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.

Yong-Taek Jung,1,2 So-Jung Kang,1 Tae-Kwang Oh,1 Jung-Hoon Yoon1 and Bong-Hee Kim2

Correspondence
Jung-Hoon Yoon
jhyoon@kribb.re.kr
Bong-Hee Kim
bhkimnh@cnu.ac.kr

1Korea Research Institute of Bioscience and Biotechnology (KORIBB), PO Box 115, Yusong, Taejon, Republic of Korea
2College of Pharmacy, Chungnam National University, Taejon 305-764, Republic of Korea

A Gram-positive to Gram-variable, motile and coccoid- or short rod-shaped bacterial strain, ISL-41T, was subjected to a polyphasic study to investigate its exact taxonomic position. Strain ISL-41T grew optimally at pH 7.0–8.0 and 30°C. It contained MK-8 and MK-7 as the predominant menaquinones and anteiso-C15:0, iso-C16:0, anteiso-C17:0 and C16:1o7c 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-41T belonged to the genus Planomicrobium. The levels of similarity between the 16S rRNA gene sequence of strain ISL-41T 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-41T 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-41T could be differentiated from recognized Planomicrobium species and Planococcus stackebrandtii. On the basis of the phenotypic, phylogenetic and genetic data, strain ISL-41T 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-41T (=KCTC 13261T=CCUG 56756T). It is also proposed that Planococcus stackebrandtii be transferred to the genus Planomicrobium as Planomicrobium stackebrandtii comb. nov. (type strain K22-03T=MTCC 6226T=DSM 16419T=JCM 12481T).

The genus Planomicrobium was proposed by Yoon et al. (2001) to accommodate a novel species, Planomicrobium koreense, and the reclassification of Planococcus okeanokoites (Nakagawa et al., 1996) and Planococcus mcmeekinii (Junge et al., 1998) as Planomicrobium okeanokoites 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-41T, which was isolated from a marine solar saltern in Korea.

Strain ISL-41T 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 S23F2T and Planomicrobium koreense JG07T were obtained from
previous studies (Junge et al., 1998; Yoon et al., 2001). Planomicrobium okeanokoites NBRC 12536T was obtained from the Institute for Fermentation, Osaka. Planomicrobium chinense DSM 17276T, Planomicrobium psychrophilum DSM 14507T and Planomicrobium stackebrandtii DSM 16419T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. Planomicrobium alkanoliclasticum NCIMB 13489T was obtained from the National Collection of Industrial, Marine and Food Bacteria, Aberdeen, UK. The morphological, physiological and biochemical characteristics of strain ISL-41T 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 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 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 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 carbencillin (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'-GAGTTGTATCCTGTCG-3' and 5'-AAAGAGGTTGATCCAGCC-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-41T 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-41T are given in the species description or are shown in Table 1. The almost-complete 16S rRNA gene sequence of strain ISL-41T determined in this study comprised 1506 nt. In the phylogenetic tree based on the neighbour-joining algorithm, strain ISL-41T fell within the clade comprising Planomicrobium species and Planococcus stackebrandtii (Fig. 1). The 16S rRNA gene of strain ISL-41T contained the same signature nucleotides as those defined for the genus Planomicrobium as described by Dai et al. (2005). Strain ISL-41T 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-41T 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-41T was comprised of the following (each constituting >0.5 % of total fatty acids): branched fatty acids anteiso-C15:0 (39.0 %), iso-C16:0 (11.5 %), anteiso-C17:0 (11.3 %), iso-C14:0 (8.0 %), iso-C15:0 (2.8 %), iso-C17:0 (2.8 %), iso-C15:0(2.8 %), iso-C15:0(1.8 %) and iso-C18:0 (1.3 %); unsaturated fatty acids C16:1ω7c alcohol (11.0 %) and C16:1ω11c (1.0 %); and summed

...
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 stackebrandii, 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. 

Table 1. Differential phenotypic characteristics of Planomicrobium species and Planococcus stackebrandii

| Taxa: | 1, strain ISL-41^T (Planomicrobium flavidum sp. nov.); 2, Planomicrobium koreense (data from Yoon et al., 2001); 3, Planomicrobium okenoikotes (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 stackebrandii (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 | C or SR | R | C |
| **Gram-stain** | Positive to variable | Positive to variable | Positive to variable | Positive to variable | Positive to variable | Positive to variable | Positive to variable | Positive to variable | Positive to variable |
| **Colony colour** | Orange | Orange | Orange | Orange | Orange | Orange | Orange | Orange | Orange |
| **Oxidase** | + | – | – | – | + | + | + | + | + |
| **Nitrate reduction to nitrite** | – | – | – | – | – | – | – | – | – |
| **Growth in:** | 7 % NaCl | + | W | – | + | + | + | – | + |
| **Hydrolysis of:** | 10 % NaCl | + | – | – | – | – | – | – | – |
| **Acid production from:** | Aesculin | + | W | – | – | + | + | + | + |
| **Casein** | – | + | W | – | + | + | + | + | + |
| **Gelatin** | – | + | W | – | + | + | + | + | + |
| **Starch** | – | – | – | – | – | – | – | – | – |
| **Twee80** | – | + | – | – | – | – | + | ND | – |
| **Predominant menaquinone(s)** | MK-8, 7 | MK-8, 7, 6 | MK-8, 7 | MK-8, 7 | MK-8, 7 | MK-8, 7 | MK-7, 8 | 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.

http://ijs.sgmjournals.org
Strain ISL-41<T> exhibited mean DNA–DNA relatedness values of 13–25 % to Planomicrobium koreense JG07<T>, Planomicrobium okeanokoiies NBRC 12536<T>, Planomicrobium mcmeekinii S23F2<T>, Planomicrobium chinense DSM 17276<T>, Planomicrobium psychrophilum DSM 14507<T>, Planomicrobium alkanolasticum NCIMB 13489<T> and Planococcus stackebrandii DSM 16419<T>. Strain ISL-41<T> was distinguishable from recognized Planomicrobium species and Planococcus stackebrandii 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 stackebrandii (Wayne et al., 1987; Stackebrandt & Goebel, 1994). Also, it appears to be appropriate that Planococcus stackebrandii should be reclassified as a member of the genus Planomicrobium. The phylogenetic trees based on 16S rRNA gene sequences showed that Planococcus stackebrandii 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 stackebrandii 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 stackebrandii 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 stackebrandii should be transferred to the genus Planomicrobium as Planomicrobium stackebrandii 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. Tewens 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 polymixin 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, z-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, x-galactosidase, β-galactosidase, β-glucuronidase, x-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, x-mannosidase and x-fucosidase are absent. Predominant menaquinones are MK-8 and MK-7. Major fatty acids (>10 % of total fatty acids) are anteiso-C₁₅:₀, iso-C₁₆:₀, anteiso-C₁₇:₀ and...
C\textsubscript{16:1}\textit{\textit{f}}7c 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\textsuperscript{T} (=KCTC 13261\textsuperscript{T}=CCUG 56756\textsuperscript{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).


The description is the same as that given by Mayilraj et al. (2005). The type strain is strain K22-03\textsuperscript{T} (=MTCC 6226\textsuperscript{T}=DSM 16149\textsuperscript{T}=JCM 12481\textsuperscript{T}).

**Acknowledgements**

This work was supported by the 21C Frontier program of Microbial Genomics and Applications (grant MG05-0401-2-0) from the Ministry of Education, Science and Technology (MEST) of the Republic of Korea.

**References**


Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro
dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* 39, 224–229.


