Planococcus stackebrandtii sp. nov., isolated from a cold desert of the Himalayas, India

S. Mayilraj,¹ G. S. Prasad,¹ K. Suresh,² H. S. Saini,³ S. Shivaji² and T. Chakrabarti¹

Correspondence

T. Chakrabarti

tapan@imtech.res.in

¹Microbial Type Culture Collection & Gene Bank (MTCC), Institute of Microbial Technology, Chandigarh, 160 036, India

²Centre for Cellular and Molecular Biology (CCMB), Hyderabad, 500 007, India

³Guru Nanak Dev University (GNDU), Amritsar, 143 005, India

The taxonomic position of a bacterium isolated from a cold desert of the Himalayas, India, was analysed by using a polyphasic approach. The isolated strain, designated K22-03^T, had phenotypic characteristics that matched those of the genus *Planococcus* and it represents a novel species. The almost-complete 16S rRNA gene sequence (1464 bases) of the novel strain was compared with those of previously studied *Planococcus* type strains and confirmed that the strain belongs to the genus *Planococcus*. 16S rRNA gene sequence analysis indicated that strain K22-03^T differs from all other species of *Planococcus* by at least 2.5%. DNA–DNA hybridization showed that it had low genomic relatedness with *Planomicrobium mcmeekinii* (MTCC 3704^T, 23%), *Planococcus psychrophilus* (MTCC 3812^T, 61%), *Planococcus antarcticus* (MTCC 3854^T, 45%) and *Planomicrobium okeanokoites* (MTCC 3703^T, 51%), the four species with which it was most closely related based on 16S rRNA gene sequence analysis (97–97.5% similarity). Therefore, strain K22-03^T should be recognized as a novel species, for which the name *Planococcus stackebrandtii* sp. nov. is proposed. The type strain is K22-03^T (=MTCC 6226^T=DSM 16419^T=JCM 12481^T).

The genus *Planococcus* was first established over 100 years ago by Migula (1894) and was later emended by Nakagawa *et al.* (1996). At present the genus comprises eight recognized species, *Planococcus citreus* (Migula, 1894), *Planococcus kocurii* (Hao & Komagata, 1985), *Planococcus alkanoclasticus* (Engelhardt *et al.*, 2001), *Planococcus antarcticus* and *Planococcus psychrophilus* (Reddy *et al.*, 2002), *Planococcus maritimus* (Yoon *et al.*, 2003), *Planococcus rifetoensis* (Romano *et al.*, 2003) and *Planococcus maitriensis* (Alam *et al.*, 2003).

A bacterial strain, designated K22-03^T, was isolated from a cold desert of the Himalayas, northern India, using a dilution plating technique on trypticase soy agar (TSA; Himedia, India) and maintained as glycerol stocks at –70 °C. The reference strains of *Planomicrobium mcmeekinii* (MTCC 3704^T), *Planomicrobium okeanokoites* (MTCC 3703^T), *Planococcus psychrophilus* (MTCC 3812^T) and *Planococcus antarcticus* (MTCC 3854^T) were obtained

from the Microbial Type Culture Collection (MTCC), Chandigarh, India.

Strain K22-03^T and the three closely related organisms given above were grown on TSA and tested for morphological, physiological and chemotaxonomic characteristics. These four strains were also checked for various biochemical properties, such as reaction to oxidase and catalase, aerobic or anaerobic growth, and hydrolysis of Tween 20, starch, casein and gelatin. Whole-cell sugars and amino acids were determined by TLC as described by Stanek & Roberts (1974). Phospholipids and menaquinones were extracted and analysed using the methods described by Minnikin & Goodfellow (1976) and Minnikin *et al.* (1978). Fatty acid methyl esters were extracted and analysed as described by Pandey *et al.* (2002).

The chromosomal DNA of strain K22-03^T was isolated according to the procedure described by Rainey *et al.* (1996). The 16S rRNA gene was amplified with primers 8–27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1500r (5'-AGAAAGGAGGTGATCCAGGC-3'). The amplified DNA fragment was separated on a 1% agarose gel, eluted from the gel and purified using the Qiaquick gel extraction kit (Qiagen). The purified PCR product was sequenced with four forward and three reverse primers: 8–27f

Published online ahead of print on 13 August 2004 as DOI 10.1099/ijs.0.63290-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Planococcus stackebrandtii* strain K22-03^T is AY437845.

(5'-AGAGTTTGATCCTGGCTCAG-3'), 357f (5'-CTCC-TACGGGAGGCAGCAG-3'), 704f (5'-TAGCGGTGAAA-TGCGTAGA-3'), 1114f (5'-GCAACGAGCGCAACC-3'), 685r (5'-TCTACGCATTTACCGCTAC-3'), 1110r (5'-GGGTTGCGCTCGTTG-3') and 1500r (5'-GAAAGGAG-GTGATCCAGGC-3') (*Escherichia coli* numbering system). The rRNA gene sequence was determined by the dideoxy chain-termination method using the BigDye terminator kit and an ABI 310 Genetic Analyser (Applied Biosystems). DNA-DNA hybridization was performed by the membrane filter method (Tourova & Antonov, 1987) as described by Shivaji *et al.* (1992) and Reddy *et al.* (2000).

The 16S rRNA gene sequence of strain K22-03^T generated here (1464 bases) was aligned with those of other *Planococcus* and *Planomicrobium* species retrieved from the GenBank database. A sequence similarity search was performed on these using BLASTN (Altschul *et al.*, 1997). Sequences of closely related taxa were retrieved and aligned using the CLUSTAL_X program (Thompson *et al.*, 1997); the alignment was corrected manually. For the neighbour-joining analysis (Saitou & Nei, 1987), distances between sequences were calculated using Kimura's two-parameter model (Kimura, 1980). Bootstrap analysis was performed to

assess the confidence limits of the branching (Felsenstein, 1985).

Colonies grown on TSA were orange-coloured and about 1–2 mm in diameter, circular, smooth and convex. Biochemical and chemotaxonomic characteristics confirmed strain K22-03^T to be a member of the genus *Planococcus*. Test reactions are given in Table 1 together with those of three closely related taxa. Strain K22-03^T differs from these three closely related species in acid production from different sugars. The diagnostic cell-wall amino acid is L-lysine and the diagnostic cell-wall sugars are D-glucose and D-ribose. The fatty acid profile of strain K22-03^T is similar to those of the three closely related species except for the presence of C_{17:0} and C_{16:1ω11c} in K22-03^T (Table 2). The major menaquinones are MK-7 and MK-8 and the strain has a type II phospholipid pattern. The DNA G+C content of strain K22-03^T is 40 mol%. To determine the phylogenetic position of strain K22-03^T, the 16S rRNA gene sequence was compared with those of type strains of species of the genera *Planococcus* and *Planomicrobium* retrieved from the GenBank database. Sequence similarity of K22-03^T with the genera *Planococcus* and *Planomicrobium* ranges from 95.4 to 97.6 %. A rooted

Table 1. Comparison of the phenotypic characteristics of strain K22-03^T (1) with those of the type strains of *Planomicrobium okeanokoites* (2), *Planomicrobium mcmeekinii* (3) and *Planococcus psychrophilus* (4)

None of the four micro-organisms produces spores, hydrolyses starch or is acid-fast. They all produce acid from fructose and galactose, but not from rhamnose or salicin. They all produce an orange pigment. +, Positive; –, negative; w, weakly positive.

Tests	1	2	3	4
Gram's staining	+	+ /Variable	+	+
Cell shape	Cocci	Cocci/rods	Cocci/rods	Rods
Cell arrangement	Tetrads	Tetrads	Tetrads	Single
Growth temperature (°C)	15–30	20–37	0–37	0–30
Acid from glucose:	–	–	+	–
Xylose	–	–	+ w	–
Lactose	+	–	+ w	–
Arabinose	–	–	+	–
Mannitol	–	+	–	–
myo-Inositol	–	–	–	+
Raffinose	+	–	+	–
Sucrose	+	+	+	–
G+C content of DNA (mol%)	40	46.3	35	44.5
Catalase	+ w	+	+	+ w
Oxidase	–	+	–	+
Nitrate reduction	–	–	+	–
Growth in:				
7 % NaCl	+	–	+	+
8 % NaCl	–	–	+	+
Hydrolysis of casein	–	+	+	–
Hydrolysis of gelatin	+ w	+	+	–
Hydrolysis of Tween 20	+	–	–	+

Table 2. Comparison of the fatty acid profile (%) of strain K22-03^T (1) with those of the type strains of *Planomicrobium okeanoikoites* (2), *Planomicrobium mcmeekinii* (3) and *Planococcus psychrophilus* (4)

Fatty acid	1	2	3	4
Straight-chain fatty acids				
C _{15:0}	5.49	1.73	4.53	Absent
C _{16:0}	Absent	1.1	1.1	2.24
C _{17:0}	2.07	Absent	Absent	Absent
Branched fatty acids				
iso-C _{14:0}	4.67	14.56	6.18	5.33
iso-C _{15:0}	2.85	5.31	4.53	5.76
anteiso-C _{15:0}	49.83	30.44	49.46	53.51
iso-C _{16:0}	5.65	18.19	6.41	7.06
iso-C _{17:0}	2.84	3.50	Absent	Absent
anteiso-C _{17:0}	4.55	3.93	5.40	4.04
iso-C _{17:1} ω10c	1.74	Absent	4.33	3.38
iso-C _{18:0}	Absent	2.26	Absent	Absent
Unsaturated fatty acids				
C _{16:1} ω7c alcohol	8.53	15.94	12.51	10.05
C _{16:1} ω11c	1.74	Absent	Absent	Absent
Summed feature 4*	8.64	2.33	11.17	8.63

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

phylogenetic tree based on the neighbour-joining method is shown in Fig. 1. Strain K22-03^T formed a separate line among the genus *Planococcus*. Strain K22-03^T is moderately related to '*Planococcus psychrotoleratus*' (97.6%), *Planomicrobium okeanoikoites* (97.5%) *Planococcus psychrophilus* (97.4%) and *Planococcus antarcticus* (97.0%), and distantly related to *Planomicrobium mcmeekinii* (96.8%), *Planococcus maritimus* (96.6%), *Planococcus alkanoclasticus* (96.5%), *Planococcus maitriensis* (96.3%), *Planococcus*

rifietoensis (96.2%), *Planococcus citreus* (95.7%) and *Planococcus kocurii* (95.4%). Genomic relatedness, as shown by DNA–DNA hybridization, of strain K22-03^T with *Planococcus mcmeekinii* is 23 %, with *Planococcus psychrophilus* is 61.0 %, with *Planococcus antarcticus* is 45 % and with *Planomicrobium okeanoikoites* is 51.0 %. At the level of 3–4 % dissimilarity in 16S rRNA gene sequences, members of other species of the genus can be excluded from DNA–DNA hybridization studies (Stackebrandt & Goebel, 1994). This conclusion is confirmed by DNA dissimilarity values distinctly lower than 70 % (Wayne *et al.*, 1987) obtained from two members each of the genera *Planococcus* and *Planomicrobium*. On the basis of morphology, growth characteristics, biochemical and chemotaxonomic characteristics, molecular systematic studies including 16S rRNA gene sequence analysis, DNA G+C content and DNA–DNA hybridization analysis, strain K22-03^T is considered to represent a novel species of the genus *Planococcus*, for which the name *Planococcus stackebrandtii* sp. nov. is proposed.

Description of *Planococcus stackebrandtii* sp. nov.

Planococcus 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).

Cells are Gram-positive, aerobic, non-spore-forming, motile and coccoid, occurring in pairs and in groups. Colonies on TSA are orange, opaque, smooth, convex, circular, entire and 1–2 mm in diameter. Growth occurs at 15–30 °C (optimum 25 °C) and at pH 5.6–11.0. Can tolerate up to 7 % NaCl. Positive for citrate utilization, Tween 20 hydrolysis, gelatin hydrolysis, ornithine decarboxylase and catalase, but negative for oxidase, casein hydrolysis, starch hydrolysis, urease production, methyl red and Voges–Proskauer reaction, hydrogen sulfide production, nitrate

Fig. 1. Neighbour-joining tree based on 16S rRNA gene (1464 bases) sequences showing the phylogenetic relationship of *Planococcus stackebrandtii* strain K22-03^T with other related species of the genera *Planococcus* and *Planomicrobium*. Bootstrap values (expressed as percentage of 1000 replications) greater than 50 % are given at the nodes. Bar, 0.5 % sequence variation.

reduction, arginine dihydrolase and lysine decarboxylase. No gas is produced from glucose medium. Acid is produced from galactose, fructose, lactose, raffinose and sucrose, but not from glucose, arabinose, mannitol, xylose, *myo*-inositol, rhamnose or salicin. As sole nitrogen source, it is negative for histidine, phenylalanine, valine, cysteine, hydroxy proline, arginine and serine. Cell-wall peptidoglycan contains L-lysine; cell-wall sugars are D-glucose and D-ribose. MK-7 and MK-8 are the major menaquinones. DNA G+C content is 40 mol%. Predominant fatty acids are anteiso-C_{15:0} and C_{16:1}ω7c alcohol, with minor amounts of C_{15:0}, C_{17:0} and C_{16:1}ω11c. Phospholipid is of type II, which has the predominant polar lipids phosphatidylglycerol, diphosphatidylglycerol and phosphatidylethanolamine.

The type strain, K22-03^T (=MTCC 6226^T=DSM 16419^T=JCM 12481^T), was isolated from soil, 0–45 m below an ice glacier, at 4200 m elevation, Kibber village, Spiti valley, Himachal Pradesh, India.

Acknowledgements

We are grateful to Dr Sanjay Kumar, IHBT, Palampur, for his help in sample collection and Dr K. Ganesan for his help with DNA sequencing. We acknowledge Professor Erko Stackebrandt for critical reading of the manuscript. We would like to thank Mr Malkit Singh for his excellent technical assistance. Financial assistance from CSIR and DBT, Government of India, is acknowledged. This is IMTECH communication number 11/2004.

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., Schaffer, 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.
- 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 beach sediment, and description of *Planococcus alkanoclasticus* sp. nov. *J Appl Microbiol* **90**, 237–247.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Hao, M. V. & Komagata, K. (1985). A new species of *Planococcus*, *P. kocurii* isolated from fish, frozen foods, and fish curing brine. *J Gen Appl Microbiol* **31**, 441–455.
- 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.
- Migula, W. (1894). Über ein neues System der Bakterien. *Arb Bakteriell Inst Karlsruhe* **1**, 235–238.
- Minnikin, D. E. & Goodfellow, M. (1976). Lipid composition in the classification and identification of *Nocardia* and related taxa. In *The Biology of the Nocardaceae*, pp. 160–219. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.
- Minnikin, D. E., Collins, M. D. & Goodfellow, M. (1978). Menaquinone patterns in the classification of nocardioform and related bacteria. *Zentbl Bakteriell Parasitenkd Infektionskr U Hyg I Abt* **1 Suppl.** **6**, 85–90.
- 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.
- Rainey, F. A., Ward-Rainey, N., Kroppenstedt, R. M. & Stackebrandt, E. (1996). The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. *Int J Syst Bacteriol* **46**, 1088–1092.
- Reddy, G. S. N., Aggarwal, R. K., Matsumoto, G. I. & Shivaji, S. (2000). *Arthrobacter flavus* sp. nov., a psychrophilic bacterium isolated from a pond in McMurdo Dry Valley, Antarctica. *Int J Syst Evol Microbiol* **50**, 1553–1561.
- 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.
- 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.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shivaji, S., Ray, M. K., Rao, N. S., Saisree, L., Jagannadham, M. V., Kumar, G. S., Reddy, G. S. N. & Bhargava, P. M. (1992). *Sphingobacterium antarcticus* sp. nov., a psychrotrophic bacterium from the soils of Schirmacher Oasis, Antarctica. *Int J Syst Bacteriol* **42**, 102–106.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Staneck, J. L. & Roberts, G. D. (1974). Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. *Appl Microbiol* **28**, 226–231.
- 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.
- Tourova, T. P. & Antonov, A. S. (1987). Identification of microorganisms by rapid DNA-DNA hybridization. *Methods Microbiol* **19**, 333–355.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- 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.