Serinibacter tropicus sp. nov., an actinobacterium isolated from the rhizosphere of a mangrove, and emended description of the genus Serinibacter

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A novel Gram-stain-positive actinobacterium, designated PS-14-7T, was isolated from the rhizosphere of a mangrove on Pramuka Island, Indonesia, and its taxonomic position was investigated using a polyphasic approach. The peptidoglycan type of strain PS-14-7T was A4\(\alpha\) and lysine was the diagnostic diamino acid of the peptidoglycan. The predominant menaquinone was MK-8(H4) and the major fatty acids were anteiso-C\(_{15:0}\), C\(_{16:0}\) and iso-C\(_{16:0}\). The DNA G+C content was 72.8 mol\%. Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain PS-14-7T was closely related to Serinibacter salmoneus Kis4-28T (99.6 %). However, DNA–DNA hybridization and phenotypic characteristics revealed that strain PS-14-7T differed from Serinibacter salmoneus. Therefore, strain PS-14-7T presents a novel species of the genus Serinibacter, for which the name Serinibacter tropicus sp. nov. is proposed. The type strain is PS-14-7T (=NBRC 110108\(^\text{T}\)=InaCC A 515\(^\text{T}\)). An emended description of the genus Serinibacter is also proposed.

The genus Serinibacter was proposed by Hamada et al. (2009) with a single species Serinibacter salmoneus, as a member of the family Beutenbergiaceae within the suborder Micrococccineae. The type strain of this species was isolated from the intestinal tract of a fish collected from Tokyo Bay, Japan. The single member of this genus has peptidoglycan type A4\(\alpha\) (Schleifer & Kandler, 1972) with lysine as the diagnostic diamino acid of the peptidoglycan. The predominant menaquinone is MK-8(H4) and the major fatty acids are anteiso-C\(_{15:0}\), C\(_{16:0}\) and iso-C\(_{16:0}\). During the course of a study on the isolation and diversity of actinobacteria from seashore environments in Indonesia, a novel actinobacterium was isolated from the rhizosphere of a mangrove. Comparative 16S rRNA gene sequence analysis revealed that the isolate, which was designated strain PS-14-7T, was phylogenetically related to the member of the genus Serinibacter. The objective of this study was to clarify the taxonomic position of the isolate by using a polyphasic approach.

Strain PS-14-7T was isolated from a sediment sample that had been collected from the rhizosphere of a mangrove (Rhizopora mucronata) growing on the seashore of Pramuka Island, DKI Jakarta, Indonesia. Approximately 1 g sample was diluted 10-, 100- and 1000-fold with artificial seawater (Nihon Pharmaceutical) before 0.2 ml each dilution was spread on plates of 0.2 × NBRC medium 802 [0.2 % (w/v) polypeptone, 0.04 % (w/v) yeast extract, 0.02 % (w/v) MgSO\(_4\) · 7H\(_2\)O and 1.5 % (w/v) agar; pH 7.0], supplemented with 5.0 % (w/v) NaCl, 0.005 % (w/v) cycloheximide and 0.002 % (w/v) nalidixic acid. After cultivation at 30 °C for 1 week and single colony isolation twice, strain PS-14-7T was obtained. As the strain did not require NaCl for growth, full-strength NBRC medium 802 [1.0 % (w/v) polypeptone, 0.2 % (w/v) yeast extract (Difco), 0.1 % (w/v) MgSO\(_4\) · 7H\(_2\)O and, if required, 1.5 % (w/v) agar; pH 7.0] was used as the basal medium for this study. Serinibacter salmoneus NBRC 104924\(^\text{T}\) was used as a reference strain in this study.

Colony appearance was examined after cultivation at 28 °C for 5 days on an agar plate of NBRC medium 802. Morphological features were observed depending on the age (up to 7 days) under a light microscope (BX-51; Olympus) and a scanning electron microscope (JSM-6060; JEOL). The temperature range and optimum temperature for growth were determined by incubating cultures at 5, 10, 15, 20, 25, 28, 37, 45 and 60 °C on agar plates of NBRC medium 802 for 4 days (15–60 °C) or 14 days (5 and 10 °C). The pH and
NaCl ranges for growth were determined by measuring the turbidity (610 nm) of 5 ml of the culture in test tubes after 1–4 days incubation at 28 °C. The pH range and optimum pH for initial growth were established by using liquid NBRC medium 802 adjusted to pH 4–10 (in 1.0 pH unit intervals) with either 4 M HCl or 5 M KOH. Tolerance to NaCl was tested using liquid NBRC medium 802 adjusted to NaCl concentrations of 1, 3, 5, 7, 10 and 15 % (w/v). Cell motility, oxidase and catalase activities, anaerobic growth and Gram staining were determined using the methods described by Hamada et al. (2012). Other physiological and biochemical tests were performed using API ZYM, API Coryne and API 50 CH systems (bioMérieux) according to the manufacturer’s instructions.

Strain PS-14-7T formed orange–yellow, circular and smooth colonies that were approximately 0.5–1.0 mm in diameter after 5 days cultivation. Cells of the strain were irregular, short rod-shaped (0.4–0.5 × 1–2 μm), Gram-stain-positive, non-motile and non-endospore-forming. Cells in older cultures tended to be shorter and rounder (Fig. S1, available in the online Supplementary Material). The strain was also catalase- and oxidase-positive. Growth occurred at 20–37 °C, and no growth was observed at 5, 10, 15, 45 or 60 °C. The pH range for growth was 6.0–9.0. Optimal growth was noted at 28 °C and pH 7.0. The strain exhibited good growth with NaCl concentrations of 0–5 % (w/v) and weak growth with 7 % NaCl; no growth was observed with 10 or 15 % NaCl. Weak growth was observed under anaerobic conditions. The results of other physiological and biochemical analyses are summarized in the species description.

DNA was isolated using PrepMan Ultra Reagent (Applied Biosystems) according to the manufacturer’s instructions. The 16S rRNA gene was amplified by PCR using KOD FX (Toyobo) with the following primer pair: 9F (5’-GAGTTTGATCCCTGCTCAAG-3’) and 1541R (5’-AAGGAGGTGATCCAGGCC-3’). The amplified 16S rRNA gene was subjected to cycle sequencing using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) with the following primers: 9F, 785F (5’-GGATTAGATACCGTGATGTC-3’), 802R (5’-TACCAAGGTATCTAATCC-3’) and 1541R. The products were analysed using an automated DNA sequencer (ABI PRISM 3730 Genetic Analyzer; Applied Biosystems). Phylogenetic neighbours were identified and pairwise 16S rRNA gene sequence similarities were calculated using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). The CLUSTAL X program (Thompson et al., 1997) was used to align the almost-complete 16S rRNA gene sequence of strain PS-14-7T (1483 nt) with corresponding sequences of members of the family Beutenbergiaceae. Evolutionary distances were calculated using Kimura’s two-parameter model (Kimura, 1980). Phylogenetic trees were reconstructed by the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) algorithms by using the MEGA 5.0 program (Tamura et al., 2011). The resultant tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) based on 1000 replicates.

Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain PS-14-7T was closely related to members of the family Beutenbergiaceae. The highest 16S rRNA gene sequence similarity value was observed with Serinibacter salmoneus Kis4-28T (99.6 %), followed by Minimonas arenae YM18-15T (97.3 %), Beutenbergia cavernae DSM 12333T (96.5 %) and Salana multivorans DSM 13521T (96.1 %). Strain PS-14-7T showed less than 95.0 % 16S rRNA gene sequence similarity with other members of the suborder Micrococcineae. In the neighbour-joining tree, strain PS-14-7T and Serinibacter salmoneus Kis4-28T formed a monophyletic cluster with a bootstrap resampling value of 100 % (Fig. 1). This cluster was also recovered in the trees generated by the maximum-likelihood and maximum-parsimony algorithms.

Biomass for chemotaxonomic studies, except for fatty acid analysis, was obtained by cultivating strains in shake flasks

![Phylogenetic tree derived from 16S rRNA gene sequences of strain PS-14-7T and members of the family Beutenbergiaceae, reconstructed with the neighbour-joining method. The 16S rRNA gene sequence of Cellulomonas flavigena DSM 20109T (GenBank accession no. X83799) was used as the outgroup. Bootstrap values (>50 %) based on 1000 replicates are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Bar, 0.01 K\textsubscript{sub} substitutions per nucleotide position.](image-url)
at 28 °C and 100 r.p.m. for 48 h. Amino acids and the isomers in cell-wall hydrolysates, cell-wall sugars, isoprenoid quinones and the DNA G+C content were determined according to the methods described by Hamada et al. (2012). Polar lipids were extracted from 100 mg freeze-dried cells, purified using the method described by Minnikin et al. (1975) and analysed by TLC using chloroform/methanol/water (65:25:4, by vol.) in the first direction and chloroform/acetate/methanol/water (80:18:12:5, by vol.) in the second. For fatty acid methyl ester analysis, strain PS-14-7T and the reference strain were cultured on tryptic soy agar (Difco) for 24 h at 28 °C. Cellular fatty acid methyl esters were analysed by GC (6890N; Agilent Technologies) according to the standard protocol of the Sherlock Microbial Identification System (Sasser, 1990) with Sherlock MIDI software (version 4.0) and a TSBA database (version 4.0).

The peptidoglycan sample of strain PS-14-7T contained alanine (Ala), glutamic acid (Glu), serine (Ser) and lysine (Lys) in a molar ratio of 1.5:2.0:1.3:1.2. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of D-Ala, D-Glu, L-Glu, L-Ser and L-Lys; however, L-Ala was absent. These data suggested that the peptidoglycan type of strain PS-14-7T was A4\(_2\), with lysine as the diagnostic cell-wall diamino acid, an interpeptide bridge comprising L-Glu, A11.54 (Schleifer & Kandler 1972; Schumann, 2011), and an L-Ser residue at position 1 of the peptide subunit. A trace amount of galactose was detected as a cell-wall sugar. The predominant menaquinone was MK-8(H4); MK-7(H4), MK-8(H6), MK-8 and MK-8(H2) were also detected as minor or trace components. The major cellular fatty acids of strain PS-14-7T were anteiso-C\(_{15}\):0 (36.7 %), C\(_{16}\):0 (29.3 %) and iso-C\(_{16}\):0 (13.2 %) (Table S1). The polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and five unidentified phospholipids. Meanwhile, the polar lipids of Serinibacter salmoneus NBRC 104924\(^T\) were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and two unidentified phospholipids (Fig. S2).

Hamada et al. (2009) reported that phosphatidylethanolamine was present as a polar lipid of Serinibacter salmoneus. However, this study revealed that phosphatidylethanolamine was absent in Serinibacter salmoneus together with strain PS-14-7\(^T\). The DNA G+C content of strain PS-14-7\(^T\) was 72.8 mol%.

The microplate hybridization method developed by Ezaki et al. (1989) was used to determine DNA–DNA relatedness. DNA–DNA hybridizations were performed using five replications. After the highest and lowest values for each sample were excluded, the mean of the remaining three values was reported as the DNA–DNA relatedness value. The DNA–DNA relatedness value between strain PS-14-7\(^T\) and Serinibacter salmoneus NBRC 104924\(^T\) was 31.2 % (reciprocal reaction, 26.2 %). This value is well below the 70 % cut-off point of DNA–DNA relatedness, which is a criterion for the assignment of bacterial strains to the same genomic species (Wayne et al., 1987).

The result of the phylogenetic analysis based on 16S rRNA gene sequences suggested that strain PS-14-7\(^T\) belonged to the genus Serinibacter, and chemotaxonomic characteristics also corresponded to those of the genus. Meanwhile, the DNA–DNA relatedness value between strain PS-14-7\(^T\) and Serinibacter salmoneus NBRC 104924\(^T\) was low, and strain PS-14-7\(^T\) differed from the type strain of Serinibacter salmoneus in the following characteristics: the presence of urease; the absence of pyrazinamidase and pyrrolidonyl arylamidase; acid production from L-rhamnose; and no acid production from N-acetylglucosamine, cellobiose, fructose, gentiobiose, D-glucose, glycogen, L-lactose, starch and sucrose (Table 1). Therefore, it is proposed that strain PS-14-7\(^T\) represents a novel species of the genus Serinibacter, with the name Serinibacter tropicus sp. nov. In addition, an emended description of the genus Serinibacter Hamada et al. 2009 is provided to correct the polar lipid profile.

### Emended description of the genus Serinibacter Hamada et al. 2009

The description is as given by Hamada et al. (2009) with the following modifications. Major polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and unidentified phospholipids. Phosphatidylethanolamine is absent.

### Description of Serinibacter tropicus sp. nov.

Serinibacter tropicus (tro’pi.cus. L. masc. adj. tropicus tropical, pertaining to the tropics).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
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<tr>
<td>Temperature range for growth (°C)</td>
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<td>10–37</td>
</tr>
<tr>
<td>NaCl range for growth (% w/v)</td>
<td>0–7</td>
<td>0–10</td>
</tr>
<tr>
<td>Pyrazinamidase</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Pyrrolidonyl arylamidase</td>
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<td>+</td>
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<tr>
<td>Urease</td>
<td>–</td>
<td>+</td>
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<tr>
<td>Acid production from (API 50 CH):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose</td>
<td>–</td>
<td>+</td>
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<td>Fructose</td>
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<td>Starch</td>
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<tr>
<td>Sucrose</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>72.8</td>
<td>70.7</td>
</tr>
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</table>

Table 1. Differential phenotypic characteristics of strain PS-14-7\(^T\) and the type strain of Serinibacter salmoneus

Strains: 1, PS-14-7\(^T\); 2, Serinibacter salmoneus NBRC 104924\(^T\). All data are from this study. +, Positive; –, negative.
Cells are irregular, short rod-shaped (0.4–0.5 × 1–2 μm), Gram-stain-positive, non-motile and non-endospore-forming. Cells in older cultures tend to be shorter and rounder. Colonies are orange–yellow, circular and smooth, after 5 days cultivation on an agar plate of NBRC medium 802. Catalase- and oxidase-positive. The temperature range for growth is 20–37 °C (optimum 28 °C). The pH range for growth is 6.0–9.0 (optimum pH 7.0). Growth occurs in NaCl concentrations of 0–7%. Weak growth occurs under anaerobic conditions. Positive results in tests for cellular phosphatase, x-galactosidase, β-galactosidase, x-glucosidase and urease, but negative results for alkaline phosphatase, chymotrypsin, cysteine arylamidase, x-fucosidase, β-gluconoridase, lipase (C14), x-mannosidase, pyrazimidase, pyrrolidonyl arylamidase, trypsin and valine arylamidase. Weakly positive results in tests for N-acetlyl-β-glucosaminidase, esterase (C4), esterase lipase (C8), β-glucosidase, leucine arylamidase and phosphohydrolase. In API 50 CH assays, acid is produced from D-galactose, glycerol, D-maltose, D-mannose, methyl x-D-glucopyranoside, methyl β-D-xylopyranoside, L-ramnose, trehalose, turanose and D-xyllose. Aesculin is hydrolysed, but gelatin is not. Nitrate is not reduced. The peptidoglycan is of the A4z type with an interpeptide bridge comprising L-Glu, and an L-Ser residue at position 1 of the peptide subunit. The cell-wall sugar is galactose. The predominant menaquinone is MK-8(H4); MK-7(H4), MK-8(H6), MK-8 and MK-8(H2) are minor or trace components. The major cellular fatty acids are anteiso-C15:0, C16:0 and iso-C16:0, followed by C14:0, iso-C14:0, anteiso-C17:0, C18:0 and C12:0. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylglynositol and five unidentified phospholipids.

The type strain, PS-14-7T (=NBRC 110108T=InaCC A 5153T), was isolated from the rhizosphere of a mangrove growing on the seashore of Pramuka Island, Indonesia. The DNA G+C content of the type strain is 72.8 mol%.

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References


