Planomicrobium soli sp. nov., isolated from soil

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A Gram-staining-positive bacterium, designated strain XN13T, was isolated from a soil sample collected from ALaShan National Geological Park in Inner Mongolia Autonomous Region, China and subjected to a taxonomic study using a polyphasic approach. Strain XN13T was found to have a range of chemical and morphological properties consistent with its classification in the genus Planomicrobium. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain XN13T was related to members of the genus Planomicrobium. The closest phylogenetic relatives were Planomicrobium okeanokoites NBRC 12536T, Planomicrobium koreense JG07T, Planomicrobium mcmeekinii S23F2T and Planomicrobium flavidum ISL-41T with 98.2 %, 97.8 %, 97.8 % and 97.7 % 16S rRNA gene sequence similarity, respectively. The major cellular fatty acids were anteiso-C15 : 0, C16 : 1ω7c alcohol, iso-C14 : 0 and C16 : 1ω10c. The predominant menaquinones were MK-8 and MK-7. The DNA G + C content was 40.3 mol%. The DNA–DNA relatedness values between strain XN13T and Planomicrobium okeanokoites KCTC 3672T, Planomicrobium koreense KCTC 3684T, P. mcmeekinii CGMCC 1.2724T, Planomicrobium flavidum KCTC 13261T, Planomicrobium chinense CGMCC 1.3454T and Planomicrobium glaciei CGMCC 1.6846T were 36 %, 30 %, 34 %, 29 %, 30 % and 31 %, respectively. The organism is different from recognized species of the genus Planomicrobium in several phenotypic characteristics. On the basis of phenotypic and genotypic properties, strain XN13T represents a novel species of the genus Planomicrobium, for which the name Planomicrobium soli sp. nov. is proposed. The type strain is XN13T (=CGMCC 1.12259T =KCTC 33047T).

The genus Planomicrobium was proposed by Yoon et al. (2001). At the time of writing, the genus Planomicrobium comprises nine species with validly published names: Planomicrobium alkanolicasticum (Engelhardt et al., 2001; Dai et al., 2005), Planomicrobium chinenense (Dai et al., 2005), Planomicrobium flavidum (Jung et al., 2009), Planomicrobium glaciei (Zhang et al., 2009), Planomicrobium mcmeekinii (Junge et al., 1998; Yoon et al., 2001), Planomicrobium okeanokoites (Nakagawa et al., 1996; Yoon et al., 2001), Planomicrobium koreense (Yoon et al., 2001), Planomicrobium psychrophilum (Reddy et al., 2002; Dai et al., 2005) and Planomicrobium stackebrandtii (Mayilraj et al., 2005; Jung et al., 2009). Members of the genus Planomicrobium are Gram-staining-positive to Gram-staining-variable, aerobic, motile and non-endospore-forming. Colony colour is yellow to orange or pale orange. The peptidoglycan type is A4α type. The menaquinone profile is characterized by the predominance of MK-8 followed by MK-7, or by the predominance of MK-8 followed by MK-7 and MK-6. The cellular fatty acids are mainly saturated, unsaturated and branched fatty acids. The G + C content of the genomic DNA is 35–49 mol% (Yoon et al., 2001; Zhang et al., 2009).

A bacterial strain, designated XN13T, was isolated from a soil sample collected from ALaShan National Geological Park (30° 27′ 24″ N 106° 12′ 03″ E, 1038 m above sea level), located in Inner Mongolia Autonomous Region, China. The pH of the soil sample was pH 6. For isolation, the soil sample (1 g) was suspended in distilled water (100 ml) and spread on plates of trypticase soy agar (TSA; Difco) after serial dilution. The plates were incubated at 30 °C for 4 days. Purification was achieved by streaking single colonies on TSA. Strain XN13T was maintained on TSA at 4 °C and as glycerol suspensions (20 %, v/v) at −20 °C. The aim of the present work was to elucidate the taxonomic position of strain XN13T by means of phenotypic, genotypic and chemotaxonomic analyses. The following type strains of species of the genus Planomicrobium were used as reference strains: Planomicrobium okeanokoites KCTC 3672T, Planomicrobium koreense KCTC 3684T and Planomicrobium flavidum KCTC 13261T, obtained from the Korean Collection for Type
Cultures, Taegon, Korea; and Planomicrobium mecekinii CGMCC 1.2724T, Planomicrobium chineense CGMCC 1.3454T and Planomicrobium glaciei CGMCC 1.6846T, obtained from China General Microbiological Culture Collection Center.

General cell morphology was studied by light microscopy (BH-2; Olympus) and transmission electron microscopy (JEM-1400; JEOL) with a 2-day-old culture of strain XN13T grown on TSA. Colonial properties were examined by using the standard Microbial Identification System (MIDI; Sasser, 1990; Kämpfer & Kroppenstedt, 1996) version 4.5. The G+C content of the DNA was determined by means of the thermal denaturation method (Marmur & Doty, 1962) with Escherichia coli K-12 as the reference.

Cells of strain XN13T contained MK-8 (73.9 %) and MK-7 (18.9 %) as the major respiratory quinones. The cell-wall composition of strain XN13T contained l-lysine, glutamic acid and alanine, and this is consistent with the A4 type (Schleifer & Kandler, 1972) cell wall of the genus Planomicrobium. The polar lipids of strain XN13T were phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol (Fig. S2). The major cellular fatty acids were anteiso-C15 : 0 (36.0 %), C16 : 1ω7c alcohol (14.3 %), iso-C14 : 0 (10.9 %) and C16 : 1ω11c (10.1 %). No pronounced differences in the fatty acid profiles of strain XN13T and the reference strains were found, although there were minor differences in the proportions of some components (Table S1). The genomic DNA G+C content of strain XN13T was 40.3 mol%.

Extraction of genomic DNA and amplification of the 16S rRNA gene were performed as described by Rainey et al. (1996). 16S rRNA gene sequences were amplified by PCR from chromosomal DNA using two universal primers as described previously (Yoon et al., 1998). The PCR product was sequenced directly using the method of Lu et al. (2001). The resultant 16S rRNA gene sequence was compared with those available in the GenBank database, using the BLAST program. Multiple alignments with closely related species were performed using the CLUSTAL X 1.8 software (Thompson et al., 1997). Sequence similarities were calculated using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/, Kim et al., 2012). The phylogenetic consensus tree was reconstructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) methods in MEGA software version 5.0 (Tamura et al., 2011), with bootstrap values based on 1000 replications. Evolutionary distances were calculated.
The 16S rRNA gene sequence of strain XN13<sup>T</sup> was a continuous stretch of 1468 bp. It was apparent from the neighbour-joining tree (Fig. 1) that strain XN13<sup>T</sup> was classically defined as belonging to the genus Planomicrobium. Strain XN13<sup>T</sup> showed 96.5–98.2 % 16S rRNA gene sequence similarities with respect to the type strains of species of the genus Planomicrobium, the highest similarity values being found with the sequences of Planomicrobium okeanokoites NBRC 12536<sup>T</sup> (98.2 %), Planomicrobium koreense JG07<sup>T</sup> (97.8 %), Planomicrobium mcmeekinii S23F2<sup>T</sup> (97.8 %), Planomicrobium flavidum ISL-41<sup>T</sup> (97.7 %), Planomicrobium chinense DX3-12<sup>T</sup> (97.7 %) and Planomicrobium glaciei 0423<sup>T</sup> (97.6 %). In addition, strain XN13<sup>T</sup> also shared high 16S rRNA gene sequence similarities with respect to the type strains of species of the genus Planococcus [Planococcus salinarum ISL-16<sup>T</sup> (98.0 %) and Planococcus plakortidis AS/ASP6 (II)<sup>T</sup> (97.9 %)]. However, from the neighbour-joining tree (Fig. 1) strain XN13<sup>T</sup> fell within the evolutionary radius of the genus Planomicrobium, and the isolate should therefore not be assigned to the genus Planococcus. The maximum-parsimony tree also supported the clustering of strain XN13<sup>T</sup> and species of the genus Planomicrobium.

DNA–DNA hybridization values were determined from the initial DNA–DNA liquid reassociation rate as described by De Ley et al. (1970) and modified by Huß et al. (1983). The tests were performed on a Lambda 35 UV/Vis spectrophotometer equipped with a temperature program controller (Perkin-Elmer). Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values. The mean DNA–DNA relatedness values between strain XN13<sup>T</sup> and Planomicrobium okeanokoites KCTC 3672<sup>T</sup>, Planomicrobium koreense KCTC 3684<sup>T</sup>, Planomicrobium mcmeekinii CGMCC 1.2724<sup>T</sup>, Planomicrobium flavidum ISL-41<sup>T</sup>, Planomicrobium chinense DX3-12<sup>T</sup> and Planomicrobium glaciei 0423<sup>T</sup> were 40.3, 46.3<sup>a</sup>, 47.0<sup>b</sup>, 35.0<sup>c</sup>, 45.9, 34.8 and 49.0<sup>d</sup>, respectively.

### Table 1. Differential properties of strain XN13<sup>T</sup> and type strains of closely related species of the genus Planomicrobium

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td>Cell shape</td>
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<td>R</td>
<td>C or SR/R</td>
<td>R/C or SR</td>
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<tr>
<td>Gram-stain</td>
<td>Positive</td>
<td>Positive to variable</td>
<td>Yellow-orange</td>
<td>Pale orange</td>
<td>Light yellow</td>
<td>Yellow-orange</td>
<td>Positive</td>
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<tr>
<td>Colony colour</td>
<td>Pale Orange</td>
<td>Bright yellow -bright orange</td>
<td>Yellow-orange</td>
<td>Pale orange</td>
<td>Light yellow</td>
<td>Yellow-orange</td>
<td>Positive</td>
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<td>Oxidase</td>
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<td>–</td>
<td>+</td>
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<td>+</td>
<td>+</td>
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<td>7 % NaCl</td>
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<td>37 °C</td>
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<td>Casein</td>
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<td>+</td>
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<td>+</td>
<td>W</td>
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<td>–</td>
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<td>Tween 80</td>
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<td>+</td>
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<td>Cellulose</td>
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<td>D-Fructose</td>
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<tr>
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<td>+</td>
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<td>Lactose</td>
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<td>Malate</td>
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<tr>
<td>Melibiose</td>
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<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Predominant menaquinone(s)</td>
<td>MK-8,7</td>
<td>MK-8,7</td>
<td>MK-8,7,6</td>
<td>MK-8,7</td>
<td>MK-8,7</td>
<td>MK-8</td>
<td>MK-8,7</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>40.3</td>
<td>46.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>45.9</td>
<td>34.8</td>
<td>49.0&lt;sup&gt;d&lt;/sup&gt;</td>
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</table>

*Data from: a, Nakagawa et al. (1996); b, Yoon et al. (2001); c, Junge et al. (1998); d, Zhang et al. (2009).
flavidum KCTC 13261^T, Planomicrobium chinense CGMCC 1.3454^T and Planomicrobium glaciei CGMCC 1.6846^T were 36 %, 30 %, 34 %, 29 %, 30 % and 31 %, respectively. These DNA–DNA relatedness values were significantly lower than the threshold value of 70 % that is commonly accepted for the definition of bacterial species (Wayne et al., 1987).

Strain XN13^T showed a range of phenotypic characteristics that differentiated it from recognized species of the genus Planomicrobium (Table 1). In addition, phylogenetic divergence (Fig. 1) and DNA–DNA relatedness values differentiate strain XN13^T from recognized species of the genus Planomicrobium. It is apparent that strain XN13^T is not affiliated to any recognized species of the genus Planomicrobium. Therefore, on the basis of the data presented above, strain XN13^T represents a novel species of the genus Planomicrobium, for which the name Planomicrobium soli sp. nov. is proposed.

**Description of Planomicrobium soli sp. nov.**

Planomicrobium soli (so’li. L. gen. n. soli of soil).

Cells are Gram-staining-positive, aerobic, cocci or short rods (0.8 × 1.0 μm) and non-endospore-forming. Colonies on TSA (Difco) are smooth, circular, convex with entire margins, and pale orange at 28 °C. Growth occurs between 4 °C and 38 °C (optimal growth at 28–30 °C) and at pH 6.0–10.0 (optimal growth at pH 7.0–8.0). Growth occurs with 0.5–7.5 % NaCl (optimal growth with 3–4 % NaCl). Does not reduce nitrate to nitrite or nitrogen gas. Oxidase-negative and catalase-positive. Degrades casein, gelatin, urea and aesculin, but not Tween 80 or starch. H2S is not produced. Citrate does not support growth. Oxidase-negative and catalase-positive. Degrades casein, gelatin, urea and aesculin, but not Tween 80 or starch. H2S is not produced. Citrate does not support growth.

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, displaying the relationships between strain XN13^T and closely related species. Filled circles indicate that the corresponding branches were also recovered in the maximum-parsimony tree. Numbers at nodes are bootstrap values (%) based on 1000 replications; only values ≥50 % are shown. Bar, 0.005 substitutions per nucleotide position.
polysaccharide is phosphatidylyethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The major cellular fatty acids are anteiso-C\(_{15:0}\), C\(_{16:1}ω7c\) alcohol, iso-C\(_{14:0}\) and C\(_{16:1}ω11c\). The cell-wall peptidoglycan type is A\(_4\) type.

The type strain, XN13\(^T\) (=CGMCC 1.12259\(^T\)=KCTC 33047\(^T\)) was isolated from a soil sample collected from ALaShan National Geologic Park, Inner Mongolia Autonomous Region, China. The DNA G+C content of the type strain is 40.3 mol%. The species description is based on this single strain and hence serves as a description of the species.

### Acknowledgements

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### References


