Planococcus columbae sp. nov., isolated from pigeon faeces

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An orange-pigmented, Gram-positive bacterial strain, designated PgEx11T, was isolated from pigeon faeces. Analysis of the 16S rRNA gene sequence of the isolate indicated that it had 94.2–98.2 % sequence identity with respect to those of seven recognized species of the genus Planococcus. The strain PgEx11T contained anteiso-C₁₅ : ₀ as a major cellular fatty acid and MK-7 and MK-8 as the major menaquinones. The DNA G+C content of strain PgEx11T was 50.5 mol%. Furthermore, analysis of the 16S rRNA gene sequence indicated high levels of similarity with Planococcus rifietoensis (98.2 %), Planococcus maitriensis (97.6 %), Planococcus citreus (97.5 %) and Planococcus maritimus (97.1 %). However, the mean value for DNA–DNA relatedness between PgEx11T and these four closely related species was in the range 45.4–16.8 %, respectively. Moreover, strain PgEx11T also differs from its close relatives with regard to biochemical and chemotaxonomic characteristics. On the basis of phenotypic, chemotaxonomic and genotypic differences, strain PgEx11T represents a novel species of the genus Planococcus, for which the name Planococcus columbae sp. nov. is proposed. The type strain is PgEx11T (= MTCC 7251T = DSM 17517T).

Members of the genus Planococcus have been isolated from diverse environments, such as seawater (Yoon et al., 2003), sediments (Romano et al., 2003), soil (Kocur et al., 1970; Mayilraj et al., 2005), cyanobacterial mats (Reddy et al., 2002; Alam et al., 2003) and fish (Hao & Komagata, 1985). The genus Planococcus was proposed by Migula (1894) to accommodate Gram-positive, aerobic, motile cocci. This genus is currently placed within the family Planococcaceae in the phylum Firmicutes. Other genera belonging to this family are Filibacter, Kurthia, Planomicrobium and Sporosarcina. The type species of the genus is Planococcus citreus. However, on the basis of phenotypic and phylogenetic analyses, a few species of this genus were transferred to the genus Planomicrobium (Yoon et al., 2001; Dai et al., 2005) and Planococcus halophilus was reclassified as Marinococcus halophilus (Hao et al., 1984). In the present communication, we describe the characterization, using a polyphasic approach, of a novel strain isolated from pigeon faeces and which we propose as a novel species of the genus Planococcus.

The bacterium PgEx11T was isolated from pigeon faeces by dilution-plating samples on nutrient agar (Himedia) and incubating the plates at 30 °C for 24 h. The dried surface of the faeces was washed with sterile 0.1 mM phosphate buffer (pH 7.2) and the core was used to make a suspension in the same buffer. An orange-pigmented colony was isolated, purified and preserved for further characterization. Reference strains Planococcus citreus JCM 2532T and Planococcus maritimus JCM 11543T were obtained from the Japan Collection for Micro-organisms (Saitama, Japan), Planococcus rifietoensis DSM 15069T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and Planococcus maitriensis MTCC 4827T was obtained from the Microbial Type Culture Collection and Gene Bank (Chandigarh, India). The cell morphology of strain PgEx11T was observed by using phase-contrast microscopy (Axiphot; Zeiss) with an oil-immersion objective (×100). Physiological tests were performed using nutrient agar medium. For the various biochemical tests mentioned in the species description, the culture was grown at 30 °C in nutrient broth (Himedia). Catalase and oxidase tests were performed according to Cowan & Steel (1965). The following determinations were performed as described by Lanyi (1987): the hydrolysis of aesculin, the indole test, the Voges–Proskauer test, the methyl red test, H₂S production, and nitrate reduction. The utilization of various carbon compounds and the hydrolysis of casein, gelatin, starch and Tween 20 were determined by...
following standard methods (Smibert & Krieg, 1994). The ability of PgEx11T to utilize various carbon compounds as sole carbon sources was checked as described previously (Suresh et al., 2006). Antibiotic discs (Himedia) placed on tryptone soya agar were used to determine the sensitivity of PgEx11T to various antibiotics. Strain PgEx11T comprises motile, aerobic, Gram-positive, catalase-positive cocci; other phenotypic and chemotaxonomic characteristics are given in the species description and in Table 1.

For cellular fatty acid analyses, cells were grown on nutrient agar with 4 % NaCl at 30 °C for 24 h; the fatty acid methyl esters were extracted (Sato & Murata, 1988) and analysed using the Microbial Identification System (MIDI) as described by Pandey et al. (2002). Isoprenoid quinones and polar lipids were extracted and analysed according to the methods described by Minnikin et al. (1984). Peptidoglycan was prepared and analysed according to the method described by Komagata & Suzuki (1987). The G + C content (mol%) of genomic DNA was estimated spectrophotometrically (Lambda 35; Perkin Elmer) as described by Mandel & Marmur (1968). DNA–DNA hybridization was performed by using the membrane filter method (Tourova & Antonov, 1987) as described by Shivaji et al. (1992).

The fatty acid profile of strain PgEx11T was similar to those of members of the genus Planococcus and contained iso-C15 : 0 and anteiso-C15 : 0 as the major fatty acids (Table 2). The polar lipids found in strain PgEx11T were phosphatidylglycerol, diphosphatidylglycerol and phosphatidylcholine, which were also reported for members of the genus Planococcus. In addition, strain PgEx11T contained phosphatidylinositol, an unknown glycolipid and two unknown phospholipids. The peptidoglycan cross-link peptide in strain PgEx11T was L-Lys–D-Glu (classified as the A4 α type by Schleifer & Kandler, 1972).
The 16S rRNA gene was amplified by using a PCR with primers 8-27f (5’- AGAGTTTGATCCTGGCTCAG-3’) and 1492r (5’- TACGGYTACCTTGTTACGACTT-3’) as described by Pandey et al. (2002). The PCR product was purified using a QIAquick PCR purification kit (Qiagen). Purified amplicon was sequenced using a BigDye terminator automatic DNA sequencer (Applied Biosystems). The Purified amplicon was sequenced using a BigDye terminator as described by Pandey et al. (2002).

Table 2. Fatty acid contents (%) of strain PgEx11T and five closely related species of Planococcus and three of Planomicrobium

<table>
<thead>
<tr>
<th>Fatty acid methyl ester</th>
<th>1*</th>
<th>2*</th>
<th>3*</th>
<th>4*</th>
<th>5*</th>
<th>6†</th>
<th>7†</th>
<th>8†</th>
<th>9†</th>
</tr>
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<tbody>
<tr>
<td>iso-C14:0</td>
<td>10.5</td>
<td>5.1</td>
<td>2.1</td>
<td>2.4</td>
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<td>13.1</td>
<td>21.0</td>
<td>40.4</td>
<td>18.1</td>
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<td>iso-C15:0</td>
<td>25.2</td>
<td>18.1</td>
<td>23.0</td>
<td>5.3</td>
<td>6.7</td>
<td>5.1</td>
<td>6.4</td>
<td>NR</td>
<td>5.3</td>
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<tr>
<td>anteiso-C15:0</td>
<td>35.1</td>
<td>43.7</td>
<td>38.0</td>
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<td>45.7</td>
<td>40.9</td>
<td>26.1</td>
<td>4.0</td>
<td>32.0</td>
</tr>
<tr>
<td>C16:1ω7cOH</td>
<td>4.7</td>
<td>4.3</td>
<td>3.3</td>
<td>2.6</td>
<td>–</td>
<td>11.4</td>
<td>23.6</td>
<td>26.1</td>
<td>22.9</td>
</tr>
<tr>
<td>C16:1ω9c</td>
<td>–</td>
<td>–</td>
<td>5.2</td>
<td>–</td>
<td>NR</td>
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</tr>
<tr>
<td>C16:1ω11c</td>
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<td>–</td>
<td>0.6</td>
<td>0.3</td>
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<td>1.7</td>
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<td>1.6</td>
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<td>1.6</td>
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<tr>
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<td>–</td>
<td>–</td>
<td>4.5</td>
<td>–</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
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<td>–</td>
<td>4.5</td>
<td>–</td>
<td>–</td>
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<td>–</td>
<td>3.1</td>
<td>–</td>
<td>–</td>
<td>4.5</td>
<td>NR</td>
<td>NR</td>
<td>3.0</td>
</tr>
</tbody>
</table>

*Strains were grown on nutrient agar with 4% NaCl at a temperature of 30°C for 24 h.
†Strains were grown on marine agar (data from Yoon et al., 2001).
‡Summed feature 4 consists of C17:1ω and anteiso C17:0.

The levels of genomic relatedness between strain PgEx11T and four closely related species of the genus Planococcus, namely Planococcus rifietoensis, Planococcus maitriensis, Planococcus citreus and Planococcus maritimus (showing 16S rRNA gene sequence similarities between 98.2 and 97.1%), were 45.4, 42.7, 27.8 and 16.8%, respectively. It is generally accepted that at more than 3% dissimilarity in the 16S rRNA gene sequences, other species of the genus can be excluded from DNA–DNA hybridization (Stackebrandt & Goebel, 1994). Moreover, although the cellular fatty acid composition and cell-wall peptidoglycan of strain PgEx11T are similar to those of members of the genus Planococcus, the cellular polar lipid profile and menaquinone composition differ (Table 1).

We have examined our data for strain PgEx11T, Planococcus rifietoensis, Planococcus maritimus, Planococcus maitriensis and Planococcus citreus and other workers’ data for different species of Planococcus and for members of the genus Planomicrobium. Strain PgEx11T formed a cluster with the species showing 16S rRNA gene sequence similarity levels above 97% (Fig. 1). A phylogenetic tree constructed using UPGMA also yielded a similar stable grouping. Strain PgEx11T exhibited the presence of the nucleotides T and A at positions 183 and 190 (according to Escherichia coli numbering) in the 16S rRNA gene; these were described as signature nucleotides for Planococcus by Dai et al. (2005) differentiating members of that genus from members of the genus Planomicrobium.
Planomicrobium (see Supplementary Table S1, available in IJSEM Online). On the basis of this comparative analysis of phenotypic, chemotaxonomic and phylogenetic characteristics, we came to the conclusion that the creation of the genus Planomicrobium cannot be justified. The genera Planococcus and Planomicrobium cannot be distinguished on the basis of phenotypic characteristics. Some species of Planococcus share characteristics mentioned in the description of the genus Planomicrobium (Yoon et al., 2001). Casein hydrolysis is negative for Planomicrobium psychrophilum and Planomicrobium chinense, but is positive in the genus description. Similarly, starch is hydrolysed in Planomicrobium alkanoclasticum, but is negative in the genus description. The only features common to the species of the genus Planomicrobium are the presence of signature nucleotides C and G at positions 183 and 190 (Dai et al., 2005).

Phylogenetically, the species of the genus Planomicrobium clusters with the genus Planococcus. The peptidoglycan cross-link is L-Lys–D-Glu in both genera, with the exception of two Planomicrobium species, in which it is L-Lys–D-Asp. In fact, the type species of Planomicrobium, Planomicrobium koreense, has the same peptidoglycan as all other Planococcus species. Menaquinoines MK-7 and MK-8 are present in all species of Planococcus and Planomicrobium. The polar lipids in Planomicrobium species, phosphatidylethanolamine, phosphatidyglycerol and diphosphatidylglycerol, are also not unique to that genus because they are also present in at least four species of the genus Planococcus. The cellular fatty acid profiles of the species of these two genera do not exhibit any qualitative differences with respect to each other. In addition, the DNA G+C contents (mol%) do not show a clear demarcation distinguishing the species of Planococcus from those of the genus Planomicrobium.

The taxonomic status of the genus Planomicrobium should therefore be revisited. We strongly feel that the species removed from the genus Planococcus should be reinstated and that the type species of the genus Planomicrobium, Planomicrobium koreense, should be considered as a species of the genus Planococcus.

On the basis of phenotypic, chemotaxonomic and phylogenetic characteristics, strain PgEx11T represents a novel species of the genus Planococcus, for which the name Planococcus columbae sp. nov. is proposed.

**Description of Planococcus columbae sp. nov.**

Planococcus columbae (co.lum’ba.e. L. gen. n. columbae of a pigeon Columba livia).

The cells of PgEx11T are aerobic, Gram-positive cocci, motile, non-sporulating and 0.8–1.0 μm in size. Colonies on nutrient agar are orange, circular, entire, smooth, convex and 1.0–2.0 mm in diameter. Strain PgEx11T grows at temperatures between 8 and 42 °C (optimum, 30 °C) but not at 4 °C, and grows at pH 6.0–11.0 (optimum, pH 7.0–8.0). Growth is observed between 0 and 14 % NaCl (optimum, 4–5 %). Positive for catalase, but negative for amylase, protease and arginine dihydrolase. Does not hydrolyse urea.

![Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between strain PgEx11T and related species of the genera Planococcus and Planomicrobium. Bootstrap values greater than 50 % (based on 1000 replicates) are given at the nodes. Bar, 0.01 substitutions per site.](http://ijs.sgmjournals.org)
or aesculin. Negative in the Voges–Proskauer test and for H2S production. Utilizes D-arabitol, L-arabinose, carboxymethylcellulose, D-cellobiose, dulcitol, D-glucose, D-galactose, D-fucose, inulin, lactose, melezitose, D-maltose, D-mannitol, D-mannose, D-ribose, L-rhamnose, N-acetylglucosamine, sodium acetate, starch, sucrose, xylan and D-xylene but not D-malic acid. Does not produce acid from glucose, galactose, inulin, i-inositol, lactose, raffinose, salicin, sorbitol or trehalose. Other phenotypic characteristics are shown in Table 1. Resistant to penicillin (1.5 U), cephaloridine (30 μg) and tobramycin (10 μg), but sensitive to rifampicin (5 μg), gentamicin (10 μg), neomycin (30 μg), streptomycin (25 μg) and chlorotetracycline (30 μg). Major fatty acids are anteiso-C15:0 (35.1 %), iso-C15:0 (25.2 %), iso-C16:0 (11.5 %) and C14:0 (10.5 %). Cell-wall peptidoglycan is L-Lys–D-Glu. The menaquinones found in strain PgEx11T are MK-7 (48 %), MK-8 (35 %) and MK-7(H2) (22 %). The DNA G+C content is 50.5 mol %.

The type strain, PgEx11T (=MTCC 7251T = DSM 17517T), was isolated from pigeon faeces.

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References


