	+	+	+	+	+	+	+	+	–	+
Methyl β-xyloside	–	–	–	–	–	–	–	(+)	–	–	–	–	–	–

diameter. Growth occurs at 15 °C but not at 45 °C. Growth occurs at 7% NaCl. Facultatively anaerobic and produces DL-lactic acid heterofermentatively with acetic acid and ethanol as other metabolites from glucose. Gas is produced from glucose and gluconate. Arginine is deaminated. Both strains produce acid from L-arabinose, D-arabitol, gluconate, N-acetylglucosamine, D-glucose, D-fructose, maltose and ribose. Negative for acid production from D-arabinose, L-arabitol, adonitol, amygdalin, arbutin, cellobiose, dulcitol, aesculin, erythritol, D-fucose, L-fucose, β -gentiobiose, 2- and 5-ketogluconate, methyl α -D-glucoside, glycerol, glycogen, inositol, inulin, D-lyxose, D-mannose, methyl α -D-mannoside, melezitose, D-raffinose, rhamnose, sucrose, salicin, starch, sorbitol, L-sorbose, D-tagatose, trehalose, D-turanose, xylitol, L-xylose and methyl β -xyloside. Results are strain-dependent for acid production from galactose, lactose, mannitol, melibiose and D-xylose. The DNA G+C content is 53–54 mol%.

The type strain, LMG 22198^T (= R-18615^T = CCM 7241^T), was isolated from a Belgian artisanal wheat sourdough.

Acknowledgements

We acknowledge the financial support of the Flemish Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT), in particular the STWW project 'Functionality of Novel Starter Cultures in Traditional Fermentation Processes' and the SBO project 'New Strategy for the Development of Functional and Performant Starter Cultures for Foods in Function of Food Qualitomics', and the LINK 2002 Action of the Brussels Capital Region. This research was also supported by the Prime Minister's Services – Federal Office for Scientific, Technical and Cultural Affairs, Belgium. We thank Spiros Paramithiotis for providing strain LMG 22199.

References

- Boot, H. J., Kolen, C. P. A. M., Pot, B., Kersters, K. & Pouwels, P. H. (1996). The presence of two S-layer-protein-encoding genes is conserved among species related to *Lactobacillus acidophilus*. *Microbiology* **142**, 2375–2384.
- Corsetti, A., Gobbetti, M., Balestrieri, F., Paoletti, F., Russi, L. & Rossi, J. (1998). Sourdough lactic acid bacteria effects on bread firmness and staling. *J Food Sci* **63**, 347–351.
- De Vuyst, L., Schrijvers, V., Paramithiotis, S., Hoste, B., Vancanneyt, M., Swings, J., Kalantzopoulos, G., Tsakalidou, E. & Messens, W. (2002). The biodiversity of lactic acid bacteria in Greek traditional wheat sourdoughs is reflected in both composition and metabolite formation. *Appl Environ Microbiol* **68**, 6059–6069.
- 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.
- Gänzle, M. G., Ehrmann, M. & Hammes, W. P. (1998). Modeling of growth of *Lactobacillus sanfranciscensis* and *Candida milleri* in response to process parameters of sourdough fermentation. *Appl Environ Microbiol* **64**, 2616–2623.
- Gevers, D., Huys, G. & Swings, J. (2001). Applicability of rep-PCR fingerprinting for differentiation of *Lactobacillus* species. *FEMS Microbiol Lett* **205**, 31–36.
- Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA–DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.
- Hammes, W. P. & Gänzle, M. G. (1998). Sourdough breads and related products. In *Microbiology of Fermented Foods*, vol. 1, pp. 199–216. Edited by B. J. B. Woods. London: Blackie.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Meroth, C. B., Hammes, W. P. & Hertel, C. (2004). Characterisation of the microbiota of rice sourdoughs and description of *Lactobacillus spicheri* sp. nov. *Syst Appl Microbiol* **27**, 151–160.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Niemann, S., Puehler, A., Tichy, H.-V., Simon, R. & Selbitschka, W. (1997). Evaluation of the resolving power of three different DNA fingerprinting methods to discriminate among isolates of a natural *Rhizobium meliloti* population. *J Appl Microbiol* **82**, 477–484.
- Pot, B., Vandamme, P. & Kersters, K. (1994). Analysis of electrophoretic whole-organism protein fingerprints. In *Chemical Methods in Prokaryotic Systematics*, pp. 493–521. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
- Rosenquist, H. & Hansen, A. (1998). The antimicrobial effect of organic acids, sour dough and nisin against *Bacillus subtilis* and *B. licheniformis* isolated from wheat bread. *J Appl Microbiol* **85**, 621–631.
- Schleifer, K. H. & Ludwig, W. (1995). Phylogeny of the genus *Lactobacillus* and related genera. *Syst Appl Microbiol* **18**, 461–467.
- Thompson, F. L., Hoste, B., Vandemeulebroecke, K. & Swings, J. (2001). Genomic diversity amongst *Vibrio* isolates from different sources determined by fluorescent amplified fragment length polymorphism. *Syst Appl Microbiol* **24**, 520–538.
- Vancanneyt, M., Mengaud, J., Cleenwerck, I. & 7 other authors (2004). Reclassification of *Lactobacillus kefirgranum* Takizawa et al. 1994 as *Lactobacillus kefirnofaciens* subsp. *kefirgranum* subsp. nov. and emended description of *L. kefirnofaciens* Fujisawa et al. 1988. *Int J Syst Evol Microbiol* **54**, 551–556.
- Vogel, R. F., Knorr, R., Müller, M. R. A., Steudel, U., Gänzle, M. G. & Ehrmann, M. (1999). Non-dairy lactic fermentations: the cereal world. *Antonie van Leeuwenhoek* **76**, 403–411.