

## *Marinilactibacillus piezotolerans* sp. nov., a novel marine lactic acid bacterium isolated from deep sub-seafloor sediment of the Nankai Trough

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A piezotolerant, mesophilic, marine lactic acid bacterium (strain LT20<sup>T</sup>) was isolated from a deep sub-seafloor sediment core collected at Nankai Trough, off the coast of Japan. Cells were Gram-positive, rod-shaped, non-sporulating and non-motile. The NaCl concentration range for growth was 0–120 g l<sup>-1</sup>, with the optimum at 10–20 g l<sup>-1</sup>. The temperature range for growth at pH 7.0 was 4–50 °C, with the optimum at 37–40 °C. The optimum pH for growth was 7.0–8.0. The optimum pressure for growth was 0.1 MPa with tolerance up to 30 MPa. The main cellular phospholipids were phosphatidylglycerols (25%), diphosphatidylglycerols (34%) and a group of compounds tentatively identified as ammonium-containing phosphatidylserines (32%); phosphatidylethanolamines (9%) were minor components. The fatty acid composition was dominated by side chains of 16:0, 14:0 and 16:1. The G+C content of the genomic DNA was 42 mol%. On the basis of 16S rRNA gene sequence analysis and the secondary structure of the V6 region, this organism was found to belong to the genus *Marinilactibacillus* and was closely related to *Marinilactibacillus psychrotolerans* M13-2<sup>T</sup> (99%), *Marinilactibacillus* sp. strain MJYP.25.24 (99%) and *Alkalibacterium olivapovliticus* strain ww2-SN4C (97%). Despite the high similarity between their 16S rRNA gene sequences (99%), the DNA–DNA hybridization levels were less than 20%. On the basis of physiological and genetic characteristics, it is proposed that this organism be classified as a novel species, *Marinilactibacillus piezotolerans* sp. nov. The type strain is LT20<sup>T</sup> (= DSM 16108<sup>T</sup> = JCM 12337<sup>T</sup>).

The occurrence of a deep bacterial biosphere in marine sediments to more than 800 m below the sea floor (Parkes *et al.*, 2000) has raised questions about what types of bacteria inhabit deeply buried sediments. Populations of viable micro-organisms have been found in deep marine deposits, but studies have focused on sulphate-reducers (Bale *et al.*,

1997) and methanogens (Mikucki *et al.*, 2003). Recently, however, autotrophic and heterotrophic bacteria belonging to the phyla *Firmicutes*, *Cytophaga*, *Spirochaetes* and *Proteobacteria* have been enriched from deep sediments of the Nankai Trough south-east of Japan (Toffin *et al.*, 2004a, b). In near-surface deep marine sediments, fermentative micro-organisms could play a significant role in the transformation of organic matter. At present, little is known about the origin and the adaptation of viable fermentative micro-organisms in such deep environments. To date, only a few types of lactic acid bacteria have been isolated from marine environments (Franzmann *et al.*, 1991), such as the two species from Ace Lake in Antarctica, namely *Carnobacterium fundicum* and *Carnobacterium alterfundicum* (Spielmeyer *et al.*, 1993). Recently, the fermentative

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Abbreviation: PLFA, phospholipid fatty acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Marinilactibacillus piezotolerans* LT20<sup>T</sup> is AY485792.

Details of the morphology, differential phenotypic characteristics and the effect of pH on glucose fermentation in strain LT20<sup>T</sup> are available as supplementary material in IJSEM Online.

marine lactic acid bacterium *Marinilactibacillus psychrotolerans* was isolated from dead and living marine organisms at Miura Peninsula in Japan (Ishikawa *et al.*, 2003). In addition, members of the genus *Marinilactibacillus* have been isolated from coastal sub-seafloor sediments of the Okhotsk Sea (Inagaki *et al.*, 2003) and *Carnobacterium*-like sequences have been retrieved from Nankai Trough (Newberry *et al.*, 2004). At present, the genus *Marinilactibacillus* comprises only one species, *M. psychrotolerans*, which has developed some adaptations to salt, temperature and pH. This species is also able to ferment a wide range of organic substrates, producing lactic acid as the main end product of metabolism.

*M. psychrotolerans* strain M13-2<sup>T</sup> (=IAM 14980<sup>T</sup>) was used as a reference strain. This strain was obtained from the IAM culture collection, Institute of Molecular and Cellular Biosciences (Tokyo University, Japan).

The novel isolate was obtained from a sediment core collected at 4.15 m below the sea floor from a water depth of 4790.7 m in the Pacific Ocean at Nankai Trough, off the coast of Japan (site 1173: 32° 14.7' N 135° 1.5' E) between 28 May and 7 June 2000 (Ocean Drilling Program, Leg 190). All details of the environmental conditions, sampling and sub-sampling procedures have been previously reported (Cragg *et al.*, 1992a, b; Parkes *et al.*, 1995; Moore *et al.*, 2001; Toffin *et al.*, 2004a).

Cultures were enriched anaerobically in 50 ml vials containing 10 ml medium MM, which consisted of the following (l<sup>-1</sup> distilled water): 23 g NaCl, 3 g MgCl<sub>2</sub>·6H<sub>2</sub>O, 4 g Na<sub>2</sub>SO<sub>4</sub>, 0.7 g KCl, 0.15 g CaCl<sub>2</sub>, 0.5 g NH<sub>4</sub>Cl, 0.27 g KH<sub>2</sub>PO<sub>4</sub>, 15 ml 1 M NaHCO<sub>3</sub>, 0.1 g yeast extract (Difco), 1 ml trace elements (Widdel & Bak, 1992), 1 ml vitamin solution (Widdel & Bak, 1992), 1 ml thiamine (0.01 %, w/v; Widdel & Bak, 1992), 1 ml vitamin B<sub>12</sub> (0.005 %, w/v; Widdel & Bak, 1992), 1.0 g sodium acetate, 2.0 g monomethylamine, 5.0 g sodium formate, 0.5 % (v/v) methanol and 0.5 mg resazurin. The pH of the medium was adjusted to 7.2 at room temperature before autoclaving. Sterile medium was reduced by adding 0.5 g sodium sulphide l<sup>-1</sup> and then distributed into serum vials before inoculation. Cultures were incubated at 25 °C in the dark. Growth of lemon-shaped, coccoid, vibrio-shaped, long rod-shaped and short rod-shaped cells was observed in enrichment cultures after 2 days incubation. Positive enrichments were sub-cultured into the same medium under anaerobic conditions. Once stable enrichment cultures had been established, subsequent enrichment cultures were grown on agar plates (1 %, w/v; Difco), incubated anaerobically at 25 °C. Small, translucent, round colonies around 1 mm in diameter were visible after 3 days and one colony was randomly picked. The dilution to extinction technique, followed by repeated streaking onto plates, was employed to obtain pure culture. The isolate was designated strain LT20<sup>T</sup> (=DSM 16108<sup>T</sup> =JCM 12337<sup>T</sup>) and the purity of the isolate was checked by means of microscopic observations and by

cloning and sequencing five independent 16S rRNA gene clones.

Isolate LT20<sup>T</sup> was routinely grown in YPG medium containing the following (l<sup>-1</sup> distilled water): 25 g NaCl, 3 g MgCl<sub>2</sub>, 0.5 g KCl, 4 g Na<sub>2</sub>SO<sub>4</sub>, 2 g glucose, 5 g yeast extract (Difco), 5 g peptone (Difco), 34.6 g PIPES and 0.05 g KH<sub>2</sub>PO<sub>4</sub>. The pH of the medium was adjusted to 7.2 before autoclaving. Unless indicated otherwise, cultures were incubated aerobically at 37 °C with shaking. Stock cultures of isolate LT20<sup>T</sup> were stored in culture medium at 4 °C. For long-term storage, pure cultures were stored at -80 °C in the same medium containing 20 % (w/v) glycerol.

Growth was monitored by measuring changes in turbidity at 600 nm using a Spectronic 401 spectrophotometer (Spectronic Instruments). Direct cell counts were determined on samples fixed with 2 % (v/v) glutaraldehyde for 1 h at 4 °C by using a counting chamber (depth 0.02 mm; Thoma). An Olympus CX40 microscope was used routinely to observe the cells. For negative staining, 20 µl bacterial suspension fixed with 2 % (w/v) glutaraldehyde was dropped onto a carbon-coated copper grid (Nishin EM). A droplet of a 1 % (w/v) neutral phosphotungstic acid solution (pH 7.6) was added to the carbon grid for 40 s and excess liquid was removed. Specimens were air-dried and electron micrographs taken using a JEOL electron microscope (JEM 1210). Gram staining, catalase, oxidase, gelatinase and amylase (1 %, w/v) reactions were tested with cells grown in YPG medium.

Cells of strain LT20<sup>T</sup> were straight to slightly curved rod-shaped cells approximately 2–2.2 µm in length and 0.3–0.35 µm wide (see the transmission electron micrograph available as a supplementary figure in IJSEM Online), but were slightly elongated in older cultures. They occurred singly, in pairs or in small chains at 37 °C and stained Gram-positive. The cells were non-motile. Endospores or spores were not observed and cells were catalase-positive and oxidase-negative.

To determine the optimum temperature, pH and NaCl, cells were grown in aerobic tubes (15 ml) containing 5 ml YPG medium with shaking (130 r.p.m.) as reported previously (Toffin *et al.*, 2004b). To determine the effect of pH on growth, YPG medium was modified with the following 100 mM buffers (Sigma): for pH 5.0, no buffer; for pH 5.5 and 6.0, MES buffer; for pH 6.5 and 7.0, PIPES buffer; for pH 7.5, HEPES buffer; for pH 8.0 and 8.5, Tris buffer; for pH 9.0, 10.0 and 11.0, no buffer.

The effect of hydrostatic pressure on growth rates of strain LT20<sup>T</sup> was determined at 0.1, 10, 20, 30, 40 and 50 MPa at 37 °C as previously described (Kato *et al.*, 1995).

The ability of strain LT20<sup>T</sup> to utilize single carbon sources was investigated by adding one of the following organic compounds to the modified YPG medium, from which carbon sources were omitted: formate (30 mM), pyruvate

(20 mM), propionate (20 mM), butyrate (10 mM), isobutyrate (5 mM), 2-methyl butyrate (5 mM), 3-methyl butyrate (5 mM), valerate (5 mM), isovalerate (5 mM), glutamate (5 mM), caproate (5 mM), malate (15 mM), succinate (10 mM), heptanoate (5 mM), acetate (10 mM), lactate (20 mM), caprylate (2.5 mM), monomethylamine (0.2%, w/v), propanol (5 mM), ethanol (0.2%, w/v) and peptone, tryptone, L-arabinose, cellobiose, fructose, galactose, lactose, maltose, D-mannitol, mannose, L-rhamnose, D-sorbitol, sucrose, xylose, glucose (each at 0.2%, w/v). Strain LT20<sup>T</sup> was additionally characterized by using the identification system API 20E (bioMérieux) at 37 °C according to the manufacturer's instructions.

The ability of the isolate to grow in the presence of different electron acceptors was tested on YP medium (Toffin *et al.*, 2004b) containing maltose (0.2%, w/v) and yeast extract (0.01%, w/v). The following electron acceptors were tested: elemental sulphur and L-cystine, each at 1% (w/v); thiosulphate, sulphate, nitrate and nitrite, each at 20 mM. The aerobic headspace in each case was replaced with N<sub>2</sub> (100%, 100 kPa). Preparation of amorphous Fe(III) oxide and Fe(II) determination were performed as described by Slobodkin *et al.* (1999). For nitrite and ammonium analysis, cultures were grown anaerobically in modified YP medium supplemented with 5 mM KNO<sub>3</sub> and under an atmosphere of N<sub>2</sub>.

Fermentation products from the sugars glucose, ribose, xylose, fructose, cellobiose, maltose, sucrose, mannitol, adonitol, L-arabinose, D-arabinose, galactose, lactose, melzitose, glycerol, sorbitol and starch were tested (each at 0.2%, w/v). Additional analysis of fermentation end products formed from glucose at different pH values was performed in modified YPG medium in which the buffer was replaced by 100 mM HEPES (Sigma) and adjusted to pH 7.0, 8.0 and 9.0 to minimize the pH decrease in media during cultivation.

The organic acid metabolic end products during fermentation and electron-acceptor tests were analysed by using HPLC (Alliance 2690; Waters) as described by Wery *et al.* (2001).

Reduction of nitrate and nitrite was determined by using the indophenol blue method of Koroleff (1969) and Solorzano (1969). H<sub>2</sub>S production was evaluated as described elsewhere (Jeanthon *et al.*, 2002).

Strain LT20<sup>T</sup> is a facultatively anaerobic and obligately heterotrophic bacterium. The isolate was able to grow on various substrates tested as sole carbon sources with O<sub>2</sub> as electron acceptor, as revealed by lactic acid production. Peptone, tryptone, cellobiose, fructose, galactose, lactose, maltose, mannitol, sorbitol, sucrose, xylose and glucose were used as sole carbon sources, but formate, pyruvate, propionate, butyrate, isobutyrate, 2-methyl butyrate, 3-methyl butyrate, valerate, isovalerate, glutamate, caproate, malate, succinate, heptanoate, caprylate, acetate, lactate,

monomethylamine, propanol, ethanol, L-arabinose, mannose and L-rhamnose were not.

The end products of a wide range of sugar fermentations were determined: lactic acid was produced as an end product with acetate, formate and ethanol (see the phenotypic characterization available as a supplementary table in IJSEM Online); at pH 7.0, lactate was strongly produced from glucose, D-xylose, D-fructose and D-mannitol, with some traces of formate, acetate and ethanol produced at various molar ratios depending on the substrate. The effect of pH on glucose fermentation was also investigated (see the data available as a supplementary table in IJSEM Online) and, despite the use of buffered media, lactic acid production correlated with pH decrease. During growth, pH values decreased by 0.3–0.5 pH units from the initial pH with buffered media. During the course of glucose fermentation at pH 9.0 using buffered medium, the pH decreased and 0.96 mol lactate was produced per mol glucose consumed (see the data available as a supplementary table in IJSEM Online). When the initial pH increased from 7.0 to 9.0, production of lactate increased, while formate, acetate and ethanol decreased, as reported previously for other lactic acid bacteria (Rhee & Pack, 1980; Franzmann *et al.*, 1991; Janssen *et al.*, 1995; Ishikawa *et al.*, 2003).

Growth was observed between 0.1 and 30 MPa at 37 °C, but no growth was observed at 40 MPa. The growth rates of the isolate under high-pressure conditions were comparable with those at atmospheric pressure ( $1/t_d = 0.3$  at 0.1 MPa, 0.28 at 10 MPa, 0.26 at 20 MPa and 0.25 at 30 MPa).

Respiratory quinones were extracted using methanol/hexane (Toffin *et al.*, 2004b). It was observed that respiratory quinones could not be detected in strain LT20<sup>T</sup>.

For phospholipid and phospholipid fatty acid (PLFA) analysis of strains LT20<sup>T</sup> and M13-2<sup>T</sup>, a modified method of Zink *et al.* (2003) was used. The lipid content of culture samples was extracted by flow-blending (Radke *et al.*, 1978) using methanol/dichloromethane (1:2, v/v). Distearoyl-D<sub>70</sub>-phosphatidylcholine (molecular mass 860.6 Da) and palmitoyl-D<sub>31</sub>-lysophosphatidylcholine (526.82 Da) were added as internal standards. The total extract was rinsed with water to remove water-soluble compounds; therefore, methanol and water (dichloromethane/methanol/water; 1:1:0.9 by vol.) were added for phase separation. Total lipids were obtained from the lower organic phase.

The extract was separated to give four fractions of different polarity modified according to Zink *et al.* (2003). Half of the fraction containing the intact phospholipids was analysed by LC-MS. The second aliquot was used for mild alkaline hydrolysis, as described by White *et al.* (1979), to obtain PLFAs, which were analysed, as their methyl esters, by using GC-MS.

Strain LT20<sup>T</sup> contains four different classes of phospholipids: phosphatidylglycerols (25% of total),

diphosphatidylglycerols (34%), tentatively identified phosphatidylserines (32%) and phosphatidylethanolamines (9%). It is noteworthy that the phosphatidylserines had an additional ammonium group linked to the head group, this being corroborated by LC-MS-MS analysis. The PLFAs obtained from GC-MS had chain lengths in the range C<sub>12</sub>–C<sub>19</sub>, the most abundant being saturated C<sub>16</sub> (44.72%), saturated C<sub>14</sub> (31.45%) and unsaturated C<sub>16</sub> (17.11%) fatty acids (Table 1). Unsaturated fatty acids only occurred as monounsaturated moieties (Table 1).

The PLFA distribution determined by GC-MS analysis corresponds with the fatty acyl side-chain composition of intact phospholipids determined by LC-MS.

The distribution of the PLFAs of strain LT20<sup>T</sup> reflects a common pattern for bacterial organisms with C<sub>16:0</sub> and C<sub>14:0</sub> as the predominant fatty acids. Thus, no significant adaptation to environmental conditions can be deduced from the PLFA composition alone. The PLFA distribution of *M. psychrotolerans* M13-2<sup>T</sup>, with C<sub>18:1</sub>, C<sub>16:0</sub>, C<sub>16:1</sub> and C<sub>18:0</sub> as the dominant fatty acids, differs significantly from the PLFA pattern of strain LT20<sup>T</sup> (Table 1) but corresponds with the findings of Ishikawa *et al.* (2003).

For determination of the G + C content, DNA was isolated after disruption of cells using a French pressure cell and then purified on hydroxyapatite (Cashion *et al.*, 1977). The DNA

**Table 1.** Relative distribution of PLFAs of *M. piezotolerans* sp. nov. LT20<sup>T</sup> and *M. psychrotolerans* M13-2<sup>T</sup>

Fatty acids are listed in order of elution and values are percentages of total fatty acids, measured as fatty acid methyl esters by using GC-MS. –, Not detected.

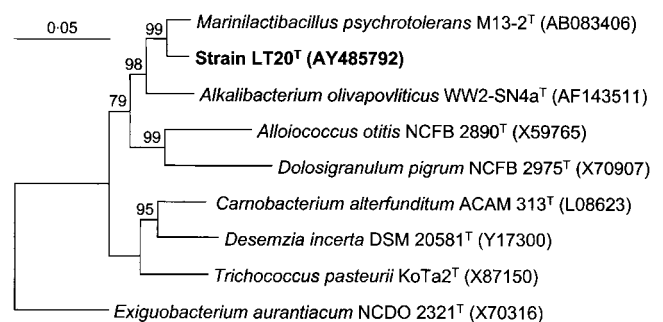
Fatty acid	LT20 <sup>T</sup>	M13-2 <sup>T</sup>
C <sub>12:0</sub>	0.81	0.24
C <sub>14:1</sub>	2.48	0.32
C <sub>14:0</sub>	31.45	2.43
C <sub>15:0</sub>	0.09	0.24
S1*		
C <sub>16:1</sub>	14.09	12.11
C <sub>16:1</sub>	3.02	2.08
C <sub>16:0</sub>	44.72	31.18
cyclo C <sub>17:0</sub> or C <sub>17:1</sub>	1.42	–
C <sub>17:0</sub>	–	0.14
S2		
C <sub>18:1</sub>	0.19	38.49
C <sub>18:1</sub>		1.67
C <sub>18:0</sub>	0.75	8.18
cyclo C <sub>19:0</sub> or C <sub>19:1</sub>	0.98	–
C <sub>20:1</sub>	–	2.92

\*S1 and S2 are deuterium-labelled fatty acids from standard phospholipids. S1 is from palmitoyl-D<sub>31</sub>-lysophosphatidylcholine and S2 is from distearoyl-D<sub>70</sub>-phosphatidylcholine.

was hydrolysed with P1 nuclease and the nucleotides were dephosphorylated with bovine alkaline phosphatase (Mesbah *et al.*, 1989). The G + C content of the genomic DNA of strain LT20<sup>T</sup> was determined by using HPLC according to the method described by Tamaoka & Komagata (1984) as 42.0 mol%.

Genomic DNA of strain LT20<sup>T</sup> for 16S rRNA gene sequence analysis and DNA–DNA hybridization was isolated by using the procedure described by Erauso *et al.* (1992). The complete 16S rRNA gene sequence of strain LT20<sup>T</sup> was determined as previously reported by Toffin *et al.* (2004b). Phylogenetic analysis was performed using the software package ARB (Ludwig *et al.*, 2004). The 16S rRNA gene sequence was aligned with FastAligner version 3.0 and aligned manually with sequences of representative related genera obtained from the Ribosomal Database Project (Maidak *et al.*, 2001) or from recent GenBank releases. The secondary structure was used as a guide to ensure that only homologous regions were compared. Phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987) with the Jukes–Cantor correction factor (Jukes & Cantor, 1969). The topologies of the trees were evaluated by using maximum parsimony (Lake, 1987) and maximum likelihood (Felsenstein, 1981). A bootstrap test of 1000 replicates was used to identify solid branches and establish a confidence level for each node (Felsenstein, 1985).

The 16S rRNA gene sequence analysis placed strain LT20<sup>T</sup> as a close relative of *M. psychrotolerans* M13-2<sup>T</sup> (Fig. 1). According to 16S rRNA gene sequences, a similarity matrix generated using the correction of Jukes & Cantor (1969) revealed that strain LT20<sup>T</sup> was 99% similar to *M. psychrotolerans* strain M13-2<sup>T</sup> isolated from living and decomposing marine organisms collected from temperate and subtropical areas in Japan (Ishikawa *et al.*, 2003), 99%



**Fig. 1.** Phylogenetic relationships of *M. piezotolerans* sp. nov. LT20<sup>T</sup> and other lactic acid bacteria produced by neighbour-joining. The sequence of the small-subunit RNA of strain LT20<sup>T</sup> was aligned with other 16S rRNA sequences from the RDP and GenBank. Numbers at branch nodes are bootstrap values based on 1000 bootstrap resamplings. This topology was confirmed by maximum-parsimony and maximum-likelihood methods. Bar, 0.05 expected changes per sequence position.

similar to *Marinilactibacillus* sp. strain OHKMJYP.25.24, isolated from sub-seafloor sediment of the Okhotsk Sea (Inagaki *et al.*, 2003), and 96.5% similar to *Alkalibacterium olivapovlitticus*, isolated from edible-olive washwaters (Ntougias & Russell, 2001). Bootstrap values using distance matrices placed strain LT20<sup>T</sup> with *M. psychrotolerans* M13-2<sup>T</sup> with 99% bootstrap resampling (Fig. 1). The predicted secondary structure of the V6 region of the 16S rRNA gene of the novel isolate was compared with those of *M. psychrotolerans* and other related genera and was found to belong to the genus *Marinilactibacillus* and to differ from the others as suggested by Ishikawa *et al.* (2003).

Levels of genetic relatedness were determined by performing DNA–DNA dot-blot hybridization experiments, as previously reported, with modifications (Marteinsson *et al.*, 1995). Probe DNA was labelled by using an Enhanced Chemi-Fluorescence random prime labelling kit (fluorescein-labelled DNA probe; Amersham International). The level of DNA–DNA hybridization is the mean level of binding for at least four replicates. No significant relatedness (<20%) was obtained between bulk cellular DNA of isolate LT20<sup>T</sup> and *M. psychrotolerans* M13-2<sup>T</sup>.

The novel marine, heterofermentative, lactic-acid strain belongs to the genus *Marinilactibacillus* on the basis of its 16S rRNA gene sequence (V6 region) and some phenotypic characteristics. However, DNA–DNA hybridization studies showed that its closest phylogenetic relative, *M. psychrotolerans*, could not be assigned to the same species (<20%) (Stackebrandt & Goebel, 1994).

Strain LT20<sup>T</sup> differs from *M. psychrotolerans* in its optimum ranges for pH and NaCl concentration. Strain LT20<sup>T</sup> can also be distinguished from *M. psychrotolerans* by the positive catalase reaction and  $\beta$ -galactosidase activity and by acetoin production. Tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, gelatinase, urease, tryptophan deaminase and H<sub>2</sub>S production from thiosulfate were negative and indole was not produced. Fermentation of sugars by strain LT20<sup>T</sup> was also slightly different from that of *M. psychrotolerans* (see the supplementary tables available in IJSEM Online). Strain LT20<sup>T</sup> differs from strain M13-2<sup>T</sup> in the PLFA distribution pattern.

Most obvious is the shift from chain lengths of 14 and 16 carbon atoms (LT20<sup>T</sup>) to 16 and 18 carbon atoms (M13-2<sup>T</sup>), and especially the high abundance of C<sub>18:1</sub> in M13-2<sup>T</sup> (Table 1). At first sight, this difference implies different responses by these species to high pressure and to low temperature in order to maintain membrane fluidity with regard to the phase transition temperature.

From the above results, we conclude that strain LT20<sup>T</sup> represents a novel *Marinilactibacillus* species. We propose to give it the name *Marinilactibacillus piezotolerans* sp. nov. to reflect its deep marine origin and its growth under a range of hydrostatic pressures.

## Description of *Marinilactibacillus piezotolerans* sp. nov.

*Marinilactibacillus piezotolerans* (pie.zo.to'le.rans. Gr. v. *piezo* to press; L. part. adj. *tolerans* tolerating, N.L. part. adj. *piezotolerans* tolerating high hydrostatic pressure).

Gram-positive. Cells are non-spore-forming, non-motile rods, 2–2.2 × 0.3–0.35  $\mu$ m. Facultative anaerobe. Grows between 4 and 50 °C, with optimum growth at around 37–40 °C; no growth is detected above 50 °C. Grows in NaCl concentrations ranging from 0 to 120 g l<sup>-1</sup>, with the optimum at approximately 10–20 g l<sup>-1</sup>; no growth detected at or above 130 g NaCl l<sup>-1</sup>. Grows at pH values between 5.5 and 10.0, with the optimum at around pH 7.0–8.0. The optimum hydrostatic pressure for growth is 0.1 MPa, with tolerance up to 30 MPa. Under optimal growth conditions (YPG medium, 37 °C, pH 7.0 and 20 g NaCl l<sup>-1</sup>), the doubling time is approximately 57 min. Sulphate, thiosulphate, elemental sulphur, L-cystine, iron oxide, nitrate and nitrite are not reduced. The main components of the lipid complex of the cells are phosphatidylglycerols (25%), diphosphatidylglycerols (34%) and tentatively identified ammonium-containing phosphatidylserines (32%); phosphatidylethanolamines are minor compounds, accounting for 9%. The most abundant fatty acyl side chains (PLFAs) of these phospholipids are 16:0 and 14:0 and 16:1, while 14:1, cyclic 17:0 or 17:1, cyclic 19:0 or 19:1, 12:0, 18:0, 18:1 and 15:0 are minor components (in decreasing intensity). Quinones are not detected. The G+C content of the DNA of the type strain is 42.0 mol% (as determined by HPLC).

The type strain is LT20<sup>T</sup> (= DSM 16108<sup>T</sup> = JCM 12337<sup>T</sup>), which was isolated from deep sub-seafloor sediment.

## Acknowledgements

The authors would like to thank the Ocean Drilling Program, Professor John Parkes and Dr Barry Cragg from Cardiff University for the Leg 190 samples. We acknowledge Dr Julian Marchesi (University of Cork) and Dr Jens Kallmeyer (Max Planck Institut für Marine Mikrobiologie, Bremen) for their help with sample handling and preparation. We also thank Dr Gordon Webster (Cardiff University) for helpful comments during preparation of this manuscript. L.T. would like to thank Dr Alexander Slobodkin for iron-reducing tests and Dr Katsuyuki Uematsu for assistance with preparation of electron micrographs. This work was funded by the European Union project DeepBUG (contract number EVK3-CT-1999-017). This research used samples and data provided by the Ocean Drilling Program, which is sponsored by the US National Science Foundation and participating countries under the management of Joint Oceanographic Institutions, Inc.

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