Chryseomicrobium imtechense gen. nov., sp. nov., a new member of the family Planococcaceae

Pankaj Kumar Arora, Archana Chauhan, Bhawana Pant, Suresh Korpole,
Shanmugam Mayilraj and Rakesh Kumar Jaint†

Correspondence

Pankaj Kumar Arora
arora484@gmail.com

Environmental Biotechnology, Microbial Type Culture Collection and Gene Bank (MTCC), Institute
of Microbial Technology (CSIR), Sector-39A, Chandigarh – 160036, India

A Gram-stain-positive, rod-shaped, yellow, non-motile, non-spore-forming, strictly aerobic bacterial strain, designated MW 10^T, was isolated from seawater of the Bay of Bengal, India, and was subjected to a polyphasic taxonomic study. Analysis of the 16S rRNA gene sequence revealed that strain MW 10^T showed highest similarity to the type strains of *Psychrobacillus psychrodurans* (96.15 %) and *Psychrobacillus psychrotolerans* (96.01 %) and showed less than 96 % similarity to members of the genera *Paenisporosarcina*, *Planococcus*, *Sporosarcina* and *Planomicrobium*. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain MW 10^T formed a clade separate from members of closely related genera. The morphological, physiological and chemotaxonomic characteristics of strain MW 10^T differed from those of members of closely related genera. The major fatty acid in strain MW 10^T was iso-C_{15:0} and the menaquinones were MK-7 (48.4 %), MK-8 (32.3 %), MK-7(H₂) (13.7 %) and MK-6 (5.6 %). The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unknown phospholipid, an unknown lipid and an unknown glycolipid. The cell-wall peptidoglycan type was L-Lys–D-Asp. The genomic DNA G+C content (53.4 mol%) of strain MW 10^T was significantly different from those of members of closely related genera. On the basis of its morphological, physiological and chemotaxonomic characteristics as well as our phylogenetic analysis, we conclude that strain MW 10^T is a member of a novel genus and species, for which the name *Chryseomicrobium imtechense* gen. nov., sp. nov., is proposed. The type strain of *Chryseomicrobium imtechense* is MW 10^T (=MTCC 10098^T =JCM 16573^T).

In the course of a study of the microbial diversity of the Bay of Bengal, India, a Gram-stain-positive, aerobic, yellow, rod-shaped, non-motile, non-spore-forming bacterium, MW 10^T, was isolated from a seawater sample collected from Sompeta district, Andhra Pradesh, at a depth of 30 m, and characterized by polyphasic approach. Strain MW 10^T was isolated on tryptone soy agar (TSA) by a dilution plating method and the plate was incubated at 30 °C for 48 h. Pure cultures were maintained as glycerol stocks at –70 °C.

Colony morphology was examined following growth of the strain on TSA at 30 °C for 48 h. Cell morphology was investigated by light microscopy (Zeiss) at ×1000 magnification. Motility was checked using the method

described by Skerman (1967). The Gram reaction was determined by using the HiMedia Gram staining kit according to the manufacturer's instructions. Spore formation was examined by heat treatment in a water bath at 80 °C for 10 min as well as by phase-contrast microscopy at the end of growth of strain MW 10^T on different sporulating media. Growth at 4, 10, 15, 20, 25, 30, 35, 40 and 45 °C was determined on TSA (pH 7.3). Growth at different pH was tested using tryptone soy broth (TSB) as growth medium adjusted to pH 4.5–9.5 (at intervals of 0.5 pH units) with acetate or citrate buffer (pH 4–6), 100 mM NaH₂PO₄/Na₂HPO₄ (pH 7–8), 100 mM NaHCO₃/Na₂CO₃ (pH 9–10) or 100 mM Na₂CO₃/NaOH (pH 11–12). The pH was adjusted prior to autoclaving. After autoclaving, the pH was rechecked and recorded as the pH of growth medium. Growth in the presence of 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 10 (w/v) NaCl was investigated in TSB. Oxidase and catalase activities were determined using oxidase discs (HiMedia) and 3 % (v/v) H₂O₂, respectively, according to methods described by Smibert & Krieg (1994). Hydrolysis of casein, gelatin, starch and egg yolk was tested on skimmed-milk

†Deceased.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MW 10^T is GQ927308.

Four supplementary figures are available with the online version of this paper.

agar, gelatin agar, starch agar and egg yolk agar, respectively. Biochemical tests such as the methyl red test and Voges–Proskauer reaction, H₂S and indole production, citrate utilization and nitrate reduction were performed according to standard procedures (Cowan & Steel, 1965). Activity of β -galactosidase was determined using *o*-nitrophenyl β -D-galactopyranoside (ONPG) discs (HiMedia). Acid production from various carbohydrates was tested as described by Claus & Berkeley (1986).

Oxidation of various substrates as sole carbon, nitrogen and energy sources was tested using the Biolog GP2 MicroPlate in accordance with the manufacturer's instructions, except that TSB was used instead of Biolog universal growth agar. The microplate, inoculated with strain MW 10^T, was incubated for 48 h and the results were read with a MicroPlate Reader using Microlog 4.2 computer software for automated reading.

For analysis of menaquinones, polar lipids and peptidoglycan, freeze-dried cells were prepared following growth of strain MW 10^T in TSB for 48 h at 30 °C. Polar lipids were extracted following the method of Bligh & Dyer (1959) and analysed by two-dimensional TLC followed by spraying with appropriate detection reagents (Komagata & Suzuki, 1987). Menaquinones were extracted by the method of Minnikin *et al.* (1984) and identified by HPLC. The peptidoglycan structure was determined by using a hydrolysate of purified cell walls, according to Schleifer & Kandler (1972). For analysis of cellular fatty acids, strain MW 10^T and a number of reference strains were grown on TSA plates at 30 °C for 48 h; fatty acid methyl esters were extracted and analysed as described by Pandey *et al.* (2002) using the Microbial Identification System (MIDI). The G+C content of genomic DNA was determined spectrophotometrically (Lambda 35; Perkin Elmer) using the thermal denaturation method (Mandel & Marmur, 1968).

The 16S rRNA gene was amplified by PCR using the universal primers 8-27F (5'-AGAGTTGATCCTGGCT-CAG-3') and 1492R (5'-TACGGYTACCTTGTTACGAC-TT-3'). Amplification and purification of the product was done as described by Pandey *et al.* (2002). The amplified 16S rRNA gene was sequenced by the dideoxy chain terminator method using a Big Dye terminator kit followed by capillary electrophoresis on an ABI 310 Genetic Analyzer (Applied Biosystems). The primers used for sequencing were 27F, 357F (5'-CTCCTACGGGAGGC-AGCAG-3'), 518R (5'-ATTACCGCGGCTGCTGG-3'), 704F (5'-GTAGCGGTGAAATGCGTAGA-3'), 1100R (5'-GGGT-TGCGCTCGTTG-3') and 1492R. The 16S rRNA gene sequences of closely related strains were retrieved from the GenBank database using BLASTN (Altschul *et al.*, 1997). Sequences were aligned with CLUSTAL W 1.8 (Thompson *et al.*, 1994). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Rzhetsky & Nei, 1992) and maximum-parsimony (Fitch, 1971) methods with the MEGA 4 program package

(Tamura *et al.*, 2007). Evolutionary distances were calculated according to the algorithm of Kimura's two-parameter model (Kimura, 1980) for the neighbour-joining method.

Strain MW 10^T formed yellow colonies and cells were Gram-stain-positive, aerobic, non-motile rods. Spores were never observed under any of the tested growth conditions. Spore staining of cells grown on sporulating agar showed no spore formation in strain MW 10^T (Supplementary Fig. S1, available in IJSEM Online). Furthermore, strain MW 10^T could not survive heat treatment at 80 °C for 10 min, confirming the absence of spore formation. Morphological, physiological, biochemical and chemotaxonomic characteristics of strain MW 10^T are given in genus and species descriptions.

The 16S rRNA gene sequence (1430 nt) of strain MW 10^T was compared with sequences of closely related type strains retrieved from the GenBank database. Strain MW 10^T showed highest 16S rRNA gene sequence similarity to type strains of the genus *Psychrobacillus* (96.15 % similarity with *Psychrobacillus psychrodurans* DSM 11713^T, 96.01 % with *Psychrobacillus psychrotolerans* DSM 11706^T and 95.24 % with *Psychrobacillus insolitus* DSM 5^T). Strain MW 10^T showed less than 96 % similarity to members of the genera *Paenisporosarcina*, *Planomicrobium*, *Planococcus* and *Sporosarcina*. Strain MW 10^T also differed from closely related genera in its phenotypic and genotypic characteristics (Table 1). Strain MW 10^T differed from all members of the genera *Psychrobacillus*, *Paenisporosarcina* and *Sporosarcina* in the absence of endospores and also differed from members of the genera *Planomicrobium* and *Planococcus* in several other phenotypic characteristics, as shown in Table 1.

Comparison of the cellular fatty acid profiles of strain MW 10^T and species of the related genera *Psychrobacillus*, *Paenisporosarcina*, *Sporosarcina*, *Planococcus* and *Planomicrobium* showed significant differences. The major fatty acid of strain MW 10^T was iso-C_{15:0}, which made up 68.57 % of the total fatty acids. Significant amounts of this fatty acid (>10 %) were present in type strains of the related genera *Psychrobacillus* and *Paenisporosarcina*; however, type strains of the genera *Sporosarcina*, *Planococcus* and *Planomicrobium* contained smaller amounts (<10 % or even trace amounts) of iso-C_{15:0} (Table 2). anteiso-C_{15:0} was present either in significant amounts (>22 %) or as a major fatty acid (26–62 %) in type strains of the genera *Psychrobacillus*, *Paenisporosarcina*, *Sporosarcina*, *Planomicrobium* and *Planococcus*, whereas only a small amount of anteiso-C_{15:0} (2.52 %) was observed in strain MW 10^T.

Analysis of polar lipids using two-dimensional TLC revealed that the polar lipid profile of strain MW 10^T included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unknown phospholipid, an unknown lipid and an unknown glycolipid (Supplementary Fig. S2). Diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine were also reported as major polar lipids in members of the genera

Table 1. Characteristics that distinguish strain MW 10^T from closely related genera

Data for reference genera were taken from Krishnamurthi *et al.* (2010) (*Psychrobacillus*), Krishnamurthi *et al.* (2009) (*Paenisporosarcina* and *Sporosarcina*), Kwon *et al.* (2007) (*Sporosarcina*), Yoon *et al.* (2010) and Nakagawa *et al.* (1996) (*Planococcus*) and Yoon *et al.* (2001) and Zhang *et al.* (2009) (*Planomicrobium*).

Characteristic	Strain MW 10 ^T	<i>Psychrobacillus</i>	<i>Paenisporosarcina</i>	<i>Sporosarcina</i>	<i>Planococcus</i>	<i>Planomicrobium</i>
Cell shape	Rod	Rod/coccus	Rod/coccus	Rod/coccus	Rod/coccus	Rod/coccus
Endospore formation	No	Yes	Yes	Yes	No	No
Menaquinone(s)	7, 8, 7(H ₂), 6	8, 7, 6, 9	7, 8	7	8, 7, 6*, 7(H ₂)*	8, 7, 6*
Polar lipids†	DPG, PG, PE, PC, PL, UL, GL	DPG, PG, PE, 2PL, 4UL	DPG, PG, PE, APL, AL, UL	DPG, PG, PE, APL, 3UL	DPG, PG, PE	DPG, PG, PE
Fatty acids‡						
>35 %	i-C _{15:0}	ai-C _{15:0}	i-C _{15:0} ^a /ai-C _{15:0} ^b §	ai-C _{15:0} and/or i-C _{15:0}	ai-C _{15:0}	ai-C _{15:0} /(i-C _{14:0}) ^c
20–34.9 %	ND	ND	ai-C _{15:0} ^a /C _{16:1} ω7c alc ^b	ND	ND	(i-C _{16:0}) ^c
5–19.9 %	C _{16:1} ω7c alc, i-C _{16:0}	i-C _{15:0} , i-C _{14:0} , (C _{16:1} ω7c alc, i-C _{16:0} , C _{16:1} ω11c, C _{16:0} , ai-C _{17:0})*	C _{16:1} ω7c alc ^a /i-C _{15:0} ^b	(ai-C _{17:0} , i-C _{15:0} , i-C _{14:0})*	(i-C _{16:0} , i-C _{14:0} , C _{15:0} , ai-C _{17:0} , C _{16:1} ω7c alc)*	i-C _{14:0} , C _{16:1} ω7c alc, i-C _{16:0} /(ai-C _{15:0} , C _{16:1} ω7c alc) ^c
Peptidoglycan type(s)	L-Lys–D-Asp	L-Orn–D-Glu	L-Lys–D-Asp	L-Lys–L-Ala–D-Asp, L-Lys–Gly–D-Glu, L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Asp, L-Lys–D-Glu
DNA G + C content (mol%)	53.4	35.7–36.6	46.0	40.0–42.0	40.0–51.2	35.0–47.0

*Component present in some members of the taxon.

†DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PL, unknown phospholipid; UL, unknown lipid; GL, unknown glycolipid; APL, unknown aminophospholipid; AL, unknown aminolipid.

‡Classified by proportion of total fatty acids; ND, none detected. ai, Anteiso-branched; alc, alcohol; i, iso-branched.

§Fatty acids present in: *a*, *Paenisporosarcina quisquiliarum* MTCC 7604^T; *b*, *Paenisporosarcina macmurdoensis* MTCC 4670^T; *c*, *Planomicrobium okeanokoites* NBRC 12536^T.

Psychrobacillus, *Paenisporosarcina*, *Sporosarcina*, *Planococcus* and *Planomicrobium* (Krishnamurthi *et al.*, 2009, 2010; Mayilraj *et al.*, 2005; Romano *et al.*, 2003; Suresh *et al.*, 2007; Yoon *et al.*, 2001, 2010; Yu *et al.*, 2008). However, phosphatidylcholine, detected in strain MW 10^T, was not found in closely related genera.

The menaquinone profile of strain MW 10^T consisted of MK-7 (48.4 %), MK-8 (32.3 %), MK-7(H₂) (13.7 %) and MK-6 (5.6 %). MK-7 and MK-8 were reported as predominant menaquinones in members of the genera *Psychrobacillus*, *Planococcus*, *Planomicrobium* and *Paenisporosarcina* (Alam *et al.*, 2003; Choi *et al.*, 2007; Dai *et al.*, 2005; Jung *et al.*, 2009; Krishnamurthi *et al.*, 2009; Zhang *et al.*, 2009) whereas, in members of the genus *Sporosarcina*, MK-7 was reported as the major menaquinone (Reddy *et al.*, 2003; Yu *et al.*, 2008). MK-6 was also present in minor amounts in some members of the genera *Planomicrobium* and *Planococcus* (Yoon *et al.*, 2001, 2010). MK-7(H₂) was reported in *Planococcus columbae* (Suresh *et al.*, 2007); however, MK-7(H₂) was not present in the genera *Psychrobacillus*, *Paenisporosarcina*, *Sporosarcina* or *Planomicrobium*.

Strain MW 10^T had cell-wall peptidoglycan type A4α (L-Lys–D-Asp), which was also reported in species of the genus *Paenisporosarcina* (Krishnamurthi *et al.*, 2009) and a few species of the genus *Planomicrobium* (Yoon *et al.*, 2001). However, the peptidoglycan type of strain MW 10^T differed from those of members of the genera *Psychrobacillus*, *Sporosarcina* and *Planococcus*.

The DNA G + C content of strain MW 10^T was 53.4 mol%. This value is significantly higher than those reported for members of the genera *Paenisporosarcina*, *Sporosarcina*, *Planomicrobium* and *Planococcus* (Table 1).

Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain MW 10^T fell within the family *Planococcaceae* and formed a clade separate from species of the genera *Planomicrobium* and *Planococcus* (Fig. 1). Strain MW 10^T was separated phylogenetically from all closely related genera of the family *Planococcaceae* (Fig. 1 and Supplementary Figs S3 and S4).

On the basis of the phenotypic characteristics and phylogenetic analysis, strain MW 10^T cannot be assigned to any recognized bacterial genus in the family

Table 2. Comparison of the fatty acid profiles of strain MW 10^T and type strains of related species

Strains: 1, strain MW 10^T; 2, *Psychrobacillus psychrotolerans* DSM 11706^T; 3, *Psychrobacillus psychrodurans* DSM 11713^T; 4, *Psychrobacillus insolitus* DSM 5^T; 5, *Paenisporosarcina quisquiliarium* MTCC 7604^T; 6, *Paenisporosarcina macmurdoensis* MTCC 4670^T; 7, *Sporosarcina ureae* DSM 2281^T (data in columns 1–7 from the present study); 8, *Planococcus citreus* DSM 20549^T (data from Yoon *et al.*, 2001); 9, *Planomicrobium koreense* JCM 10704^T (Zhang *et al.*, 2009). Major differences are highlighted in bold. ND, Not detected/not reported; tr, trace.

Fatty acid	1	2	3	4	5	6	7	8	9
Saturated fatty acids									
C _{14:0}	ND	0.78	0.84	ND	0.90	0.61	0.99	ND	ND
C _{15:0}	ND	ND	ND	ND	ND	ND	ND	6.7	ND
C _{16:0}	1.51	0.81	3.48	11.91	1.98	ND	2.57	ND	2.8
C _{17:0}	ND	ND	ND	ND	ND	ND	ND	2.3	ND
C _{18:0}	ND	ND	1.93	ND	1.19	2.57	ND	ND	ND
Unsaturated fatty acids									
C _{16:1} ω7c alcohol	10.88	8.67	4.54	8.92	9.47	20.29	3.26	9.8	9.6
C _{16:1} ω11c	1.86	0.75	0.93	11.61	1.87	1.48	4.37	2.5	4.0
Branched fatty acids									
iso-C _{14:0}	2.81	12.45	5.05	12.97	4.52	0.61	3.58	8.8	16.2
iso-C _{15:0}	68.57	13.62	10.52	9.17	41.45	10.38	7.32	ND	6.3
anteiso-C _{15:0}	2.52	51.60	48.09	35.60	22.65	38.85	61.81	52.1	43.6
iso-C _{16:0}	6.42	4.23	8.38	9.80	3.28	2.66	1.36	8.1	6.8
iso-C _{17:0}	3.00	ND	ND	ND	1.29	ND	ND	ND	tr
anteiso-C _{17:0}	ND	3.01	13.48	ND	2.56	1.09	8.67	5.2	2.9
iso-C _{17:1} ω10c	2.43	ND	ND	ND	2.52	ND	0.86	ND	ND
iso-C _{18:0}	ND	0.40	ND	ND	ND	ND	ND	ND	ND
Summed feature 4*	ND	2.85	2.81	ND	5.92	7.98	4.40	4.5	ND

*Summed feature 4 represents iso-C_{17:1} I and/or anteiso-C_{17:1} B, which could not be separated by GLC with the MIDI system.

Planococcaceae. Therefore, we propose a new genus and species, *Chryseomicrobium imtechense* gen. nov., sp. nov., to accommodate strain MW 10^T.

Description of *Chryseomicrobium* gen. nov.

Chryseomicrobium (Chry'se.o.mi.cro'bi.um. Gr. adj. *chru-seos* golden; N.L. neut. n. *microbium* microbe from Gr. adj. *mikros* small and Gr. n. *bios* life; N.L. neut. n. *Chryseomicrobium* yellow microbe).

Cells are Gram-stain-positive, aerobic, non-motile, non-spore-forming rods. Catalase-positive and oxidase-negative. The major fatty acid is iso-C_{15:0}. The menaquinones are MK-7, MK-8, MK-7(H₂) and MK-6. The cell-wall peptidoglycan is of the A4α type with an interpeptide bridge L-Lys-D-Asp. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, an unknown phospholipid, an unknown lipid and an unknown glycolipid. The genomic DNA G+C content of the type strain of the type species is 53.4 mol%. The type species is *Chryseomicrobium imtechense*.

Description of *Chryseomicrobium imtechense* sp. nov.

Chryseomicrobium imtechense [im.tech.en'se. N.L. neut. adj. *imtechense* pertaining to the Institute of Microbial

Technology (IMTECH), where the type strain was characterized].

Displays the following properties in addition to those described for the genus. Forms yellow colonies. Cells are 0.3–0.7 µm wide and 1.7–2.9 µm long. Grows at 4–45 °C, pH 6–9 and 0–6 % NaCl. Casein is hydrolysed but gelatin, starch and aesculin are not. Egg yolk reaction and oxidase test are negative. H₂S production, indole formation and urease test are negative. Methyl red test, Voges–Proskauer test and oxidation/fermentation test are negative. Does not hydrolyse ONPG. No growth on MacConkey or Simmons' citrate agar. Nitrate is not reduced to nitrite. Acid is produced from glucose, salicin, fructose, maltose, sucrose, inulin, melibiose and cellobiose. In the Biolog GP2 system, positive for oxidation of α-ketovaleric acid, glycerol, γ-hydroxybutyric acid, pyruvic acid, L-serine, D-ribose, pyruvic acid methyl ester, L-alanine, 2-deoxyadenosine and L-arabinose and negative for oxidation of α- and β-cyclodextrin, glycogen, inulin, mannan, lactose, methyl β-D-glucoside, L-alaninamide, trehalose, D-lactic acid methyl ester, D-alanine, arbutin, L-lactic acid, xylitol, D- and L-malic acid, raffinose, L-asparagine, L-fucose, L-rhamnose, adenosine 5'-monophosphate, melezitose, α-hydroxybutyric acid, glycyl L-glutamic acid, Tweens 40 and 80, D-galacturonic acid, melibiose, salicin, propionic acid, L-pyroglutamic acid, uridine 5'-monophosphate, gentiobiose, methyl α-D-galactoside, sedoheptulosan, D-fructose 6-phosphate, N-acetyl-β-glucosamine,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain MW 10^T and related taxa. Bootstrap values (expressed as percentages of 1000 replications) >70 % are shown at branch points. Bar, 0.005 substitutions per nucleotide position.

D-gluconic acid, methyl β -D-galactoside, D-sorbitol, *p*-hydroxyphenylacetic acid, succinamic acid, putrescine, α -D-glucose 1-phosphate, amygdalin, *myo*-inositol, methyl α -D-glucoside, sucrose, *N*-acetyl L-glutamic acid and DL- α -glycerol phosphate. The cellular fatty acid profile includes C_{16:0}, C_{16:1} ω 7c alcohol, C_{16:1} ω 11c, iso-C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0} and iso-C_{17:1} ω 10c.

The type strain, MW 10^T (=MTCC 10098^T =JCM 16573^T), was isolated from a seawater sample collected from the Bay of Bengal, India.

Acknowledgements

The authors are grateful to the Council of Scientific and Industrial Research (CSIR), Government of India, for financial support.

References

- Alam, S. I., Singh, L., Dube, S., Reddy, G. S. N. & Shivaji, S. (2003). Psychrophilic *Planococcus maitriensis* sp. nov. from Antarctica. *Syst Appl Microbiol* **26**, 505–510.
- Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.

Choi, J.-H., Im, W.-T., Liu, Q.-M., Yoo, J.-S., Shin, J.-H., Rhee, S.-K. & Roh, D.-H. (2007). *Planococcus donghaensis* sp. nov., a starch-degrading bacterium isolated from the East Sea, South Korea. *Int J Syst Evol Microbiol* **57**, 2645–2650.

Claus, D. & Berkeley, R. C. W. (1986). Genus *Bacillus* Cohn 1872, 174^{AL}. In *Bergey's Manual of Systematic Bacteriology*, vol. 2, pp. 105–1139. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins.

Cowan, S. T. & Steel, K. J. (1965). *Manual for the Identification of Medical Bacteria*. London: Cambridge University Press.

Dai, X., Wang, Y.-N., Wang, B.-J., Liu, S.-J. & Zhou, Y.-G. (2005). *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. *Int J Syst Evol Microbiol* **55**, 699–702.

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Jung, Y.-T., Kang, S.-J., Oh, T.-K., Yoon, J.-H. & Kim, B.-H. (2009). *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. *Int J Syst Evol Microbiol* **59**, 2929–2933.

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.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

- Krishnamurthi, S., Bhattacharya, A., Mayilraj, S., Saha, P., Schumann, P. & Chakrabarti, T. (2009). Description of *Paenisporosarcina quisquiliarum* gen. nov., sp. nov., and reclassification of *Sporosarcina macmurdoensis* Reddy *et al.* 2003 as *Paenisporosarcina macmurdoensis* comb. nov. *Int J Syst Evol Microbiol* **59**, 1364–1370.
- Krishnamurthi, S., Ruckmani, A., Pukall, R. & Chakrabarti, T. (2010). *Psychrobacillus* gen. nov. and proposal for reclassification of *Bacillus insolitus* Larkin & Stokes, 1967, *B. psychrotolerans* Abd-El Rahman *et al.*, 2002 and *B. psychrodurans* Abd-El Rahman *et al.*, 2002 as *Psychrobacillus insolitus* comb. nov., *Psychrobacillus psychrotolerans* comb. nov. and *Psychrobacillus psychrodurans* comb. nov. *Syst Appl Microbiol* **33**, 367–373.
- Kwon, S.-W., Kim, B.-Y., Song, J., Weon, H.-Y., Schumann, P., Tindall, B. J., Stackebrandt, E. & Fritze, D. (2007). *Sporosarcina koreensis* sp. nov. and *Sporosarcina soli* sp. nov., isolated from soil in Korea. *Int J Syst Evol Microbiol* **57**, 1694–1698.
- Mandel, M. & Marmur, J. (1968). Use of ultraviolet absorbance-temperature profile for determining the guanine plus cytosine content of DNA. *Methods Enzymol* **12B**, 195–206.
- 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.
- Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.
- Nakagawa, Y., 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.
- Pandey, K. K., Mayilraj, S. & Chakrabarti, T. (2002). *Pseudomonas indica* sp. nov., a novel butane-utilizing species. *Int J Syst Evol Microbiol* **52**, 1559–1567.
- Reddy, G. S. N., Matsumoto, G. I. & Shivaji, S. (2003). *Sporosarcina macmurdoensis* sp. nov., from a cyanobacterial mat sample from a pond in the McMurdo Dry Valleys, Antarctica. *Int J Syst Evol Microbiol* **53**, 1363–1367.
- Romano, I., Giordano, A., Lama, L., Nicolaus, B. & Gambacorta, A. (2003). *Planococcus rifietensis* sp. nov., isolated from algal mat collected from a sulfurous spring in Campania (Italy). *Syst Appl Microbiol* **26**, 357–366.
- Rzhetsky, A. & Nei, M. (1992). A simple method for estimating and testing minimum evolution trees. *Mol Biol Evol* **9**, 945–967.
- 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.
- Skerman, V. B. D. (1967). *A Guide to the Identification of the Genera of Bacteria*, 2nd edn. Baltimore: Williams & Wilkins.
- Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerhardt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for Microbiology.
- Suresh, K., Mayilraj, S., Bhattacharya, A. & Chakrabarti, T. (2007). *Planococcus columbae* sp. nov., isolated from pigeon faeces. *Int J Syst Evol Microbiol* **57**, 1266–1271.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.
- Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
- Yoon, J.-H., Kang, S.-S., Lee, K.-C., Lee, E. S., Kho, Y. H., Kang, K. H. & 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., Kang, S.-J., Lee, S.-Y., Oh, K.-H. & Oh, T.-K. (2010). *Planococcus salinarum* sp. nov., isolated from a marine solar saltern, and emended description of the genus *Planococcus*. *Int J Syst Evol Microbiol* **60**, 754–758.
- Yu, Y., Xin, Y.-H., Liu, H.-C., Chen, B., Sheng, J., Chi, Z.-M., Zhou, P.-J. & Zhang, D.-C. (2008). *Sporosarcina antarctica* sp. nov., a psychrophilic bacterium isolated from the Antarctic. *Int J Syst Evol Microbiol* **58**, 2114–2117.
- Zhang, D.-C., Liu, H.-C., Xin, Y.-H., Yu, Y., Zhou, P.-J. & Zhou, Y. G. (2009). *Planomicrobium glaciei* sp. nov., a psychrotolerant bacterium isolated from a glacier. *Int J Syst Evol Microbiol* **59**, 1387–1390.