Planococcus columbae sp. nov., isolated from pigeon faeces

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An orange-pigmented, Gram-positive bacterial strain, designated PgEx11^T, 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 PgEx11^T contained anteiso-C_{15:0} as a major cellular fatty acid and MK-7 and MK-8 as the major menaquinones. The DNA G+C content of strain PgEx11^T 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 PgEx11^T and these four closely related species was in the range 45.4–16.8%, respectively. Moreover, strain PgEx11^T also differs from its close relatives with regard to biochemical and chemotaxonomic characteristics. On the basis of phenotypic, chemotaxonomic and genotypic differences, strain PgEx11^T represents a novel species of the genus *Planococcus*, for which the name *Planococcus columbae* sp. nov. is proposed. The type strain is PgEx11^T (=MTCC 7251^T = DSM 17517^T).

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 GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain PgEx11^T is AJ966515.

Differential phenotypic and chemotaxonomic characteristics for strain PgEx11^T with respect to species of the genera *Planococcus* and *Planomicrobium* are shown in a supplementary table available with the online version of this paper.

The bacterium PgEx11^T 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 2532^T and *Planococcus maritimus* JCM 11543^T were obtained from the Japan Collection for Micro-organisms (Saitama, Japan), *Planococcus rifietoensis* DSM 15069^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) and *Planococcus maitriensis* MTCC 4827^T was obtained from the Microbial Type Culture Collection and Gene Bank (Chandigarh, India). The cell morphology of strain PgEx11^T was observed by using phase-contrast microscopy (Axiophot; 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 PgEx11^T 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 PgEx11^T to various antibiotics. Strain PgEx11^T 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 PgEx11^T was similar to those of members of the genus *Planococcus* and contained iso-C_{15:0} and anteiso-C_{15:0} as the major fatty acids (Table 2). The polar lipids found in strain PgEx11^T were phosphatidylglycerol, diphosphatidylglycerol and phosphatidylcholine, which were also reported for members of the genus *Planococcus*. In addition, strain PgEx11^T contained phosphatidylinositol, an unknown glycolipid and two unknown phospholipids. The peptidoglycan cross-link peptide in strain PgEx11^T was L-Lys–D-Glu (classified as the A4α type by Schleifer & Kandler, 1972).

Table 1. Differential phenotypic and chemotaxonomic characteristics of strain PgEx11^T with respect to closely related species

Taxa: 1, strain PgEx11^T (data from present study); 2, *Planococcus rifetoensis* (Romano *et al.*, 2003; present study); 3, *Planococcus maritimus* (Yoon *et al.*, 2003; present study); 4, *Planococcus maitriensis* (Alam *et al.*, 2003; present study); 5, *Planococcus citreus* (Hao & Komagata, 1985; present study); 6, *Planococcus kocurii* (Hao & Komagata, 1985); 7, *Planomicrobium koreense* (Yoon *et al.*, 2001); 8, *Planomicrobium okeanokoites* (Nakagawa *et al.*, 1996; Yoon *et al.*, 2001); 9, *Planomicrobium mcmeekinii* (Junge *et al.*, 1998; Yoon *et al.*, 2001). +, Present; –, absent; w, weakly positive; NR, not reported.

Characteristic	1	2	3	4	5	6	7	8	9
Cell shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci/rods	Rods	Rods
Growth at:									
4 °C	–	+	+	+	–	+	+	–	+
42 °C	+	+	–	–	–	–	–	–	–
Oxidase	+	+	–	–	–	–	–	w	–
Hydrolysis of:									
Casein	–	–	+	–†	+	NR	+	+	+
Tween 20	+	–	–	–	–	NR	NR	NR	NR
Tween 40	+	+	–	–	+	NR	NR	NR	NR
Gelatin	–	+	–*	–†	+	+	+	+	+
Citrate utilization	–	+	–	–†	+	NR	NR	NR	NR
Nitrate reduction	+	–	–	+	–	–	–	–	+
Utilization of:									
D-Arabinose	+	+	–	–	+	NR	NR	NR	NR
D-Fructose	+	+	–	+	+	NR	NR	NR	+
Acid production from:									
Fructose	–	–	–*	+	–	NR	–	+	+
Mannose	–	–	–*	+	–	NR	–	NR	NR
Sucrose	+	–	–	w	+	NR	–	–	NR
Peptidoglycan type	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Asp	L-Lys–D-Asp
Menaquinones	MK-7, MK-7(H ₂), MK-8	MK-8	MK-6, MK-7, MK-8	MK-7, MK-8	MK-6, MK-7, MK-8	MK-7, MK-8	MK-6, MK-7, MK-8	MK-7, MK-8	MK-7, MK-8

*Data differed from that in Yoon *et al.* (2003).

†Data differed from that in Alam *et al.* (2003).

Table 2. Fatty acid contents (%) of strain PgEx11^T and five closely related species of *Planococcus* and three of *Planomicrobium*

Taxa: 1, strain PgEx11^T (data from present study); 2, *Planococcus rifietoensis* (present study); 3, *Planococcus maritimus* (present study); 4, *Planococcus maitriensis* (present study); 5, *Planococcus citreus* (present study); 6, *Planococcus kocurii* (Yoon *et al.*, 2001); 7, *Planomicrobium koreense* (Yoon *et al.*, 2001); 8, *Planomicrobium okeanokoites* (Yoon *et al.*, 2001); 9, *Planomicrobium mcmeekinii* (Yoon *et al.*, 2001). –, Absent; NR, not reported.

Fatty acid methyl ester	1*	2*	3*	4*	5*	6†	7†	8†	9†
iso-C _{14:0}	10.5	5.1	2.1	2.4	9.4	13.1	21.0	40.4	18.1
iso-C _{15:0}	25.2	18.1	23.0	5.3	6.7	5.1	6.4	NR	5.3
anteiso-C _{15:0}	35.1	43.7	38.0	31.4	45.7	40.9	26.1	4.0	32.0
C _{16:1} ω7cOH	4.7	4.3	3.3	2.6	–	11.4	23.6	26.1	22.9
C _{16:1} ω9c	–	–	–	5.2	–	NR	NR	NR	NR
C _{16:1} ω11c	–	–	0.6	0.3	9.8	2.5	1.7	2.1	2.3
iso-C _{16:0}	11.5	9.3	4.1	9.2	8.6	7.2	11.1	24.5	8.7
C _{16:0}	1.5	2.8	4.5	6.8	19.6	1.4	NR	1.1	NR
iso-C _{17:0}	5.0	8.8	7.6	–	–	1.7	1.6	NR	1.3
anteiso-C _{17:0}	4.3	7.6	7.0	7.2	–	2.6	1.6	NR	1.2
C _{17:0}	1.8	–	–	4.5	–	NR	NR	NR	NR
iso-C _{17:1} ω10c	–	–	4.5	–	–	2.5	1.9	NR	2.4
Summed feature 4‡	–	–	3.1	–	–	4.5	NR	NR	3.0

*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 C_{17:1} iso I/anteiso B, which could not be separated by MIDI.

The 16S rRNA gene was amplified by using a PCR with primers 8-27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTACGACTT-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 cycle sequencing kit and an ABI PRISM model 3130xl automatic DNA sequencer (Applied Biosystems). The sequence of the 16S rRNA gene (a 1481 bp continuous stretch) was aligned with closely related sequences using CLUSTAL_X (Thompson *et al.*, 1997) and was edited manually. Phylogenetic distances were calculated using the Kimura two-parameter model (Kimura, 1980) and trees were constructed using the neighbour-joining and UPGMA methods in the PHYLIP package (Felsenstein, 1993). A phylogenetic tree was also constructed using TREECON (version 1.3b) (Van de Peer & De Wachter, 1997). The stability of the tree was assessed by performing 1000 replicates. *Paenibacillus polymyxa* (X60632) was used as an outgroup.

Analysis of the 16S rRNA gene sequence of strain PgEx11^T indicated that it is closely related to *Planococcus rifietoensis* DSM 15069^T (98.2% identity); it exhibited lower levels of identity with respect to *Planococcus* species such as *Planococcus maitriensis* MTCC 4827^T (97.6%), *Planococcus citreus* NCIMB 1493^T (97.5%), *Planococcus maritimus* KCCM 41587^T (97.1%), *Planococcus kocurii* (95.0%) and *Planococcus stackebrandtii* MTCC 6226^T (94.2%). It showed less than 96% sequence similarity with respect to species of the

genus *Planomicrobium*. Strain PgEx11^T 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 PgEx11^T 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*.

The levels of genomic relatedness between strain PgEx11^T 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 PgEx11^T 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 PgEx11^T, *Planococcus rifietoensis*, *Planococcus maritimus*, *Planococcus maitriensis* and *Planococcus citreus* and other workers' data for different species of *Planococcus* and for members of the genus

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationship between strain PgEx11^T 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.

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. Menaquinones MK-7 and MK-8 are present in all species of *Planococcus* and *Planomicrobium*. The polar lipids in *Planomicrobium* species, phosphatidylethanolamine, phosphatidylglycerol 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 PgEx11^T 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 PgEx11^T 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 PgEx11^T 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

or aesculin. Negative in the methyl red test, in the test for indole production, 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-xylose 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 chlortetracycline (30 µg). Major fatty acids are anteiso-C_{15:0} (35.1%), iso-C_{15:0} (25.2%), iso-C_{16:0} (11.5%) and C_{14:0} (10.5%). Cell-wall peptidoglycan is L-Lys–D-Glu. The menaquinones found in strain PgEx11^T are MK-7 (48%), MK-8 (35%) and MK-7(H₂) (22%). The DNA G + C content is 50.5 mol%.

The type strain, PgEx11^T (= MTCC 7251^T = DSM 17517^T), was isolated from pigeon faeces.

Acknowledgements

We would like to thank Mr Deepak Bhat (IMTECH), for his help with DNA sequencing, and Ms Gabi Poetter (Deutsche Sammlung von Mikroorganismen und Zellkulturen) for her help with some chemotaxonomic analyses. This work was supported by the Department of Biotechnology (DBT), Government of India and Council of Scientific and Industrial Research (CSIR). A. B. is a recipient of a CSIR fellowship. This is IMTECH communication number 37/2006.

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