Planomicrobium chinense sp. nov., isolated from coastal sediment, and transfer of *Planococcus psychrophilus* and *Planococcus alkanoclasticus* to *Planomicrobium* as *Planomicrobium psychrophilum* comb. nov. and *Planomicrobium alkanoclasticum* comb. nov.

Xin Dai, Ya-Nan Wang, Bao-Jun Wang, Shuang-Jiang Liu and Yu-Guang Zhou

Correspondence
Shuang-Jiang Liu
shuangjiang@hotmail.com

State Key Laboratory of Microbial Resources at the Institute of Microbiology, Chinese Academy of Sciences, Zhong-Guan-Cun, Haidian, Beijing 100080, PR China

An aerobic, motile, Gram-positive strain, DX3-12^T, was isolated from coastal sediment of the Eastern China Sea in Fujian Province, China, and was identified by means of polyphasic taxonomy. On the basis of 16S rRNA gene sequence similarity, strain DX3-12^T was closely related to members of the genera *Planomicrobium* and *Planococcus*. 16S rRNA gene sequence similarities between strain DX3-12^T and *Planomicrobium mcmeekinii*, *Planomicrobium koreense*, *Planomicrobium okeanokoites*, *Planococcus psychrophilus* and *Planococcus alkanoclasticus* were 97.8, 97.8, 97.1, 97.5 and 96.4 %, respectively. Chemotaxonomic and physiological properties of strain DX3-12^T supported its affiliation to the genus *Planomicrobium*. Strain DX3-12^T and *Planomicrobium mcmeekinii* exhibited common phenotypic properties such as the ability to reduce nitrate, but they could be distinguished from each other by physiological and biochemical tests, e.g. acid production from glucose, growth temperature and NaCl concentration requirements. DNA–DNA hybridization further distinguished strain DX3-12^T from *Planomicrobium mcmeekinii* at the species level. Therefore, it is proposed that strain DX3-12^T (= AS 1.3454^T = JCM 12466^T) represents a novel species, *Planomicrobium chinense* sp. nov. Based on 16S rRNA gene sequence similarities, signature nucleotides, cell morphology and physiological and biochemical properties, *Planococcus psychrophilus* and *Planococcus alkanoclasticus* have been transferred to the genus *Planomicrobium* as *Planomicrobium psychrophilum* comb. nov. (type strain DSM 14507^T = MTCC 3812^T) and *Planomicrobium alkanoclasticum* comb. nov. (type strain NCIMB 13489^T = CIP 107718^T), respectively.

Aerobic, Gram-positive, motile cocci or rods were classified as members of the genus *Planococcus* before the genus *Planomicrobium* was established (Nakagawa *et al.*, 1996). At present, the genus *Planomicrobium* includes the following species: *Planomicrobium mcmeekinii* (Junge *et al.*, 1998), *Planomicrobium okeanokoites* (Nakagawa *et al.*, 1996) and *Planomicrobium koreense* (Yoon *et al.*, 2001). Two species belonging to the genus *Planococcus*, *Planococcus psychrophilus* (Reddy *et al.*, 2002) and *Planococcus alkanoclasticus* (Engelhardt *et al.*, 2001), may also be members of the genus *Planomicrobium* (Yoon *et al.*, 2003). During an ecological survey of the microbial diversity of coastal

sediments from the Eastern China Sea in Fujian Province, China, a nitrate-reducing isolate, strain DX3-12^T, was obtained. Characterization and classification of this isolate is described.

Strain DX3-12^T was isolated from coastal sediment of the Eastern China Sea. Samples (4–6 cm beneath surface) were diluted with 9 ml sterile saline solution and 10^{−3} and 10^{−4} dilutions (0.1 ml) were plated onto artificial sea water basal medium with 1 % peptone and 0.5 % yeast extract (Eguchi *et al.*, 1996). Routine cultivation of strain DX3-12^T was done at 30 °C in marine broth 2216 (MB; Difco). Observation of the morphology of strain DX3-12^T was carried out with optical (Olympus BX51) and scanning electron (Hitachi S-570) microscopes. Physiological and biochemical tests, including catalase and oxidase activities, decomposition of

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain DX3-12^T is AJ697862.

gelatin and casein and hydrolysis of starch were determined by the methods of Cowan & Steel (1965). Nitrate reductase production was evaluated as described by Lanyi (1987). NaCl tolerance was investigated by supplementing MB with various NaCl concentrations. The temperature and pH ranges for growth were determined in MB.

Biomass for chemotaxonomic analysis was harvested from MB cultures on a rotary shaker (100 r.p.m., 30 °C). Preparation of cell walls and determination of peptidoglycan structure were carried out using the methods of Schleifer & Kandler (1972). Menaquinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989) with *Planomicrobium mcmeekinii* as the reference. The fatty acid profile of whole cells was analysed by GC with a model HP6890 equipped with a hydrogen ionization detector (FID). Peaks were identified with pre-installed software, HPCHEM-STATION (version A5.01).

Genomic DNA of strain DX3-12^T was extracted according to Marmur (1961) and G + C content (mol%) was determined by thermal denaturation, with *Escherichia coli* K-12 as the reference. The 16S rRNA gene of strain DX3-12^T was amplified and sequenced as described previously (Zhang *et al.*, 2003). Alignments of 16S rRNA gene sequences of strain DX3-12^T and other type strains of the genera *Planomicrobium* and *Planococcus* were carried out with CLUSTAL X version 1.8 (Thompson *et al.*, 1997). Evolutionary distance calculations were performed according to Kimura (1980). Phylogenetic trees were constructed according to the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony tree-making algorithms in the PHYLIP software package (Felsenstein, 1993). Positions 31–1431 (*E. coli* numbering) were used in all phylogenetic analyses. DNA relatedness was determined by the method of De Ley *et al.* (1970).

Cells of strain DX3-12^T were cocci or short rods, 0·8 × 1·0 µm. Detailed phenotypic properties are given in the species description. Physiological and biochemical characteristics of this strain that enable it to be distinguished from

other type strains of *Planomicrobium* species are listed in Table 1. The cell wall composition of strain DX3-12^T was determined and contained L-lysine, L-aspartic acid, L-glutamic acid and alanine, but not L-ornithine; this is consistent with the A4α type (Schleifer & Kandler, 1972) cell wall of *Planomicrobium*. The major menaquinones were MK-8 and MK-7, with a peak area ratio of 55:8. Cells of strain DX3-12^T grown in MB contained anteiso-C_{15:0} (49·7 %) as the major fatty acid and significant amounts of C_{16:1ω7c} alcohol (12·1 %). The genomic DNA G + C content was 34·8 mol%.

The nearly complete 16S rRNA gene (1510 bp) of strain DX3-12^T was cloned and sequenced. Phylogenetic analysis based on 16S rRNA gene sequence similarity showed that strain DX3-12^T was closely related to members of the genus *Planomicrobium* [*Planomicrobium mcmeekinii* (97·8 % similarity), *Planomicrobium koreense* (97·8 %) and *Planomicrobium okeanokoites* (97·1 %)] and to members of the genus *Planococcus* [*Planococcus psychrophilus* (97·5 %), *Planococcus alkanoclasticus* (96·4 %) and *Planococcus stackebrandtii* (96·3 %) (Mayilraj *et al.*, 2005)]. These species gathered and formed a cluster with moderate bootstrap support, as indicated by either neighbour-joining (Fig. 1) or maximum-parsimony (data not shown). Strain DX3-12^T could be distinguished phenotypically from other *Planomicrobium* species (Table 1). DNA relatedness to *Planomicrobium mcmeekinii* DSM 13963^T, a phylogenetically related and phenotypically similar species, was 35·4 %. DNA–DNA hybridization to other species of *Planomicrobium* was not attempted because of the large differences in G + C content between these species and strain DX3-12^T (11·5–12·2 %; Table 1). Based on the above characteristics, strain DX3-12^T was identified as a representative of a novel species of *Planomicrobium*; the name *Planomicrobium chinense* sp. nov. is proposed.

16S rRNA gene sequence analyses of this and previously studied strains (Yoon *et al.*, 2003) showed that two members

Table 1. Differential phenotypic properties of strain DX3-12^T and other type strains of *Planomicrobium* species

Taxa: 1, DX3-12^T; 2, *Planomicrobium koreense*; 3, *Planomicrobium mcmeekinii*; 4, *Planomicrobium okeanokoites*; 5, *Planomicrobium alkanoclasticum* comb. nov.; 6, *Planomicrobium psychrophilum* comb. nov. (data from references listed). +, Positive; –, negative; w, weakly positive; NR, not reported.

Property	1	2	3	4	5	6
Cell shape	Coccoid/short rods	Coccoid/rods	Coccoid/rods	Rods	Rods	Rods
Growth at/in:						
Temperature (°C)	12–43	4–38	0–37	20–37	15–41	0–30
NaCl (%)	0–10	0–7	0–7	0–7	0·8–6	0–12
Oxidase	–	–	–	w	–	+
Nitrate reduction	+	–	+	–	–	–
Aesculin hydrolysis	–	+	NR	–	NR	+
Acid production from glucose	–	w	+	–	–	–
G + C content (mol%)	34·8	47	35	46·3	45·3	44·5

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequence analysis showing phylogenetic relationships between strain DX3-12^T, other related species of the genera *Planomicrobium* and *Planococcus*, and related reference organisms. Bootstrap confidence values greater than 500 obtained from 1000 bootstrap replications are given at the nodes. Bar, 0.02 substitutions per nucleotide position.

of the genus *Planococcus*, *Planococcus psychrophilus* and *Planococcus alkanoclasticus*, clustered with members of the genus *Planomicrobium*, rather than with members of the genus *Planococcus*. This clustering is consistently supported by different tree construction methods. When the 16S rRNA gene sequences were further examined, these two species were shown to have sequence signatures at positions 183 and 190 (*E. coli* numbering) indicative of *Planomicrobium* rather than *Planococcus* (Table 2). In addition, cells of these two species are rods, unlike members of the genus *Planococcus*, which have coccoid cells. Thus, these two species are here transferred to the genus *Planomicrobium* as *Planomicrobium psychrophilum* comb. nov. and *Planomicrobium alkanoclasticum* comb. nov. The very recently described species *Planococcus stackebrandtii* (Mayilraj *et al.*, 2005) also clustered within the *Planomicrobium* group (Fig. 1) and had *Planomicrobium* signature nucleotides at positions 183 and 190; this species may possibly be a member of the genus *Planomicrobium*.

Description of *Planomicrobium chinense* sp. nov.

Planomicrobium chinense (chin.en'se. N.L. neut. adj. *chinense* pertaining to China, where the type strain was isolated and studied).

Cells are coccoid or short rods, $0.8 \times 1.0 \mu\text{m}$. Gram-positive and non-spore-forming. Motile by polar flagella. Strictly aerobic. Colonies are smooth, circular, low-convex and yellow to orange in colour when cultivated on MA. Growth occurs at 10–45 °C; optimum growth at 30–35 °C and no growth above 46 °C. Optimal pH for growth is 6.0–7.0; no growth occurs below pH 5.0 or above pH 10.0. Growth occurs in 0–10% NaCl. Gelatin is hydrolysed, but not casein, starch, Tween 80 or aesculin. Acid is produced from glucose, but not from sucrose, raffinose, lactose, arabinose, cellobiose, xylose, rhamnose, melibiose, mannose or mannitol. Citrate does not support growth. Nitrate is reduced. Cell wall is type A4x. Cells contain MK-8 as major

Table 2. Sequence signatures of 16S rRNA genes for type strains of species of the genera *Planomicrobium* and *Planococcus*

Common nucleotides shared by species of the genus *Planomicrobium* or *Planococcus* are shown in bold.

Organism	183	190
<i>E. coli</i>	C G T C G C A A G A C	
Strain DX3-12 ^T	C C T C C T G G T G G	
<i>Planomicrobium koreense</i> (AF144750)	C C T C C T G G G G C	
<i>Planomicrobium okeanokoites</i> (D55729)	C C T C C T G G – G N	
<i>Planomicrobium mcmeekinii</i> (AF041791)	C C T C C T G G G G C	
<i>Planomicrobium psychrophilum</i> (AJ314746)	C C T C C T G G C G A	
<i>Planomicrobium alkanoclasticum</i> (AF029364)	C C T C C T G G G G C	
<i>Planococcus citreus</i> (X62172)	T C T C A T G A G G C	
<i>Planococcus kocurii</i> (X62173)	T C T C C T G A G G C	
<i>Planococcus maitriensis</i> (AJ544622)	T C T C A T G A G G C	
<i>Planococcus rifietoensis</i> (AJ493659)	T C T C A T G A G G C	
<i>Planococcus maritimus</i> (AF500007)	T C T C A T G A G G C	
<i>Planococcus antarcticus</i> (AJ314745)	T C T C C T G A G G C	

menaquinone. The cellular fatty acid profile is as follows: iso-C_{14:0} (10.4 %), anteiso-C_{15:0} (49.7 %), iso-C_{15:0} (3.8 %), C_{15:0} (4.9 %), C_{16:1ω7c} alcohol (12.1 %), iso-C_{16:0} (4.1 %), C_{16:1ω11c} (4.5 %), C_{16:0} (1.5 %), iso-C_{17:1ω10c} (1.7 %), iso-C_{17:1} I (4.4 %) and anteiso-C_{17:0} (2.8 %).

The type strain, DX3-12^T (=AS 1.3454^T=JCM 12466^T), was isolated from coastal sediment from the Eastern China Sea in Fujian Province, China. The DNA G+C content of the type strain is 34.8 mol%.

Description of *Planomicrobium psychrophilum* comb. nov.

Planomicrobium psychrophilum (psy.chro.phi'lum. Gr. n. *psychros* cold; Gr. adj. *philos* loving; N.L. neut. adj. *psychrophilum* cold-loving).

Basonym: *Planococcus psychrophilus* Reddy *et al.* 2002.

The description is as given previously by Reddy *et al.* (2002). The type strain is DSM 14507^T (=MTCC 3812^T).

Description of *Planomicrobium alkanoclasticum* comb. nov.

Planomicrobium alkanoclasticum (al.kan.o.cla'sti.cum. N.L. n. *alkanum* alkane; Gr. adj. *clastos* broken; N.L. neut. adj. *alkanoclasticum* breaking alkanes).

Basonym: *Planococcus alkanoclasticus* Engelhardt *et al.* 2001.

The description is as given previously by Engelhardt *et al.* (2001). The type strain is NCIMB 13489^T (=MAE2^T=CIP 107718^T).

Acknowledgements

This work was supported by grants from National Natural Science Foundation of China and Chinese Academy of Sciences (KSCX2-SW-113). We thank Professor H. Trüper at the University of Bonn, Germany, for his help with the nomenclature and etymology.

References

- Collins, M. D. (1985). Isoprenoid quinone analysis in classification and identification. In *Chemical Methods in Bacterial Systematics*, pp. 267–287. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.
- Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 143–153.
- Eguchi, M., Ishikawa, T., MacDonald, K., Cavicchioli, R., Gottschal, J. C. & Kjelleberg, S. (1996). Response to stress and nutrient availability by the marine ultramicrobacterium *Sphingomonas* sp. strain RB2256. *Appl Environ Microbiol* **62**, 1287–1294.
- Engelhardt, M. A., Daly, K., Swannell, R. P. J. & Head, I. M. (2001). Isolation and characterization of a novel hydrocarbon-degrading, Gram-positive bacterium, isolated from intertidal sediment, and description of *Planococcus alkanoclasticus* sp. nov. *J Appl Microbiol* **90**, 237–247.
- Felsenstein, J. (1993). PHYLIP (phylogenetic inference package), version 3.5c. Department of Genetics, University of Washington, Seattle, WA, USA.
- Junge, K., Gosink, J. J., Hoppe, H.-G. & Staley, J. T. (1998). *Arthrobacter*, *Brachybacterium* and *Planococcus* isolates identified from Antarctic sea ice brine. Description of *Planococcus mcmeekinii* sp. nov. *Syst Appl Microbiol* **21**, 306–314.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Lanyi, B. (1987). Classical and rapid identification methods for medically important bacteria. *Methods Microbiol* **19**, 1–67.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Mayilraj, S., Prasad, G. S., Suresh, K., Saini, H. S., Shivaji, S. & Chakrabarti, T. (2005). *Planococcus stackebrandtii* sp. nov., isolated from a cold desert of the Himalayas, India. *Int J Syst Evol Microbiol* **55**, 91–94.
- Nakagawa, N., Sakane, T. & Yokota, A. (1996). Emendation of the genus *Planococcus* and transfer of *Flavobacterium okeanokoites* Zobell and Upham 1944 to the genus *Planococcus* as *Planococcus okeanokoites* comb. nov. *Int J Syst Bacteriol* **46**, 866–870.
- Reddy, G. S. N., Prakash, J. S. S., Vairamani, M., Prabhakar, S., Matsumoto, G. I. & Shivaji, S. (2002). *Planococcus antarcticus* and *Planococcus psychrophilus* spp. nov. isolated from cyanobacterial mat samples collected from ponds in Antarctica. *Extremophiles* **6**, 253–261.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Wu, C., Lu, X., Qin, M., Wang, Y. & Ruan, J. (1989). Analysis of menaquinone compound in microbial cells by HPLC. *Microbiology* (English translation of *Mikrobiologiya*) **16**, 176–178.
- Yoon, J.-H., Kang, S.-S., Lee, K.-C., Lee, E.-S., Kho, Y. H., Kang, K. E. & Park, Y.-H. (2001). *Planomicrobium koreense* gen. nov., sp. nov., a bacterium isolated from the Korean traditional fermented seafood jeotgal, and transfer of *Planococcus okeanokoites* (Nakagawa *et al.* 1996) and *Planococcus mcmeekinii* (Junge *et al.* 1998) to the genus *Planomicrobium*. *Int J Syst Evol Microbiol* **51**, 1511–1520.
- Yoon, J.-H., Weiss, N., Kang, K.-H., Oh, T.-K. & Park, Y.-H. (2003). *Planococcus maritimus* sp. nov., isolated from sea water of a tidal flat in Korea. *Int J Syst Evol Microbiol* **53**, 2013–2017.
- Zhang, D., Yang, H., Zhang, W., Huang, Z. & Liu, S.-J. (2003). *Rhodocista pekingensis* sp. nov., a cyst-forming phototrophic bacterium from a municipal wastewater treatment plant. *Int J Syst Evol Microbiol* **53**, 1111–1114.