Planococcus salinarum sp. nov., isolated from a marine solar saltern, and emended description of the genus *Planococcus*

Jung-Hoon Yoon, So-Jung Kang, Soo-Young Lee, Ki-Hoon Oh and Tae-Kwang Oh

Korea Research Institute of Bioscience and Biotechnology (KRIBB), PO Box 115, Yusong, Taejeon, Republic of Korea

Correspondence

Jung-Hoon Yoon
jhyoon@kribb.re.kr

A Gram-positive, non-motile and coccoid-, short rod- or rod-shaped bacterial strain, ISL-16^T, was isolated from a marine solar saltern in Korea and its taxonomic position was investigated using a polyphasic taxonomic approach. Strain ISL-16^T grew optimally at pH 7.0–8.0, at 30 °C and in the presence of 2 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain ISL-16^T joined the cluster comprising species of the genus *Planococcus*. Its 16S rRNA gene sequence contained the same signature nucleotides as those defined for the genus *Planococcus*. Strain ISL-16^T exhibited 16S rRNA gene sequence similarity values of 96.9–98.2 % to the type strains of species of the genus *Planococcus*. Strain ISL-16^T contained MK-8 and MK-7 as the predominant menaquinones and anteiso-C_{15:0}, C_{16:1}ω7c alcohol and anteiso-C_{17:0} as the major fatty acids. The DNA G + C content was 48.3 mol%. DNA–DNA relatedness values between strain ISL-16^T and the type strains of species of the genus *Planococcus* were 15–28 %. Differential phenotypic properties, together with its phylogenetic and genetic distinctiveness, enabled strain ISL-16^T to be differentiated from recognized species of the genus *Planococcus*. On the basis of the data presented, strain ISL-16^T is considered to represent a novel species of the genus *Planococcus*, for which the name *Planococcus salinarum* sp. nov. is proposed. The type strain is ISL-16^T (=KCTC 13584^T=CCUG 57753^T). An emended description of the genus *Planococcus* is also given.

The genus *Planococcus* was first proposed by Migula (1894) but the name was not validly published for many years. *Planococcus citreus*, the type species, was proposed by Kocur *et al.* (1970) and, subsequently, additional *Planococcus* species were described. However, five species of the genus *Planococcus*, namely *Planococcus okeanokoites*, *Planococcus mcmeekinii*, *Planococcus alkanoclasticus*, *Planococcus psychrophilus* and *Planococcus stackebrandtii*, have since been transferred to the genus *Planomicrobium* (Yoon *et al.*, 2001; Dai *et al.*, 2005; Jung *et al.*, 2009). At the time of writing, the genus *Planococcus* comprises eight additional recognized species, *Planococcus citreus* (Kocur *et al.*, 1970), *Planococcus kocurii* (Hao & Komagata, 1985), *Planococcus antarcticus* (Reddy *et al.*, 2002), *Planococcus rifietoensis* (Romano *et al.*, 2003), *Planococcus maritimus* (Yoon *et al.*, 2003a), *Planococcus maitriensis* (Alam *et al.*, 2003), *Planococcus columbae* (Suresh *et al.*, 2007) and *Planococcus donghaensis* (Choi *et al.*, 2007). In this study, a bacterial strain, ISL-16^T, which is phylogenetically related to members of the genera *Planococcus* and *Planomicrobium*,

is described. The aim of the present work was to determine the exact taxonomic position of strain ISL-16^T by using a polyphasic approach that included the determination of phenotypic and chemotaxonomic properties and a detailed phylogenetic investigation based on 16S rRNA gene sequences.

Bacterial strains were isolated from sediment collected from a marine solar saltern at Byunsan on the west coast of Korea. Isolation of strain ISL-16^T was performed by using the standard dilution plating technique at 30 °C on marine agar 2216 (MA; Difco) supplemented with 6 % (w/v) NaCl. Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultures grown at 30 °C in marine broth 2216 (MB; Difco). The type strains of species of the genus *Planococcus* were used as reference strains for DNA–DNA hybridization. *Planococcus antarcticus* DSM 14505^T, *Planococcus citreus* DSM 20549^T, *Planococcus columbae* DSM 17517^T, *Planococcus kocurii* DSM 20747^T, *Planococcus maitriensis* DSM 15305^T and *Planococcus rifietoensis* DSM 15069^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany. *Planococcus dong-*

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain ISL-16^T is FJ765415.

haensis KCTC 13050^T was obtained from the Korean Collection for Type Cultures, Taejeon, Korea. *Planococcus maritimus* TF-9^T was obtained from the study of Yoon *et al.* (2003a).

Morphological, physiological and biochemical characteristics of strain ISL-16^T were investigated using routine cultivation at 30 °C on MA. Cell morphology and flagellation were determined as described by Yoon *et al.* (2001). Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. The pH range for growth was determined in MB that was adjusted to various pH values (pH 4.5–9.5 at intervals of 0.5 pH units) by the addition of HCl or Na₂CO₃. Growth in the presence of 0, 0.5, 1.0, 2.0 and 3.0 % (w/v) NaCl was investigated on trypticase soy broth, supplemented with 0.45 % (w/v) MgCl₂·6H₂O and in the absence of MgCl₂·6H₂O or with 0.06 % (w/v) KCl, and prepared according to the formula of the Difco medium except that NaCl was excluded. Growth at various NaCl concentrations (2.0–15.0 %, w/v, in increments of 1.0 %) was investigated in MB. Growth at various temperatures (4, 10, 20, 25, 28, 30, 35, 37, 38, 39, 40 and 45 °C) was measured on MA. Biochemical and physiological properties were investigated as described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea, and nitrate reduction were investigated as described previously (Lányi, 1987) with the modification that artificial seawater was used for preparation of media. The artificial seawater contained (l⁻¹ distilled water): 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂·6H₂O, 5.94 g MgSO₄·7H₂O and 1.3 g CaCl₂·2H₂O (Bruns *et al.*, 2001). H₂S production was tested as described previously (Bruns *et al.*, 2001).

Susceptibility to antibiotics was investigated on MA plates by using antibiotic discs containing the following: polymyxin B, 100 U; streptomycin, 50 µg; penicillin G, 20 U; chloramphenicol, 100 µg; ampicillin, 10 µg; cephalothin, 30 µg; gentamicin, 30 µg; novobiocin, 5 µg; tetracycline, 30 µg; kanamycin, 30 µg; lincomycin, 15 µg; oleandomycin, 15 µg; neomycin, 30 µg; and carbenicillin, 100 µg. Acid production from carbohydrates was tested as described by Leifson (1963). Enzyme activities were determined by using the API ZYM system (bioMérieux). Morphological, cultural, physiological and biochemical characteristics of strain ISL-16^T are given in the species description (see below) or in Table 1.

Chromosomal DNA was isolated and purified according to the method described by Yoon *et al.* (1996), with the exception that RNase T1 was used in combination with RNase A to minimize the contamination of RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phy-

logenetic analysis were performed as described by Yoon *et al.* (2003b). The almost-complete 16S rRNA gene sequence of strain ISL-16^T determined in this study comprised 1506 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. In the phylogenetic tree based on the neighbour-joining algorithm, strain ISL-16^T joined the clade comprising species of the genus *Planococcus* and this cluster joined the clade comprising species of the genus *Planomicrobium* with a bootstrap resampling value of 100 % (Fig. 1). The relationship between strain ISL-16^T and species of the genus *Planococcus* was also found in trees constructed using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). The 16S rRNA gene of strain ISL-16^T contained the same signature nucleotides (T and A at positions at 183 and 190, respectively; *E. coli* 16S rRNA numbering) as those defined for the genus *Planococcus* as described by Dai *et al.* (2005). Strain ISL-16^T exhibited 16S rRNA gene sequence similarity values of 96.9–98.2 % to the type strains of species of the genus *Planococcus*, 96.7–98.2 % to the type strains of species of the genus *Planomicrobium* and less than 94.7 % to other species used in the phylogenetic analysis.

Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. The isoprenoid quinone profile of strain ISL-16^T was characterized by the predominance of menaquinone MK-8 (approx. 63 %) followed by MK-7 (approx. 29 %) and MK-6 (approx. 8 %). For cellular fatty acid analysis, cell mass of strain ISL-16^T was harvested from MA plates after cultivation for 3 days at 30 °C. Fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The fatty acid profile consisted of the following (each constituting >0.5 % of total fatty acids): branched fatty acids anteiso-C_{15:0} (44.8 %), anteiso-C_{17:0} (10.8 %), iso-C_{16:0} (7.5 %), iso-C_{14:0} (4.2 %), iso-C_{15:0} (2.7 %), iso-C_{17:0} (2.5 %), iso-C_{17:1ω10c} (2.0 %) and iso-C_{18:0} (0.5 %); unsaturated fatty acids C_{16:1ω7c} alcohol (16.7 %) and C_{16:1ω11c} (1.4 %); and summed feature 4 (iso-C_{17:1} and/or anteiso-C_{17:1}, 6.0 %). This fatty acid profile was similar to those of species of the genus *Planococcus*, although there were differences in the proportions of some fatty acids, probably due to differences in cultivation conditions and extraction procedures (Romano *et al.*, 2003; Yoon *et al.*, 2003a; Choi *et al.*, 2007). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with the modification that DNA was hydrolysed using nuclease P1 (Sigma) and the resultant nucleotides were analysed by reversed-phase HPLC. The DNA G+C content of strain ISL-16^T was 48.3 mol%. Chemotaxonomic data for strain ISL-16^T, which were similar to those for species of the genus *Planococcus*, supported the results of the phylogenetic analysis, i.e. that strain ISL-16^T is a member of the genus *Planococcus*. The cell morphology of strain ISL-16^T

Table 1. Differential phenotypic characteristics of strain ISL-16^T and other species of the genus *Planococcus*

Taxa: 1, ISL-16^T; 2, *P. citreus* (data from Hao & Komagata, 1985); 3, *P. kocurii* (Hao & Komagata, 1985); 4, *P. antarcticus* (Reddy *et al.*, 2002); 5, *P. maritimus* (Yoon *et al.*, 2003a); 6, *P. rifietoensis* (Romano *et al.*, 2003; Suresh *et al.*, 2007); 7, *P. maitriensis* (Alam *et al.*, 2003; Suresh *et al.*, 2007); 8, *P. columbae* (Suresh *et al.*, 2007); 9, *P. donghaensis* (Choi *et al.*, 2007). +, Positive; w, weakly positive; –, negative; v, variable; ND, not determined. All taxa are positive for Gram staining and catalase (data for *P. donghaensis* from this study). All taxa are negative for spore formation and urease (data for *P. rifietoensis* from this study).

Characteristic	1	2	3	4	5	6	7	8	9
Cell morphology	Cocci, short rods or rods	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Motility	–	+	+	+	+	ND	+	+	+
NaCl requirement	–*	–	–	–	–	–	+	–	+†
Growth in:									
15 % NaCl	–	+	–	–	+	+	–	–	–
17 % NaCl	–	–†	–†	–	+	–	–	–	–
Oxidase	+	–	–	–	–	+	–	+	+
Nitrate reduction	–	–	–	–	–	–	+	+	–†
Hydrolysis of:									
Gelatin	–	+	v	+	+	+	+	–	–
Aesculin	–	–	–	–	–	–	+	–	+
Starch	–	–	–	–	–	–	–	–	+
Acid production from:									
Cellobiose	–	–	–	–	–	–	–	–	+†
Fructose	+	+†	w†	–†	+	–	+	–	+†
Galactose	–	–†	–†	–†	–	–	–	–	–†
D-Glucose	–	+	v	+	+	–	–	–	–
Inositol	–	–	–	–†	–	–	–	–	–
Lactose	–	–	–	–	–	–	–	–	–†
D-Mannitol	–	–	–	–†	+	–†	–†	–†	+
D-Mannose	–	–	–	–†	–	–	+	–	–†
Raffinose	–	–	–	–†	–	–	–	–	–†
D-Sorbitol	–	–	–	–†	–	–	–	–	–
Sucrose	–	–	–	+	–	–	w	+	+
Trehalose	–	–	–	–†	–	–	–	–	+†
Predominant menaquinone(s)	MK-8, MK-7	MK-8, MK-7	MK-8, MK-7	MK-7, MK-8	MK-8, MK-7, MK-6	MK-8	MK-7, MK-8	MK-7, MK-8, MK-7(H ₂)	MK-7, MK-8
DNA G + C content (mol%)	48.3	48.0–51.2	39.6–42.9	41.5	48	47.9	39	50.5	47

*Grows in 0 % NaCl when Mg²⁺ ions are added.

†Data from this study for the type strains of reference species.

differed from that of other species of the genus *Planococcus* in that short rod- and rod-shaped cells were also present (Table 1). However, it is likely that cell morphology is no longer a suitable characteristic to differentiate the genera *Planococcus* and *Planomicrobium* or on which to assign a novel isolate to one of these two genera. The genus *Planomicrobium* also consists of species that form rod-shaped and/or coccoid-shaped cells (Mayilraj *et al.*, 2005; Jung *et al.*, 2009; Zhang *et al.*, 2009).

DNA–DNA hybridization was performed fluorometrically by the method of Ezaki *et al.* (1989) using photobiotin-labelled DNA probes and microdilution wells. 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. Strain ISL-16^T exhibited DNA–DNA relatedness values of 15–28 % to the type strains of species of the genus *Planococcus*: *Planococcus antarcticus* DSM 14505^T (19 %), *Planococcus citreus* DSM 20549^T (23 %), *Planococcus columbae* DSM 17517^T (17 %), *Planococcus kocurii* DSM 20747^T (25 %), *Planococcus maitriensis* DSM 15305^T (28 %), *Planococcus rifietoensis* DSM 15069^T (15 %), *Planococcus donghaensis* KCTC 13050^T (23 %) and *Planococcus maritimus* TF-9^T (21 %). Strain ISL-16^T could be differentiated from species of the genus *Planococcus* by some phenotypic characteristics, including cell morphology, motility, halotolerance, and hydrolysis of and acid production from several substrates (Table 1). The phylogenetic and genetic distinctiveness and differential phenotypic properties support the classification of strain ISL-16^T as a member of a species that is separate from recognized species of the genus

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Planococcus salinarum* sp. nov. ISL-16^T and related taxa. Bootstrap values (expressed as percentages of 1000 replications) of $\geq 50.0\%$ are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in both trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood algorithm. *Exiguobacterium aurantiacum* NCDO 2321^T (GenBank accession no. X70316) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Planococcus (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain ISL-16^T is considered to represent a novel species of the genus *Planococcus*, for which the name *Planococcus salinarum* sp. nov. is proposed. An emended description of the genus *Planococcus* is also presented.

Emended description of the genus *Planococcus* Migula 1894 (Approved Lists 1980) emend. Nakagawa *et al.* 1996

The description of the genus *Planococcus* is as given by Nakagawa *et al.* (1996) with the following amendments. Cells are cocci, short rods or rods. Cells can be motile or non-motile. The DNA G + C content is 39.0–51.2 mol%.

Description of *Planococcus salinarum* sp. nov.

Planococcus salinarum (sa.li.na'rum. L. pl. gen. n. *salinarum* of salterns).

Cells are Gram-stain-positive and non-motile, occurring as cocci, short rods or rods ($0.4\text{--}0.8 \times 0.8\text{--}5.0\text{ }\mu\text{m}$). Colonies on MA are circular, raised to slightly convex, glistening, smooth, pale yellow in colour and 1.0–2.0 mm in diameter after incubation for 3 days at 30 °C. Growth occurs at 4 and 38 °C, but not at 39 °C. Optimal pH for growth is between 7.0 and 7.5; growth occurs at pH 6.0, but not at pH 5.5. Optimal growth occurs in the presence of 2% (w/v) NaCl; growth occurs in 0–13.0% (w/v) NaCl. Mg^{2+} ions are required for growth. Anaerobic growth does not occur on MA or on MA supplemented with nitrate. H_2S is not produced. Casein and Tweens 20, 40, 60 and 80 are hydrolysed, but hypoxanthine, xanthine and L-tyrosine are not. Acid is not produced from L-arabinose, maltose,

melezitose, melibiose, L-rhamnose, D-ribose or D-xylose. Susceptible to ampicillin, carbenicillin, cephalothin, chloramphenicol, gentamicin, kanamycin, lincomycin, neomycin, novobiocin, oleandomycin, penicillin G and streptomycin, but not to polymyxin B or tetracycline. In assays with the API ZYM system, leucine arylamidase and cystine arylamidase are present and esterase (C4) and esterase lipase (C8) are weakly present, but alkaline phosphatase, lipase (C14), valine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase are absent. The predominant menaquinones are MK-8 and MK-7. The major fatty acids ($>10\%$ of total fatty acids) are anteiso- $\text{C}_{15:0}$, $\text{C}_{16:1\omega7c}$ alcohol and anteiso- $\text{C}_{17:0}$. Other phenotypic characteristics are given in Table 1.

The type strain is ISL-16^T (=KCTC 13584^T=CCUG 57753^T), isolated from a marine solar saltern of the Yellow Sea, Korea. The DNA G + C content of the type strain is 48.3 mol% (determined by HPLC).

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