Planomicrobium flavidum sp. nov., isolated from a marine solar saltern, and transfer of *Planococcus stackebrandtii* Mayilraj *et al.* 2005 to the genus *Planomicrobium* as *Planomicrobium stackebrandtii* comb. nov.

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A Gram-positive to Gram-variable, motile and coccoid- or short rod-shaped bacterial strain, ISL-41^T, was subjected to a polyphasic study to investigate its exact taxonomic position. Strain ISL-41^T grew optimally at pH 7.0–8.0 and 30 °C. It contained MK-8 and MK-7 as the predominant menaquinones and anteiso-C_{15:0}, iso-C_{16:0}, anteiso-C_{17:0} and C_{16:1ω7c} alcohol as the major fatty acids. The DNA G + C content was 45.9 mol%. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain ISL-41^T belonged to the genus *Planomicrobium*. The levels of similarity between the 16S rRNA gene sequence of strain ISL-41^T and those of the type strains of recognized *Planomicrobium* species and *Planococcus stackebrandtii* were 97.4–98.6 %. Mean DNA–DNA relatedness values between strain ISL-41^T and the type strains of *Planomicrobium* species and *Planococcus stackebrandtii* were 13–25 %. Differential phenotypic properties, together with the phylogenetic and genetic distinctiveness, showed that strain ISL-41^T could be differentiated from recognized *Planomicrobium* species and *Planococcus stackebrandtii*. On the basis of the phenotypic, phylogenetic and genetic data, strain ISL-41^T is considered to represent a novel species within the genus *Planomicrobium*, for which the name *Planomicrobium flavidum* sp. nov. is proposed. The type strain is ISL-41^T (=KCTC 13261^T=CCUG 56756^T). It is also proposed that *Planococcus stackebrandtii* be transferred to the genus *Planomicrobium* as *Planomicrobium stackebrandtii* comb. nov. (type strain K22-03^T=MTCC 6226^T=DSM 16419^T=JCM 12481^T).

The genus *Planomicrobium* was proposed by Yoon *et al.* (2001) to accommodate a novel species, *Planomicrobium koreense*, and the reclassification of *Planococcus okeanoikoites* (Nakagawa *et al.*, 1996) and *Planococcus mcmeekinii* (Junge *et al.*, 1998) as *Planomicrobium okeanoikoites* and *Planomicrobium mcmeekinii*, respectively. Subsequently, *Planococcus alkanoclasticus* (Engelhardt *et al.*, 2001) and *Planococcus psychrophilus* (Reddy *et al.*, 2002) were transferred to the genus *Planomicrobium* as *Planomicrobium alkanoclasticum* and *Planomicrobium psychrophilum*, respectively, and two other *Planomicrobium* species, *Planomicrobium chinense* (Dai *et al.*, 2005) and *Planomicrobium glaciei* (Zhang *et al.*, 2009), were described. Members of the genus *Planomicrobium* have

been isolated from fermented seafood, marine mud, Antarctic samples, intertidal sediments and glacier (Nakagawa *et al.*, 1996; Junge *et al.*, 1998; Engelhardt *et al.*, 2001; Yoon *et al.*, 2001; Reddy *et al.*, 2002; Dai *et al.*, 2005; Zhang *et al.*, 2009). In this study, we report the taxonomic characterization of a *Planomicrobium*-like bacterial strain, ISL-41^T, which was isolated from a marine solar saltern in Korea.

Strain ISL-41^T was isolated by means of the standard dilution plating technique at 30 °C on marine agar 2216 (MA; Difco) supplemented with 8 % (w/v) NaCl. The type strains of recognized *Planomicrobium* species and *Planococcus stackebrandtii* were used as reference strains for DNA–DNA hybridization and phenotypic characterization. *Planomicrobium mcmeekinii* S23F2^T and *Planomicrobium koreense* JG07^T were obtained from

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain ISL-41^T is FJ265708.

previous studies (Junge *et al.*, 1998; Yoon *et al.*, 2001). *Planomicrobium okeanokoites* NBRC 12536^T was obtained from the Institute for Fermentation, Osaka. *Planomicrobium chinense* DSM 17276^T, *Planomicrobium psychrophilum* DSM 14507^T and *Planococcus stackebrandtii* DSM 16419^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. *Planomicrobium alkanoclasticum* NCIMB 13489^T was obtained from the National Collection of Industrial, Marine and Food Bacteria, Aberdeen, UK. The morphological, physiological and biochemical characteristics of strain ISL-41^T were investigated using routine cultivation at 30 °C on MA. Cell morphology was examined by using light microscopy (Nikon E600) and transmission electron microscopy. Flagellation was determined using a Philips CM-20 transmission electron microscope with cells from exponentially growing cultures. For this purpose, the cells were negatively stained with 1% (w/v) phosphotungstic acid and the grids were examined after being air-dried. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and 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 marine broth 2216 (MB; Difco) 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 absence of NaCl was investigated using trypticase soy broth prepared according to the formula of the Difco medium except that NaCl was excluded from the medium formula. Growth at various NaCl concentrations (0.5%, w/v, and 1.0–15.0%, w/v, in 1.0% increments) was investigated in MB or trypticase soy broth (Difco). Growth at various temperatures (4, 10, 20, 25, 28, 30, 35, 37, 40 and 45 °C) was measured on MA. Catalase and oxidase activities and hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations 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 with the following concentrations: 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).

Cell biomass for DNA extraction and for isoprenoid quinone analysis was obtained from cultures grown at 30 °C in MB. 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, 5'-GAGTTTGATCCTGGCTCAG-3' and 5'-AGAAAGGAGGTGATCCAGCC-3', as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon *et al.* (2003). Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For cellular fatty acid analysis, cell mass of strain ISL-41^T was harvested from MA plates after cultivation for 3 days at 30 °C. The 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 DNA G+C content was determined by using 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. DNA–DNA hybridization was performed fluorometrically by using the method of Ezaki *et al.* (1989) with 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.

Morphological, cultural, physiological and biochemical characteristics of strain ISL-41^T are given in the species description or are shown in Table 1. The almost-complete 16S rRNA gene sequence of strain ISL-41^T determined in this study comprised 1506 nt. In the phylogenetic tree based on the neighbour-joining algorithm, strain ISL-41^T fell within the clade comprising *Planomicrobium* species and *Planococcus stackebrandtii* (Fig. 1). The 16S rRNA gene of strain ISL-41^T contained the same signature nucleotides as those defined for the genus *Planomicrobium* as described by Dai *et al.* (2005). Strain ISL-41^T exhibited 16S rRNA gene sequence similarity values of 97.4–98.6% to the type strains of *Planomicrobium* species and *Planococcus stackebrandtii*, and 96.5–97.9% to the type strains of other *Planococcus* species.

The menaquinone profile of strain ISL-41^T was characterized by the predominance of MK-8 (approx. 72%), followed by MK-7 (approx. 21%) and MK-6 (approx. 5%). The fatty acid profile of strain ISL-41^T was comprised of the following (each constituting >0.5% of total fatty acids): branched fatty acids anteiso-C_{15:0} (39.0%), iso-C_{16:0} (11.5%), anteiso-C_{17:0} (11.3%), iso-C_{14:0} (8.0%), iso-C_{17:0} (2.8%), iso-C_{17:1}ω10c (2.8%), iso-C_{15:0} (1.8%) and iso-C_{18:0} (1.3%); unsaturated fatty acids C_{16:1}ω7c alcohol (11.0%) and C_{16:1}ω11c (1.0%); and summed

Table 1. Differential phenotypic characteristics of *Planomicrobium* species and *Planococcus stackebrandtii*

Taxa: 1, strain ISL-41^T (*Planomicrobium flavidum* sp. nov.); 2, *Planomicrobium koreense* (data from Yoon *et al.*, 2001); 3, *Planomicrobium okeanokoites* (Nakagawa *et al.*, 1996; Yoon *et al.*, 2001); 4, *Planomicrobium mcmeekinii* (Junge *et al.*, 1998; Yoon *et al.*, 2001); 5, *Planomicrobium chinense* (Dai *et al.*, 2005); 6, *Planomicrobium psychrophilum* (Reddy *et al.*, 2002; this study); 7, *Planomicrobium alkanoclasticum* (Engelhardt *et al.*, 2001; this study); 8, *Planomicrobium glaciei* (Zhang *et al.*, 2009); 9, *Planococcus stackebrandtii* (Mayilraj *et al.*, 2005). +, Positive reaction; –, negative reaction; w, weakly positive reaction; ND, not determined. All species are positive for motility and catalase activity (not determined for *Planomicrobium chinense*).

Characteristic	1	2	3	4	5	6	7	8	9
Cell morphology*	C or SR	C or SR/R	R	R/C or SR	C or SR	R	R	C or SR	C
Gram-stain	Positive to variable	Positive to variable	Positive to variable	Positive	Positive	Positive	Positive or variable	Positive	Positive
Colony colour†	Light yellow	Yellow–orange	Bright yellow–bright orange	Pale orange	Yellow–orange	Orange	Orange	Yellow–orange	Orange
Oxidase	+	–	w	–	–	+	–	–	–
Nitrate reduction to nitrite	–	–	–	+	+	–	–	+	–
Growth in:									
7 % NaCl	+	w	–	+	+	+	–	+	+
10 % NaCl	+	–	–	+	+	+	–	+	–
Hydrolysis of:									
Aesculin	–	+	–	–	–	+	ND	ND	ND
Casein	w	+	+	+	–	ND	ND	+	–
Gelatin	–	+	+	+	+	+	+	+	+
Starch	–	–	–	–	–	–	+	–	–
Tween 80	+	–	–	–	–	+	ND	–	ND
Acid production from:‡									
L-Arabinose	–	–	–	–	–	–	–	ND	–
Cellobiose	–	+	–	–	–	–	+	ND	ND
D-Fructose	+	–	+	+	ND	+	+	ND	+
D-Glucose	–	w	–	+	+	–	+	ND	–
Lactose	–	+	–	–	–	–	–	ND	+
Maltose	–	+	–	w	ND	–	–	ND	ND
D-Mannose	–	–	–	–	–	–	–	ND	ND
Melibiose	–	+	–	–	–	–	–	ND	ND
Raffinose	–	–	–	–	–	–	–	ND	+
L-Rhamnose	–	–	–	–	–	–	–	ND	–
Ribose	–	–	+	–	ND	–	–	+	ND
Sucrose	–	–	–	–	–	–	–	ND	+
D-Xylose	–	–	+	–	–	–	–	ND	–
Mannitol	–	–	–	–	–	–	+	ND	–
Predominant mena-quinone(s)	MK-8, 7	MK-8, 7, 6	MK-8, 7	MK-8, 7	MK-8	MK-7, 8	ND	MK-7, 8	MK-7, 8
DNA G + C content (mol%)	45.9	47	46	35	34.8	44.5	45.3 ± 0.4	49	40

*C, Cocci; R, rods; SR, short rods.

†Differences may be caused by different cultivation conditions.

‡Data for *Planomicrobium psychrophilum* and *Planomicrobium alkanoclasticum* are from this study.

feature 4 (iso-C_{17:1} I and/or anteiso-C_{17:1} B; 8.4 %). This fatty acid profile was similar to those of *Planomicrobium* species and *Planococcus stackebrandtii*, although there were differences in the proportions of some fatty acids, probably because of differences in cultivation conditions and extraction procedures (Engelhardt *et al.*, 2001; Reddy

et al., 2002; Mayilraj *et al.*, 2005; Dai *et al.*, 2005). The DNA G + C content of strain ISL-41^T was 45.9 mol%. The results obtained from chemotaxonomic analyses showed the properties that are shared by *Planomicrobium* species and thereby support the result of the phylogenetic analysis, i.e. that strain ISL-41^T belongs to the genus *Planomicrobium*.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain ISL-41^T (*Planomicrobium flavidum* sp. nov.) and other related taxa. Bootstrap values (expressed as percentages of 1000 replications) of >40% are shown at branch points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Exiguobacterium aurantiacum* NCDO 2321^T (GenBank accession number X70316) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Strain ISL-41^T exhibited mean DNA–DNA relatedness values of 13–25% to *Planomicrobium koreense* JG07^T, *Planomicrobium okeanokoites* NBRC 12536^T, *Planomicrobium mcmeekinii* S23F2^T, *Planomicrobium chinense* DSM 17276^T, *Planomicrobium psychrophilum* DSM 14507^T, *Planomicrobium alkanoclasticum* NCIMB 13489^T and *Planococcus stackebrandtii* DSM 16419^T. Strain ISL-41^T was distinguishable from recognized *Planomicrobium* species and *Planococcus stackebrandtii* by differences in several phenotypic characteristics as listed in Table 1. The phylogenetic and genetic distinctiveness and differential phenotypic properties of strain ISL-41^T were sufficient to categorize it as a member of a species that is distinct from the recognized *Planomicrobium* species and *Planococcus stackebrandtii* (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994). Also, it appears to be appropriate that *Planococcus stackebrandtii* should be reclassified as a member of the genus *Planomicrobium*. The phylogenetic trees based on 16S rRNA gene sequences showed that *Planococcus stackebrandtii* K22-03^T fell within the cluster comprising *Planomicrobium* species, not *Planococcus* species (Fig. 1; Dai *et al.*, 2005; Zhang *et al.*, 2009). In the neighbour-joining phylogenetic tree, the relationship between the cluster comprising *Planomicrobium* species and *Planococcus stackebrandtii* K22-03^T and the cluster comprising other *Planococcus* species was maintained by a bootstrap resampling value of 100% (Fig. 1; Dai *et al.*, 2005; Zhang *et al.*, 2009). The 16S rRNA gene of *Planococcus stackebrandtii* K22-03^T was found to have signature nucleotides at positions at 183 and 190 (*Escherichia coli* 16S rRNA numbering) characteristic of the genus *Planomicrobium* rather than the genus *Planococcus* as described by Dai *et al.* (2005). Accordingly, it is proposed that *Planococcus stackebrandtii* should be transferred to the genus *Planomicrobium* as *Planomicrobium stackebrandtii* comb. nov. In addition, on the basis of the data presented, strain ISL-41^T should be classified as representing a novel

species of the genus *Planomicrobium*, for which the name *Planomicrobium flavidum* sp. nov. is proposed.

Description of *Planomicrobium flavidum* sp. nov.

Planomicrobium flavidum (fla'vi.dum. L. neut. adj. *flavidum* pale yellow).

Cells are Gram-positive to Gram-variable and cocci or short rods (0.4–0.8 × 0.4–1.6 µm); a few cells are rods (2.7–3.3 µm in length) in young cultures. Motile by means of a single polar flagellum. Colonies on MA are circular, raised to slightly convex, glistening, smooth, light yellow in colour and 1.2–2.0 mm in diameter after incubation for 3 days at 30 °C. Growth occurs at 4 and 37 °C, but not at 40 °C. Optimal pH for growth is between 7.0 and 8.0; growth occurs at pH 6.0, but not at pH 5.5. Growth occurs in the presence of 13% (w/v) NaCl, but not in the absence of NaCl or in the presence of more than 14% (w/v) NaCl. Anaerobic growth does not occur on MA and on MA supplemented with nitrate. H₂S is not produced. Tweens 20, 40 and 60 are hydrolysed, but hypoxanthine, xanthine and L-tyrosine are not. Acid is not produced from D-galactose, melezitose, trehalose, *myo*-inositol and D-sorbitol. 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, esterase (C4) and esterase lipase (C8) are present, but alkaline phosphatase, lipase (C14), leucine arylamidase, valine arylamidase, cystine 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. Predominant menaquinones are MK-8 and MK-7. Major fatty acids (>10% of total fatty acids) are anteiso-C_{15:0}, iso-C_{16:0}, anteiso-C_{17:0} and

C_{16:1ω7c} alcohol. The DNA G+C content of the type strain is 45.9 mol% (determined by HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, ISL-41^T (=KCTC 13261^T=CCUG 56756^T), was isolated from a marine solar saltern of the Yellow Sea, Korea.

Description of *Planomicrobium stackebrandtii* Mayilraj *et al.* 2005 comb. nov.

Planomicrobium stackebrandtii (sta.cke.brand.ti'i. N.L. gen. n. *stackebrandtii* of Stackebrandt, to honour Erko Stackebrandt, a German microbiologist, for his valuable contributions to microbial taxonomy and molecular systematics).

Basonym: *Planococcus stackebrandtii* Mayilraj *et al.* 2005.

The description is the same as that given by Mayilraj *et al.* (2005). The type strain is strain K22-03^T (=MTCC 6226^T=DSM 16419^T=JCM 12481^T).

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