Spirosoma lacussanchae sp. nov., a phosphate-solubilizing bacterium isolated from a freshwater reservoir

Yong Li,1 Meng-Jie Ai,2 Ye Sun,2 Yu-Qin Zhang2,* and Jian-Qiang Zhang1,*

Abstract

A phosphate-solubilizing bacterium, designated CPCC 100624T, was isolated from a freshwater reservoir in south-west China. The 16S rRNA gene sequence comparison of strain CPCC 100624T with the available sequences in the GenBank database showed that the isolate was closely related to members of the genus Spirosoma. In the phylogenetic tree based on 16S rRNA gene sequences, strain CPCC 100624T formed a stable phylogenetic subclade with Spirosoma soli MIMBqt12T within the genus Spirosoma, which indicated that strain CPCC 100624T could be identified as a member of the genus Spirosoma. The strain grew at 10–40 °C (optimum 30 °C), at pH 6.5–8.0 (optimum pH 7.0–7.5) and in the presence of 0–5 % (w/v) NaCl (optimum 0–1 %). MK-7 was detected as the main menaquinone, with a minor amount of MK-7(H6) in its menaquinone system. Cells contained summed feature 3 (C16:1ω6c and/or C16:1ω7c), C16:1ω5c and iso-C15:0 as the major fatty acids. The polar lipids of strain CPCC 100624T contained phosphatidylethanolamine, one unidentified aminolipid, two aminophospholipids and three unidentified lipids. The genomic DNA G+C content was 49.3 mol%. On the basis of the above taxonomic data and differences in physiological characteristics from the closely related type strains, strain CPCC 100624T represents a novel species of the genus Spirosoma, for which the name Spirosoma lacussanchae sp. nov. is proposed. The type strain is CPCC 100624T (=NBRC 111852=DSM 101771T).

Sanchahu Reservoir, located at Jianyang city in Sichuan Province, south-west China, is the second biggest freshwater lake in Sichuan Province, which is well known as Tianfu Pearl. In recent years, cyanobacterial blooms were found to frequently occur in this reservoir. In order to demonstrate the relationship of the soluble phosphate with the cyanobacterial blooms, we analysed the bacterial community structure in different seasons in this aquatic ecosystem. As a result, diverse phosphate-solubilizing bacteria (PSB) were obtained and characterized, among which, one PSB strain, CPCC 100624T, was found to represent a novel species of the genus Spirosoma.

The genus Spirosoma was proposed by Migula [1] as a member of the family Cytophagaceae. At the time of writing, there were 13 species of this genus with validly published names, including three novel names that appeared in IJSEM in 2016 (www.bacterio.net/spirosoma.html) [2–4]. These species share the common chemotaxonomic characteristics of containing MK-7 as the predominant quinone, phosphatidylethanolamine as the major polar lipid, C16:1ω6c/C16:1ω7c, C16:1ω5c, iso-C15:0 and C16:0 as the major cellular fatty acids, and genomic G+C contents of 47.2–57.0 mol% [5, 6]. Members of the genus Spirosoma were found in a wide variety of environments, such as soil, freshwater, Arctic glacial till, air and soil crust in desert. Here, we report the taxonomic study using a polyphasic approach on another member of the genus Spirosoma member, strain CPCC 100624T, discovered from a sediment sample collected from a reservoir. The phenotypic and genotypic characteristics of strain CPCC 100624T approved it as a novel species of the genus Spirosoma.

The surface sediment samples for isolation of PSB were collected from Sanchahu Reservoir (30°15′–30°20’ N 104°15’–104°16’ E, depth 10–23 m). About 20 g mud from each sampling site was placed in a sterilized tube following collection, taken to the laboratory and then processed for isolation immediately after arriving at the laboratory. Strain CPCC 100624T was recovered on the isolation plate containing (l−): glucose, 10 g; l-α-phosphatidylcholine, 2.0 g; (NH4)2SO4, 0.5 g; MgSO4, 7H2O, 0.3 g; NaCl, 0.3 g; KCl, 0.3 g; FeSO4. 4H2O, 0.036 g; MnSO4, 4H2O, 0.03 g; agar, 15 g; distilled water, 1000 ml; pH 7.2. The purified culture...
was maintained on YM slants at 4 °C and also as glycerol suspensions (20%, v/v) at −80 °C and in liquid nitrogen. The YM medium contained (L−1): yeast extract (Difco), 4.0g; malt extract (Difco), 10.0 g; glucose (Difco), 4.0g; agar, 15.0g; distilled water 1000 ml; pH 7.2.

Colonial morphology was determined after 3 days at 30 °C on YM agar medium. Cellular morphology was studied using a JEOL JEM-1010 electron microscope (transmission electron microscopy mode) with cells from exponentially growing cultures on YM agar. Before cells were mounted on formvar-coated copper grids (Electron Microscopy Science), they were negatively stained with 2% (w/v) uranyl acetate for 15 s. Motility of cells was examined on YM swarming agar (0.3 %, w/v), and then checked using the electron microscope. Gram staining was carried out by the standard Gram reaction and observed by light microscopy (BH-2; Olympus).

The temperature range for growth was tested at 0, 4, 10, 25, 28 to 37 °C (at intervals of 1.0 °C), and 40 °C in YM and TSB (DSMZ 545 medium) media using multi-thermo incubators (Eyela, MTI-20; Tokyo Rikakikai). The pH range for growth was tested at different pH (5.0–11.5, at intervals of 0.5 pH units), using the following buffers to adjust the pH: 0.1 M citric acid/0.1 M sodium citrate for pH 5.0, 0.1 M KH2PO4/0.1 M NaOH for pH 6.0–8.0, 0.1 M NaHCO3/0.1 M Na2CO3 for pH 9.0–10.0, and 0.1 M Na2HPO4/0.1 M NaOH for pH 11.0–11.5. Tolerance to NaCl was examined on YM agar with different NaCl concentrations (0, 1, 3, 5, 7 and 10 %, w/v). Catalase activity was determined by observing the production of bubbles after the addition of a drop of 3 % H2O2 to the cells. Metabolic characters were determined by Biolog GEN III MicroPlate, and API 50CH and API ZYM test kits (bioMeriéux). Oxidase activity was detected using API oxidase reagent (bioMeriéux) according to the manufacturer’s instructions. Hydrolisis of gelatin and starch, and production of H2S were tested as described by Smibert and Krieg [7]. Susceptibility to antibiotics was determined following the procedure described previously [8]. The ability to solubilize insoluble phosphate was examined on plates using phosphate-solubilizing media [(L−1): glucose, 10 g; (NH4)2SO4, 0.5 g; MgSO4, 7H2O, 0.3 g; NaCl, 0.3 g; KCl, 0.3 g; FeSO4. 4H2O, 0.036 g; MnSO4. 4H2O, 0.03 g; Ca3(PO4)2, 10 g or l-α-phosphatidylcholine, 2.0 g; distilled water, 1000 ml; pH 7.0], and further confirmed using a liquid medium system with a final cell density of (ml−1) 1×106 c.f.u. The culture broth was centrifuged (4860 g, 20 min) on the fifth day of incubation, and the amount of available phosphorus in the supernatant (measured as phosphate equivalent) was determined colorimetrically according to the protocol described by Zhang et al. [9].

Growth of strain CPCC 100624T was observed to occur at 10–40 °C (optimum 30 °C), at pH 6.5–8.0 (optimum pH 7.0–7.5), and in the presence of 0–5 % (w/v) NaCl (optimum 0–1 %) in YM and TSB media. Colonies were pale yellow, circular, convex and smooth. Cells were Gram-stain-negative, aerobic, non-motile and rod-shaped (Fig. S1, available in the online Supplementary Material). No visible halo zone for solubilization of Ca3(PO4)2 while a weak halo zone for solubilization of l-α-phosphatidylcholine in plates was observed around strain CPCC 100624T. In the liquid culture assay, strain CPCC 100624T showed the ability to solubilize l-α-phosphatidylcholine with a detection of additional available phosphorus of 1.9±0.12 mg L−1 more than the negative control. However, the strain was not able to solubilize Ca3(PO4)2. Strain CPCC 100624T was resistant to (µg ml−1): ampicillin (10), bacitracin (10), neomycin (10), nystatin (10), penicillin G (10), spectinomycin (10), streptomycin (30) and tetracycline (30). Other physiological characteristics are summarized in the species description. Selected characteristics that differentiate strain CPCC 100624T from related species of the genus Spirosoma are shown in Table 1.

Biomass of strain CPCC 100624T for chemical and molecular studies was obtained at the stationary growth phase by cultivation in TSB at 30 °C in shaken flasks (about 150 r.p.m.).

Polar lipids were extracted and examined by two-dimensional TLC and identified using the previously described procedures [10, 11]. The respiratory quinone was isolated, purified and analysed as described by Lee et al. [12]. Analysis of the whole cell fatty acids pattern followed the described methods using the MIDI system (Microbial ID, Inc.) [13, 14]. The Microbial Identification Standard software package MIDI version 6 and the database TSBA 6 were used in this study.

Phosphatidylethanolamine, one unidentified aminolipid, two aminophospholipids and three unidentified lipids were detected in the polar lipids extract from cells of strain CPCC 100624T (Fig. S2). MK-7 (96.3 %) was identified as the main menaquinone, with a minor amount of MK-7(H0) (3.7 %) also present. Cells were found to contain summed feature 3 (C16:1ω6c and/or C16:1ω7c), C16:1ω5c and iso-C15:0 as the major fatty acids; detailed fatty acids composition is given in Table S1. These chemotaxonomic data supported the placement of strain CPCC 100624T in the genus Spirosoma.

Extraction of genomic DNA and PCR amplification of the 16S rRNA gene were done as described by Li et al. [15]. The obtained sequence was compared with available 16S rRNA gene sequences from the GenBank database using the BLAST program and the EzTaxon-e server (www.ezbiocloud.net; [16]) to determine an approximate phylogenetic affiliation. Multiple alignments with sequences of the most closely related taxa and calculations of levels of sequence similarity were carried out using MEGA software package version 5 [17]. Phylogenetic trees were reconstructed using the neighbour-joining method [18] with K_{sub} values [19, 20] and complete deletion gaps, and maximum-parsimony [21] and maximum-likelihood [22] methods. The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein [23] with 1000 replicates. The G+C
Table 1. Differential characteristics of *Spirosoma lacussanctae* sp. nov. CPCC 100624\(^T\) and other species of the genus *Spirosoma*

Strains: 1. CPCC 100624\(^T\) (data from this study); 2. *Spirosoma solii* MIMBtg12\(^T\) [3]; 3. *Spirosoma aerophilum* 5516 J-17\(^T\) [2]; 4. *Spirosoma arcticum* R2-35\(^T\) [25]; 5. *Spirosoma endophyticum* EX36\(^T\) [26]; 6. *Spirosoma fluviale* MSd3\(^T\) [27]; 7. *Spirosoma linguale* DSM 74\(^T\) [1]; 8. *Spirosoma luteum* DSM 19990\(^T\) [6]; 9. *Spirosoma oryzae* KACC 17324\(^T\) [5]; 10. *Spirosoma panacterae* Gsoil 1519\(^T\) [28]; 11. *Spirosoma rigui* KACC 13387\(^T\) [29]; 12. *Spirosoma spitsbergense* DSM 19989\(^T\) [6]; 13. *Spirosoma aerolatum* PR1012K\(^T\) [30]; 14. *Spirosoma swuense* JBM2-3\(^T\). +, Positive; −, negative; w, weakly positive; nd, not determined; Al, aminolipid; APL, aminophospholipid; DPG, diphosphatidylglycerol; L, unidentified polar lipid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; GL, glycolipid, PL, unidentified phospholipid.

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<td><strong>DNA G+C content (mol%)</strong></td>
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<td>49.5</td>
<td>55.7</td>
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content of the genomic DNA was determined using the thermal denaturation method [24]. The almost-complete 16S rRNA gene sequence (1509 bp) of strain CPCC 100624<sup>T</sup> was obtained. The results of 16S rRNA gene sequence comparison indicated that strain CPCC 100624<sup>T</sup> was a member of the family Cytophagaceae, phylum Bacteroidetes, with 16S rRNA gene sequence similarities of 91.6–93.2% with members of the genus Spirosoma. Strain CPCC 100624<sup>T</sup> fell in the genus Spirosoma lineage and formed a stable phylogenetic subclade with Spirosoma soli MIMBbqt12<sup>T</sup> with a high bootstrap value (92, i.e. 92%) in neighbour-joining tree (Fig. 1), and the same topology was observed in maximum-parsimony and and maximum-likelihood trees. The genomic DNA G+C content of strain CPCC 100624<sup>T</sup> was determined to be 49.3 mol%.

The result of 16S rRNA gene sequence comparisons and the chemotaxonomic data demonstrated that strain CPCC 100624<sup>T</sup> was a member of the genus Spirosoma. The fact that the novel isolate CPCC 100624<sup>T</sup> forms a subclade with Spirosoma soli MIMBbqt12<sup>T</sup> within the genus Spirosoma (Fig. 1), with low 16S rRNA gene sequence similarities (91.6–93.2%) with other members of the genus Spirosoma suggested that strain CPCC 100624<sup>T</sup> is a new member of this genus. Additionally, strain CPCC 100624<sup>T</sup> differs from other members of the genus Spirosoma in some physiological and enzymatic properties (Table 1), and clearly differs from its closest phylogenetic neighbour Spirosoma soli MIMBbqt12<sup>T</sup> by fatty acid profiles (Table S1). Therefore, based on the above phenotypic and genotypic data, strain CPCC 100624<sup>T</sup> should be classified as a representative of a novel species of the genus Spirosoma, for which the name Spirosoma lacussanchae sp. nov. is proposed.

**DESCRIPTION OF SPIROSOMA LACUSSANCHAE SP. NOV.**

*Spirosoma lacussanchae* (la.cus san’chae. L. n. lacus -us lake; Sancha name of the reservoir Sancha; N.L. gen. n. lacussen-chae from Sancha Lake where the bacterium was isolated).

Colonies are pale yellow, smooth, convex, entire and opaque. Cells are Gram-stain-negative, non-motile and rod-shaped with size of 0.6–0.8 µm in width and 1.8–2.3 µm in length. Tolerates up to 5% (w/v) NaCl. Growth occurs at pH 6.5–8.0 and 10–40°C, with optimum growth at 30°C and pH 7.0–7.5. Can use d-fuctose 6-phosphate, d-fucose,

**Fig. 1.** Neighbour-joining tree derived from aligned 16S rRNA sequences, showing the position of strain CPCC 100624<sup>T</sup> among the phylogenetically nearest neighbours. Numbers at nodes indicate percentage levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets. Single closed circles indicate the corresponding nodes were also recovered by maximum-parsimony (MP) or maximum-likelihood (ML) algorithm, and double closed circles indicate the corresponding nodes were recovered by both maximum-parsimony and maximum-likelihood algorithms. Bar, 0.02 substitutions per nucleotide position.
D-galactose, D-glucose 6-phosphate, D-gluconic acid, melibiose, dextrin, glucuronamide, L-fucose, L-malic acid, L-rhamnose and α-L-dulcitol, rhamnose and α-L-rhamnose as the sole carbon sources for energy and growth in Biolog Gen III MicroPlate. Acid is produced from D-arabinose, D-fucose, D-lyxose, D-sorbitose, dulcitol, L-arabinose, L-rhamnose and L-xyllose in API 50CH array. Positive for gelatin hydrolysis, catalase activity, α-galactosidase, β-chymotrypsin, N-acytyle-β-glucosaminidase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase, but negative for reduction of nitrate, α-fucosidase, β-galactosidase, β-glucosidase, β-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase, oxidase, methyl red and Voges–Proskauer tests, casein and starch degradation, and H₂S production. The predominant menaquinone is MK-7, with a minor amount of MK-7(H₄). The polar lipid profile contains significant amounts of unidentified aminolipid and unidentified aminophospholipids, and minor amounts of unidentified lipids. The major fatty acids are summed feature 3 (C₁₆:1ω₆c and/or C₁₆:1ω7c), C₁₆:1ω5c and iso-C₁₅:0.

The type strain, CPCC 100624T (=NBRC 111852T =DSM 101771T), was isolated from surface sediment samples collected from a freshwater reservoir in south-west China. The genomic DNA G+C content of the type strain is 49.3 mol%.

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**Conflicts of interest**
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Ethical statement**
This research did not contain any studies with animals performed by any of the authors.

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