Lysinibacillus acetophenoni sp. nov., a solvent-tolerant bacterium isolated from acetophenone

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A Gram-stain-positive, solvent-tolerating, aerobic, rod-shaped bacterium that formed terminal endospores was isolated from the organic solvent acetophenone. The strain, designated JC23ᵀ, was oxidase- and catalase-positive. The strain grew in the presence of a wide range of organic solvents with partition coefficients (log p values) between 1 and 4, which are exceptionally toxic to micro-organisms. Based on 16S rRNA gene sequence analysis, strain JC23ᵀ was identified as belonging to the genus Lysinibacillus and was most closely related to Lysinibacillus manganicus Mn1-7ᵀ (98.5 % similarity), L. massiliensis 440831ᵀ (97.2 %) and L. chungukyangi 2RL3-2ᵀ (96.8 %). DNA–DNA relatedness of strain JC23ᵀ with the type strains of the closest species was <39 %. Strain JC23ᵀ grew chemo-organoheterotrophically with optimal growth at pH 7 (range pH 6–9) and at 35 °C (range 25–40 °C). The DNA G+C content was 41 mol%. Major cellular fatty acids of strain JC23ᵀ were iso-C₁₅:₀, iso-C₁₆:₀, anteiso-C₁₅:₀and anteiso-C₁₇:₀. The cell-wall peptidoglycan type was determined to be A4g (L-Lys–D-Asp), which is in agreement with the cell-wall characteristics of the genus Lysinibacillus. The predominant quinone system was MK-7. Polar lipids of strain JC23ᵀ included diphosphatidylglycerol, phosphatidylglycerol, two unidentified glycolipids, β-gentiobiosyldiacylglycerol, two unidentified phospholipids and two unidentified lipids. On the basis of our morphological, physiological, genetic, phylogenetic and chemotaxonomic analyses, we conclude that strain JC23ᵀ should be assigned to a novel species of the genus Lysinibacillus, for which the name Lysinibacillus acetophenoni sp. nov. is proposed. The type strain is strain JC23ᵀ (=CCUG 57911ᵀ=KCTC 13605ᵀ=NBRC 105754ᵀ=DSM 23939ᵀ).

The omnipresence of bacteria is driven by their wide metabolic diversity and their capability to adapt to changes in the environment, because of which they are found in all niches explored on Earth. Solvent-tolerant bacteria are a novel group of extremophilic bacteria that thrive in the presence of high concentrations of toxic organic solvents, by evading the deleterious effects of the latter (Segura et al., 2012; Torres et al., 2011; Zahir et al., 2006; Nielsen et al., 2005). Such solvent-tolerant bacteria are being explored for their potential use in industrial and environmental biotechnology, since their enzymes are likely to be steady and active even in the presence of toxic solvents (Torres et al., 2011). Most reported solvent-tolerating bacteria have been isolated either from soils (Zahir et al., 2006) or from oil fly larval guts (Nielsen et al., 2005).

During our studies on solvent-tolerant bacteria, strain JC23ᵀ was isolated from the organic solvent acetophenone and was characterized by a polyphasic taxonomic approach. Based on 16S rRNA gene sequence analysis, the strain belonged to the genus Lysinibacillus, which is a member of the phylum Firmicutes. The genus is represented by its type species Lysinibacillus boronitolerans and is characterized by spore-forming, motile rods, with A4g-type (Lys–Asp) cell-wall peptidoglycan and iso-C₁₅:₀ or anteiso-C₁₅:₀ as the major fatty acids. The dominant respiratory lipoquinone system is menaquinone 7 (MK-7). Diphosphatidyglycerol, phosphatidylglycerol and phosphatidylethanolamine are the predominant polar lipids. However, some species of the genus Lysinibacillus do not contain phosphatidylethanolamine, but unidentified glycolipids are present as major polar lipids (Jung et al., 2012). To date, there are 21 species (with validly/effectively published names) in the genus Lysinibacillus, with Lysinibacillus...
L. manganicus were achieved by using the EzTaxon-e server (Kim et al., 2013). Calculation of pairwise 16S rRNA gene sequence similarity was done as described previously (Subhash et al., 2013). JC23T and the reference strains CCUG 49529T and CCTCC AB 2012916T, L. massiliensis CCUG 49529T and L. boronitolerans DSM 17140T were grown in nutrient broth unless otherwise mentioned. Growth was measured turbidometrically at 540 nm in a colorimeter (Systronics) after 48 h of incubation.

Strain JC23T was isolated from acetophenone after filtering through a PVDF membrane (47 mm diameter; 0.22 μm; Millipore) using a glass membrane filtration unit. The membrane was then placed onto a solvent-saturated (50 mM) nutrient agar (g l⁻¹: peptone, 5; yeast extract, 1.5; beef extract, 1.5; NaCl, 5; pH 7.0) plate and incubated at 35 °C for 7 days. Colonies obtained were purified by repeated streaking on acetophenone-saturated nutrient agar plates. A pure culture of the strain was preserved through lyophilization. Unless otherwise mentioned, strain JC23T and the reference strains Lysinibacillus manganicus CCTCC AB 2012916T, L. massiliensis CCUG 49529T and L. boronitolerans DSM 17140T were grown in nutrient broth or on nutrient agar.

Genomic DNA was extracted and purified from strains JC23T, L. manganicus CCTCC AB 2012916T, L. massiliensis CCUG 49529T and L. boronitolerans DSM 17140T according to the method of Marmur (1961) and the G+C Lysinibacillus manganicus CCTCC AB 2012916T, L. massiliensis CCUG 49529T and L. boronitolerans DSM 17140T content of the DNA was determined by HPLC (Mesbah et al., 1989). 16S rRNA gene amplification and sequencing was done as described previously (Subhash et al., 2013). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarity were achieved by using the EzTaxon-e server (Kim et al., 2012). The CLUSTAL W algorithm of MEGA 5.2 was used for sequence alignments and MEGA 5.2 (Tamura et al., 2011) software was used for phylogenetic analysis of the individual sequences. Distances were calculated by using Kimura’s correction in a pairwise deletion manner (Kimura, 1980). The neighbour-joining (NJ), maximum-likelihood (ML) and minimum-evolution (ME) methods in the MEGA 5.2 software were used to reconstruct phylogenetic trees. Percentage support values were obtained using a bootstrap procedure based on 1000 replications.

The taxonomic relationships between strain JC23T and its closest phylogenetic neighbours were examined using DNA–DNA hybridization studies. Genomic relatedness was determined by the membrane filter technique as described previously (Chakravarthy et al., 2012). Hybridization was performed with three replications for each sample (as a control, the strains used for binding and labelling were reversed).

Morphological properties (cell shape, cell size, motility) of strain JC23T grown in nutrient broth were observed directly or after Gram-staining using an Olympus BH-2 phase-contrast microscope. The hanging-drop method was also employed to test motility. Malachite green was used for spore staining as described by Schaeffer & Fulton (1933).

Growth was examined at pH 4–10 (at intervals of 0.5 pH units) with sodium acetate/acetate acid buffer for pH 4–5, K₂HPO₄/KH₂PO₄ buffer for pH 5.5–8 and NaHCO₃/NaOH buffer for pH 8.5–10, at 0–50 °C (at intervals of 5 °C) and in the presence of 0–10 % (w/v) NaCl (at intervals of 1 %) in nutrient broth unless otherwise mentioned. Growth was measured turbidometrically at 540 nm in a colorimeter (Systronics) after 48 h of incubation.

Various biochemical tests such as hydrolysis of starch, casein, Tween 80, gelatin and urea, activities of oxidase and catalase, reduction of nitrate and nitrite, the methyl red and Voges–Proskauer tests, production of H₂S, utilization of citrate and acid and gas production from carbohydrates were carried out in the prescribed media as outlined by Cappuccino & Sherman (1998). Aesculin hydrolysis and activities of arginine dihydrolase, phenylalanine deaminase, ornithine decarboxylase and lysine decarboxylase were determined as described by Smibert & Krieg (1981). Other biochemical tests were performed by using the GP2 MicroPlate (Biolog) in accordance with the manufacturer’s instructions.

Utilization of organic carbon compounds as carbon and energy sources for organoheterotrophic growth was tested in a mineral medium as described previously (Lakshmi et al., 2011) with specific organic compounds (0.35 %, w/v or v/v) replacing sodium pyruvate. The organic compounds tested were adipec, D-arabinose, benzoate, caproate, citrate, ethanol, formate, D-fructose, fumarate, D-glucose, glycerol, glycolate, glutonate, L-glutamate, lactate, melibiose, malate, D-mannitol, N-acetylglucosamine, phenylacetate, proline, pyruvate, sodium acetate, succinate, D-sorbitol, sucrose, thioglycolate, tartrate, valerate and L-xylene. Growth was measured turbidometrically (OD₅₄₀) after 48 h of incubation. Nitrogen source utilization was tested by replacing ammonium chloride with different nitrogen sources (NaNO₃, NaNO₂, L-glutamate, L-aspartate, L-glutamine and urea). Vitamin requirements were tested by replacing yeast extract in the medium with a solution containing a mixture of vitamins (thiamine, riboflavin, niacin, pantothenate, pyridoxal phosphate, p-aminobenzoate, biotin, vitamin B₁₂) except the one under test.

Compositions of cellular fatty acids, polar lipids and quinones were analysed from cultures that had attained 70 % of maximal optical density (late exponential growth phase) for all the strains tested, to ensure identical growth phase (Sasser, 1990). Polar lipids were extracted from 0.1 g freeze-dried cells with methanol/chloroform/saline [0.3 % (w/v) NaCl solution] (2 : 1 : 0.8, by vol.) and the lipids were separated using silica gel TLC (Kieselgel 60 F254; Merck) by two-dimensional chromatography using chloroform/methanol/water (30 : 10 : 1, by vol.) in the first dimension and chloroform/methanol/acetic acid/water (35 : 76 : 1.5, by vol.) in the second dimension (modified after Oren et al., 1996).
Total polar lipids were detected by spraying with 5% ethanolic molybdophosphoric acid and the polar lipid profile was further characterized by spraying with ninhydrin (for amino groups), molybdenum blue (for phosphates), Dragendorff reagent (for quaternary nitrogen) or α-naphthol (for sugars) (Kates, 1972; Oren et al., 1996).

Cell-wall samples were prepared from approximately 250 mg wet cells. Peptidoglycan was isolated after disruption of the cells by shaking with glass beads and subsequent trypsin digestion, according to the method of Schleifer (1985). The cell wall was hydrolysed (6 M HCl, 120 °C, 16 h) for amino acid analyses as described by Schleifer & Kandler (1972). Amino acids in cell-wall hydrolysates were analysed by HPLC (McKerrow et al., 2000). Cellular fatty acids were methylated, separated and identified according to the instructions for the Microbial Identification System (Microbial ID) (MIDI 6.0 version; Agilent 6850); peak identification was done based on the RTSBA6 database (Sasser, 1990) (revised protocol at http://www.midi-inc.com). This analysis was outsourced to Royal Research Labs, Secunderabad, India. Respiratory quinones were analysed as described by Xie & Yokota (2003).

Tolerance of organic solvents by strain JC23T and its closest phylogenetic neighbours was monitored in the presence of solvents having a range of partition coefficients: acetophenone (log p = 1.5), benzene (log p = 2.0), toluene (log p = 2.5), xylene (log p = 3.2) and hexane (log p = 3.4). Strain JC23T, L. manganicus CCTCC AB 2012916T and L. massiliensis CCUG 49529T were grown in 250 ml Erlenmeyer flasks containing 100 ml nutrient liquid medium overlaid with 20% (v/v) organic solvent and incubated at 35 °C for 24, 48 and 60 h. Flasks were plugged with butyl rubber stoppers to prevent solvent evaporation. Cultures growing in the absence of organic solvent under similar conditions served as a positive control. Cell growth was monitored by measuring the dry cell weight. To measure the dry cell mass, solvent-grown culture broth was centrifuged at 10,000 g at 4 °C for 10 min to pellet the cell mass. The pellet was then washed twice with sterile distilled water and dried under vacuum. For transmission electron microscopy, cells of strain JC23T, grown for 48 h in nutrient liquid medium in the presence and absence of acetophenone (20%, v/v), were harvested and washed with 0.1 M phosphate buffer (pH 7.4). Cells were processed as described previously (Sarkar & Ghosh, 2012) and examined with a Hitachi model H-7500 transmission electron microscope.

Antibiotic resistance of strain JC23T was determined using the disc diffusion method with commercial antibiotic-impregnated discs (BBL Becton Dickinson). The results were interpreted according to the guidelines set down by the CLSI (2003).

EzTaxon-e server search analysis revealed that strain JC23T was most closely related to members of genus Lysinibacillus, and the highest sequence similarity was observed to L. manganicus Mn1-7T (98.5%), L. massiliensis 440831T (97.2%), L. chungkukjangi 2RL3-2T (96.8%) and other members of the genus Lysinibacillus (≤ 96%).

The results of phylogenetic analysis of 16S rRNA gene sequences (1472 bp for strain JC23T) suggested that strain JC23T clustered consistently with the genus Lysinibacillus and formed a single clade along with the most closely related strains L. manganicus Mn1-7T and L. massiliensis 4400831T (Fig. 1); the sequence similarities to the nearest phylogenetic neighbours are in agreement with the EzTaxon-e server result. Further characterization of strain JC23T was done according to the appropriate recommended minimal standards (Logan et al., 2009) together with the closely related strains L. manganicus CCTCC AB 2012916T, L. massiliensis CCUG 49529T and L. boronitolerans DSM 17140T (representing the type species of the genus Lysinibacillus).

DNA–DNA reassociation between strain JC23T and L. manganicus CCTCC AB 2012916T was 39 ± 1%, while that between strain JC23T and L. massiliensis CCUG 49529T was only 36 ± 0.8%; these values are well within the recommended standards to delineate a bacterial species based on DNA–DNA reassociation (Stackebrandt & Goebel, 1994). The DNA G+C content of strain JC23T was 41 mol% (determined by HPLC).

On nutrient agar, colonies of JC23T were irregular, 1–3 mm in diameter, convex and brownish white. Cells were straight rods, 2–10 μm long and 0.3–0.5 μm wide (Fig. S1, available in the online Supplementary Material), that were Gram-stain-positive, divided by binary fission and formed terminal spherical endospores. While cells of the other members of the genus Lysinibacillus are motile, strain JC23T was non-motile.

NaCl was not required for growth of strain JC23T, but the strain tolerated up to 2% (w/v) NaCl, while the type strains of L. manganicus and L. massiliensis were more tolerant. Strain JC23T grew at pH 6–9 with optimum growth at pH 7; this differs from L. manganicus CCTCC AB 2012916T and L. massiliensis CCUG 49529T, which have pH ranges of 5–10 and 6–8, respectively. The temperature range for growth further differentiates strain JC23T from its closest phylogenetic neighbours (Table 1).

Strain JC23T, L. manganicus CCTCC AB 2012916T, L. massiliensis CCUG 49529T and L. boronitolerans DSM 17140T were positive for catalase and oxidase activities but negative for reduction of nitrate and nitrite and production of indole and H2S. Arginine dihydrolase, lysine decarboxylase, phenylalanine deaminase and ornithine decarboxylase activities were not detected in strain JC23T or its closest phylogenetic neighbours. Other phenotypic and biochemical characteristics of strain JC23T are presented in the species description and Table 1.

Strain JC23T utilized fructose, fumarate, melibiose, pyruvate, sorbitol and xylose as carbon and energy sources (Table 1). Sodium acetate, adipate, arabinose, benzoate, caproate, citrate, ethanol, glycerol, malate, phenylacetate,
proline, succinate, sucrose, thioglycolate, tartrate and valerate could not be utilized. Ammonium salts were the most suitable nitrogen sources. Though L-glutamate, L-glutamine and L-aspartate could not be utilized as carbon sources, they could support growth of the strain when used as sole nitrogen sources. Yeast extract with vitamin B12 are required for growth of strain JC23T but not for the type strains of L. manganicus and L. massiliensis.

The polar lipids of strain JC23T included diphosphatidylglycerol, phosphatidylglycerol, two unknown glycolipids (GL1, GL2), glycolipid GL3, which showed chromatographic motility comparable to that of β-gentiobiosylglycerol reported to be present in Bacillus subtilis (Kämpfer et al., 2006) and staphylococcal species (Nahaie et al., 1984), two unknown phospholipids (PL1, PL2) and two lipids L1 and L2 that, based on their staining behaviour, did not contain either a sugar moiety or a phosphate or amino group (Fig. S2A). The polar lipid profile of strain JC23T was in agreement with those of the type strains of L. manganicus and L. massiliensis, which contained diphasphatidylglycerol, phosphatidylglycerol, GL1, GL2 and β-gentiobiosylglycerol as the major compounds. The polar lipids of strain JC23T differed from those of L. manganicus CCTCC AB 2012916T in the presence of PL1, PL2 and L1 and the absence of L4 (Fig. S2B). The polar lipids of strain JC23T also differed from those of L. massiliensis CCUG 49529T in the presence of PL1, PL2, L1 and L2 and the absence of L3 and L5 (Fig. S2C). Though strain JC23T shared the presence of diphasphatidylglycerol and phosphatidylglycerol with the type strain of the

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**Fig. 1.** NJ tree based on 16S rRNA gene sequences showing phylogenetic relationships between strain JC23T and closely related phylogenetic neighbours. The tree was reconstructed with MEGA version 5.2 (Tamura et al., 2011) and rooted by using Paenibacillus polymyxa DSM 36T as the outgroup. Numbers at nodes represent bootstrap percentages. GenBank accession numbers for 16S rRNA gene sequences are shown in parentheses. Bar, 0.01 substitutions per nucleotide position. Nodes that were obtained by all three treeing methods (NJ, ME and ML) are represented by filled circles, and empty circles represent nodes that were recovered by the NJ and ME methods.
Table 1. Differentiating characteristics between strain JC23\(^T\) and the type strains of its closest phylogenetic neighbours and the type species of the genus Lysinibacillus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolation source</td>
<td>Solvent</td>
<td>Mining soil(^a)</td>
<td>Human cerebrospinal fluid(^b)</td>
<td>Soil(^c)</td>
</tr>
<tr>
<td>Cell size ((\mu)m)</td>
<td>2–10 × 0.3–0.5</td>
<td>1.7–2.5 × 0.3–0.7(^a)</td>
<td>1.5–4 × 0.3–0.5(^b)</td>
<td>3.0–5.0 × 0.8–1.5(^c)</td>
</tr>
<tr>
<td>Gram staining</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Motility</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth in 3 % NaCl</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Temperature for growth (°C)</td>
<td>25–40</td>
<td>15–45</td>
<td>25–45</td>
<td>16–45</td>
</tr>
<tr>
<td>pH range for growth (°C)</td>
<td>6–9</td>
<td>5–10</td>
<td>6–8</td>
<td>5.5–9.5</td>
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<tr>
<td>Vitamin B(_4) requirement</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Voges–Proskauer test</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
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<tr>
<td>Aesculin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Gelatin</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Urea</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Organic substrates utilized for growth</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Sodium acetate</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Fumarate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Sorbitol</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Oxidation of carbon sources</td>
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<tr>
<td>Adenosine</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>l-Alanine</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>2′-Deoxyadenosine</td>
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<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Glycyl l-glutamate</td>
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<td>+</td>
<td>–</td>
<td>+</td>
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<td>(\beta)-Hydroxybutyrate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
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<tr>
<td>Fructose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Melibiose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Xylose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Gas production from xylose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Characteristic polar lipid(s)†</td>
<td>GL1, GL2, GL3, PL1, PL2, L, L2</td>
<td>GL1, GL2, GL3, L2, L4</td>
<td>GL1, GL2, GL3, L3, L5</td>
<td>PE</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>41</td>
<td>42</td>
<td>45</td>
<td>38</td>
</tr>
</tbody>
</table>

*Data taken from: \(^a\), Liu et al. (2013); \(^b\), Glazunova et al. (2012); \(^c\), Ahmed et al. (2007).
†PE, Phosphatidylethanolamine; GL, unidentified glycolipid (GL3 shows chromatographic behaviour consistent with \(\beta\)-gentiobiosyldiacylglycerol); PL, unidentified phospholipid; L, unidentified lipid.

The fatty acid profile of strain JC23\(^T\) was characterized by saturated branched fatty acids (Table S1) such as iso-C\(_{15:0}\) (23.4 %), anteiso-C\(_{17:0}\) (16.7 %), anteiso-C\(_{15:0}\) (15.6 %) and iso-C\(_{16:0}\) (13 %). Though strain JC23\(^T\) shared the major fatty acids with the type strains of the closely related L. manganicus and L. massiliensis, the fatty acids C\(_{16:1\omega7c}\), iso-C\(_{17:1\omega7c}\) and C\(_{18:0}\) could be detected only in the former. Fatty acids iso-C\(_{14:0}\) and C\(_{15:0}\) which were otherwise present in significant amounts in the remaining type strains of the genus, were not detected in strain JC23\(^T\), and this characteristic can be employed to differentiate the novel strain from related species (Kämper et al., 1994; Ahmed et al., 2007). The quinone system of JC23\(^T\) was
composed of menaquinones MK-7 (89%), MK-6 (11%) and MK-8 (<1%). MK-7 is observed as the major respiratory lipopiguenone system among members of the genus Lysinibacillus.

Despite the toxicity exerted by the solvents, strain JC23<sup>T</sup> and <i>L. massiliensis</i> CCUG 49529<sup>T</sup> could grow in the presence of acetophenone, benzene and xylene, among the solvents tested in the present study. In addition, strain JC23<sup>T</sup> could also grow in the presence of toluene and hexane. <i>L. manganicus</i> CCUG 57911<sup>T</sup> could not resist the deleterious effects of any of the solvents tested in the present study. Dry cell weight of strain JC23<sup>T</sup> was highest in a medium containing acetophenone [from which it was isolated (log p = 1.5)] compared with other solvents like benzene, toluene, xylene and hexane. However, growth of strain JC23<sup>T</sup>, <i>L. manganicus</i> CCUG 2012916<sup>T</sup> and <i>L. massiliensis</i> CCUG 49529<sup>T</sup> in a medium containing organic solvents was considerably less than the growth in their absence. Accumulation of solvent was clearly seen from cells of strain JC23<sup>T</sup> grown in the presence of organic solvent (Fig. S4A, B), which was similar to some previous reports when bacteria were grown in p-xylene (Cruden et al., 1992) or cyclohexane (Gupta & Khare, 2006; Sarkar & Ghosh, 2012). However, the adaptation strategy of strain JC23<sup>T</sup> to toxic organic solvents is yet to be understood.

Strain JC23<sup>T</sup> was resistant to nalidixic acid (30 μg) alone, while it was sensitive to gentamicin (120 μg), kanamycin (30 μg), vancomycin (30 μg), streptomycin (30 μg), tetracycline (30 μg), chloramphenicol (30 μg), ampicillin (30 μg) and penicillin G (30 μg).

Strain JC23<sup>T</sup> can be differentiated from the phylogenetically closest related type strains of <i>L. manganicus</i> and <i>L. massiliensis</i> on the basis of motility, temperature range for growth, NaCl tolerance, vitamin requirement, polar lipid composition and the presence/absence and amount of various fatty acids (Tables 1 and S1, Fig. S2). Its distinct phylogenetic, genotypic, chemotaxonomic and phenotypic properties thus justify the description of a novel species of the genus to accommodate strain JC23<sup>T</sup>, for which the name <i>Lysinibacillus acetophenoni</i> sp. nov. is proposed.

**Description of Lysinibacillus acetophenoni sp. nov.**

<i>Lysinibacillus acetophenoni</i> (a.ce.to.phe.no’ni. N.L. n. acetophenonum acetophenone; N.L. gen. n. acetophenoni of/from acetophenone).

Cells are Gram-stain-positive, non-motile, straight rods (2–10 × 0.3–0.5 μm). Obligate aerobic. On nutrient agar, colonies are irregular, 1–3 mm in diameter, convex and brownish white. Tolerates organic solvents with a range of partition coefficients. Temperature optimum for growth is 35 °C (range 25–40 °C). Tolerates pH 6–9 (optimum pH 7). NaCl is not required for growth, but is tolerated up to 2% (w/v). Positive for catalase and oxidase activities; negative for hydrolysis of aesculin, gelatin, Tween 80, starch, casein and urea. Tests for reduction of nitrate and nitrite, activities of arginine dihydrodrolase, lysine decarboxylase, phenylalanine deaminase and ornithine decarboxylase, citrate utilization and production of H₂S and the methyl red and Voges–Proskauer tests are negative. Indole is not produced from L-tryptophan. Acid is produced from D-fructose, melibiose and L-xylose. Negative for acid and gas production from L-arabinose, cellobiose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, raffinose, salicin, sucrose and trehalose. In the Biolog GP2 MicroPlate, oxidizes adenosine, L-alanine, 2’-deoxyadenosine, β-hydroxybutyrate and pyruvate, but not acetate, 2,3-butanediol, citrate, α-cyclodextrin, dextrin, glycerol 1-glutamic acid, glycerol, inosine, myo-inositol, lactose, lactulose, melezitose, palatinose, D-psicose, D-rhamnose, sedoheptulose, stachyose or xyliol. Utilizes D-fructose, fumarate, melibiose, pyruvate, D-sorbitol and L-xylene as sole sources of carbon and energy. Ammonium chloride, L-glutamate, L-glutamine and L-aspartate are used as nitrogen sources. Yeast extract and vitamin B₁₂ are required for growth. The quinone system is composed primarily of MK-7, with moderate amounts of MK-6. Diphosphatidylglycerol, phosphatidylglycerol, GL3 (β-gentiobiosyldiacylglycerol), unidentified glycolipids GL1 and GL2, phospholipids PL1 and PL2 and unknown lipids L1 and L2 are the polar lipids. Predominant amounts of iso-C₁₅:0 anteiso-C₁₇:0 anteiso-C₁₅:0 and iso-C₁₆:0 along with the presence of C₁₆:1ω7c, iso-C₁₇:0 ω6c, C₁₆:0 and iso-C₁₇:1ω9c in moderate quantities are the fatty acids. Cell-wall peptidoglycan contains L-lysine, L-alanine, D-alanine, D-aspartic acid and D-glutamic acid as the diagnostic amino acids (type A4x-L-Lys–D-Asp).

The type strain, JC23<sup>T</sup> (=CCUG 57911<sup>T</sup> = KCTC 13605<sup>T</sup> = NBRC 105754<sup>T</sup> = DSM 23394<sup>T</sup>), was isolated from the organic solvent acetophenone. The DNA base composition of the type strain is 41 mol% G+C.

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**References**


Lysinibacillus acetophenoni sp. nov.


