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G.S.N. Reddy · J.S.S. Prakash · M. Vairamani · S. Prabhakar
G.I. Matsumoto · S. Shivaji

Planococcus antarcticus and *Planococcus psychrophilus* spp. nov. isolated from cyanobacterial mat samples collected from ponds in Antarctica

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Abstract Thirteen orange-pigmented bacteria associated with cyanobacterial mat samples collected from four different lakes in McMurdo, Antarctica, were isolated. Twelve of the isolates, which were coccoid in shape, were very similar and possessed all the characteristics of the genus *Planococcus* and represented a new species, which was assigned the name *Planococcus antarcticus* sp. nov. (CMS 26or^T). Apart from the phenotypic differences, *P. antarcticus* differed from all reported species of *Planococcus* by more than 2.5% at the 16S rRNA gene sequence level. In addition, at the DNA–DNA hybridization level, it exhibited very little similarity either with *P. mcmeekinii* (30%–35%), *P. okeanokoites* (26%–29%), or CMS 53or^T (15%–25%), the three species with which it is closely related at the rRNA gene sequence level (2.5%–2.9%). *P. antarcticus* also showed only 2.5% difference in its 16S rRNA gene sequence compared with the *P. alkanoclasticus* sequence. But it was distinctly different from *P. alkanoclasticus*, which exists only as rods, is mesophilic and phosphatase positive, can hydrolyze starch, cannot utilize succinate, glutamate, or glucose, and cannot acidify glucose. Most important, *P. antarcticus* and *P. alkanoclasticus* varied distinctly in their fatty acid composition in that $C_{15:0}$, $C_{15:1}$, $C_{16:0}$, iso-$C_{16:1}$, and $C_{17:0}$ were present only in *P. antarcticus* but absent in *P. alkanoclasticus*. CMS 53or^T, the thirteenth isolate, was also identified as a new species of *Planococcus* and was assigned the name *Planococcus psychrophilus* sp. nov. This species was distinctly different from all the reported species, including the new species *P. antarcticus*, with respect to a number of phenotypic characteristics. At the 16S rRNA gene sequence level, it was closely related to *P. okeanokoites* (98.1%) and *P. mcmeekinii* (98%), but with respect to the DNA–DNA hybridization, the similarity was only 35%–36%. The type strain of *P. antarcticus* is CMS 26or^T (MTCC 3854; DSM 14505), and that of *P. psychrophilus* is CMS 530r^T (MTCC 3812; DSM 14507).

Key words *Planococcus* · Psychrophile · Antarctica · Halotolerant · Orange pigment · Cyanobacterial mat

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G.S.N. Reddy · J.S.S. Prakash · S. Shivaji (✉)
Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India
Tel. +91-40-7192504; Fax +91-40-7160591
e-mail: Shivas@gene.ccmbindia.org

M. Vairamani · S. Prabhakar
Indian Institute of Chemical Technology, Hyderabad, India

G.I. Matsumoto
Department of Environmental and Information Science, Otsuma Women's University, Tokyo, Japan

Introduction

The continent of Antarctica is almost totally covered by a thick ice sheet, except for a small portion (<2% of the total area), which is ice free. These ice-free areas are considered the coldest and driest deserts on Earth and do not support the growth of vascular plants, but mosses, lichens, algae, fungi, cyanobacteria, and bacteria constitute the predominant biomass. The McMurdo Dry Valleys form the largest ice-free area (2,500 km^2) in Antarctica, and many lakes and ponds are distributed throughout the region. These water bodies are permanently ice-covered (3–5 m thick), small (<200 m diameter), and shallow (<3 m) (Matsumoto 1993). Many aspects of the McMurdo Dry Valleys lakes, such as the general features, major ionic components, nutrient load, organic constituents, and physicochemical properties, (Matsumoto 1993) have been studied. In addition, it has been observed that these perennial Antarctic ice lakes support a complex assemblage of mat-forming cyanobacteria (Parker and Wharton 1985; Vincent et al. 1993). Associated with these mats is a large biomass of heterotrophic bacteria, which contributes to the dynamics of the nutrient cycle in the lake ecosystems. However, very few of these cyanobacterial mat-associated heterotrophic bacteria have been identified (Reddy et al. 2000). In this study, 13 orange-pigmented bacteria were isolated from four different cyanobacterial mat samples from four lakes in the McMurdo Dry Valleys, Antarctica, and studied in detail

with respect to their phenotypic characteristics and their phylogenetic relationships. On the basis of these studies, the 13 isolates could be divided into four distinct Groups (I to IV). The isolates of Groups I, II, and IV were closely related and represented a new species, which was assigned the name *Planococcus antarcticus* sp. nov. The lone isolate in Group III (CMS 53or^T) was distinctly different from the other isolates and from all reported species of *Planococcus* and was designated as a new species with the name *Planococcus psychrophilus* sp. nov.

Materials and methods

Source of the organism, media, and growth conditions

Thirteen bacterial cultures (CMS 2or, CMS 3or, CMS 20or to 26or^T, CMS 53or^T, CMS 82or, CMS 84or, and CMS 88or) were isolated from four different cyanobacterial mat samples collected from four different lakes, namely, Balham Lake (77°24′S, 161°40′E), E4 (77°31′4″S, 160°46′E), Lake Canopus (77°1'33″S, 161°6′E), and SF1 (77°33′41″S, 161°4′E) located in Victoria Valley (Balham Lake) and Wright Valley (E4, Lake Canopus, and SF1) in McMurdo, Antarctica (Matsumoto 1993; Matsumoto et al. 1993). CMS 2or and CMS 3or were isolated from a cyanobacterial mat sample from Balham Lake, CMS 20or–CMS 26or^T were from E4, CMS 53or^T was from Lake Canopus, and CMS 82or, CMS 84or, and CMS 88or were from SF1.

Pure cultures of the heterotrophic bacteria were set up as described earlier (Reddy et al. 2000). Briefly, about 200 mg of the cyanobacterial mat samples was suspended in 1 ml of sterile saline (150 mM NaCl), and an aliquot (100 µl) was plated on Antarctic bacterial medium (ABM) plates containing 0.5% (w/v) peptone, 0.2% (w/v) yeast extract, and 1.5% (w/v) agar (pH 6.9) and incubated at 5°C (Shivaji et al. 1988, 1989a, 1989b, 1991, 1992; Reddy et al. 2000). The appearance of colonies was monitored on a regular basis, and pure cultures of the bacteria were established by repeatedly streaking single colonies on fresh media plates. The optimum temperature, pH, and salt concentration for the growth of the cultures were determined using ABM plates.

Morpholoogy, motility, and biochemical characteristics

Bacterial cultures in the lag, log, and stationary phase of growth were observed under a phase contrast microscope (1,000×) to ascertain their shape and motility. All the biochemical tests listed in Table 1 and described in the Results

Table 1. Characteristics by which CMS 26or^T and CMS 53or^T differ from one another and reported species of *Planococcus*

Characteristics	CM6 26or^T	*P. mcmeekinii*[a]	*P. okeanokoites*[b]	*P. citreus*[c,d]	*P. kocurii*[c,d]	*P. alkanoclasticus*[e]	CMS 53or^T
Colony color	Orange	Orange	Orange	Orange/ Yellow	Orange/Yellow	Orange	Orange
Cell shape	Cocci	Cocci/rods	Rods	Cocci	Cocci	Rods	Rods
Cell arrangement	Tetrads, single, pairs, groups of three	Tetrads, single pairs	NR	Tetrads, single, pairs	Tetrads, single, pairs	Single	Single
Gram staining	+	+	+/variable	+	+	+/variable	+
Growth temperature (°C)	0–30	0–37	Good growth 20–37	4–37	4–37	15–41	0–30
Oxidase	–	–	+	–	–	–	+
Phosphatase	–	NR	NR	–	–	+	–
Nitrate reduction	–	+	–	–	–	–	–
Hydrolysis of							
Esculin	+/–	NR	–	–	–	NR	+
Tween 80	+/–	–	–	–	–	NR	+
Starch	–	–	–	–	–	+	–
Sole carbon source							
Succinate	+	–	NR	+	+	–	+
Glutamate	+	–	NR	–	–	–	+
Glucose	+	–	NR	–	–	–	–
NaCl requirement	No	No	No	No	No	Yes	No
NaCl tolerance (%)	12	7	15	10	3.3	12	12
Acid from glucose	+	+	–	+	+	–	–
Mol% G+C of DNA	41.5	35.0	46.3	47.4	47.4	45.3	44.5

+, positive; –, negative; +/–, some strains were positive; NR, not reported; No, not required; Yes, required

[a]Junge et al. (1998)

[b]Nakagawa et al. (1996)

[c]Kocur (1986)

[d]Claus et al. (1992)

[e]Engelhardt et al. (2001)

and discussion section were performed by growing the cultures at 20°C in the appropriate medium (Hugh and Leifson 1953; Stanier et al. 1966; Holding and Collee 1971; Stolp and Gadkari 1981). Further, the ability of the cultures to utilize a carbon compound as the sole carbon source, the sensitivity to different antibiotics, the isolation of DNA, and the mol% G+C content of the DNA were determined as described previously (Shivaji et al. 1988, 1989a, 1989b, 1991, 1992; Reddy et al. 2000).

DNA–DNA hybridization

DNA–DNA hybridization was performed by the membrane filter method (Tourova and Antonov 1987) as described by us previously (Shivaji et al. 1992; Reddy et al. 2000).

Identification of fatty acids

Bacterial cell pellets free of media constituents were used for the preparation of fatty acid methyl esters (FAME) (Sato and Murata 1988), which were then separated by gas chromatography on a DB-23 capillary column (30 m by 0.25 mm) (J and W Scientific, Folsom, California, USA). The initial run temperature was maintained at 175°C for 8 min and then raised to 200°C at a rate of 4°C per min and held at 200°C for 5 min. The injector port and flame ionization detector temperatures were 200°C and 240°C, respectively. The stationery phase was 0.25 μm thick, and N_2 (1 ml/min) was used as the carrier gas. The fatty acids were identified by comparison with fatty acid standards run under similar gas chromatography conditions and also by mass spectrometry (Shivaji et al. 1992; Reddy et al. 2000).

Analysis of isoprenoid quinones, polar lipids, and peptidoglycan

Menaquinones were extracted according to Collins et al. (1977), separated by thin-layer chromatography (TLC) using petroleum ether and diethyl ether (85 : 15 v/v) (Dumphy et al. 1971), and identified by mass spectrometry (Reddy et al. 2000).

Polar lipids were extracted from lyophilized cell pellets and identified by TLC (Minnikin et al. 1975) using pure lipids as standards.

Peptidoglycan was prepared according to the method of Rosenthal and Dziarski (1994) and hydrolyzed with 4 N HCl at 120°C for 60 min, and then the composition of the main chain was determined according to the method of Schleifer and Kandler (1972).

Bacterial pigment analysis

Pigments were extracted from lyophilized bacterial cell pellets with methanol and centrifuged at 10,000 g, and then the clear pigmented supernatant was recovered and the absorption spectra recorded by a Hitachi 330 spectrophotometer (Chauhan and Shivaji 1994; Shivaji et al. 1992; Jagannadham et al. 1991, 2000).

16S rRNA gene sequencing

Two primers, 16S1 (5′ AGTTTGATCCTGGCTCA 3′) and 16S2 (5′ACGGCTA CCTTGTTACGACTT 3′), corresponding to positions 9–27 and 1,477–1,498, respectively, of the *Escherichia coli* 16S rRNA gene (Lane 1991) were used to amplify the 16S rRNA gene by using 0.5 μg of bacterial DNA as described recently (Reddy et al. 2000; Shivaji et al. 2000). The amplified DNA fragment of 1.5 kb was separated on 1% agarose gel, eluted from the gel, purified using a Clean Genei Kit (Bangalore Genei, Bangalore, India), and sequenced with the 16S1 and 16S2 primers, and, in addition, with a set of five forward primers, pB [(TAACACAT GCAAGTCGAACG, (50–70)], pC [(CTACGGGAGGCA GCAGTGGG, (341–361)], pD [(CAGCAGCCGCGGTAA TAC, (518–536)], pE [(AAACTCAAAGGAATTGACGG, (908–928)], and pF [(CATGGCTGTCGTCAGCTCGT, (1,053–1,073)], and three reverse primers, pC° [(CCCACTG CTGCCTCCCGTAG, (361–341)], pE° [(CCGTCAATTC CTTTGAGTTT, (928–908)], and pH° [(AAGGAGGTGA TCCAGCCGCA, (1,542–1,522)] (Woese et al. 1983).

Phylogenetic analysis

The 16S rDNA sequences of the 13 bacteria were aligned with the reference sequences of all five known species of *Planococcus* from the European Molecular Biology Laboratory (EMBL) database using the multiple sequence alignment program Clustal V (Huggins et al. 1992). The aligned sequences were then manually checked for gaps. The DNADIST program with Kimura-2 factor was used to compute the pairwise evolutionary distances for the above aligned sequences (Kimura 1980). Further, the original sequence data set was resampled 1,000 times using SEQBOOT and subjected to bootstrap analysis to obtain the confidence values for the rDNA sequence on the basis of genetic affiliations. The multiple distance matrices thus obtained were used to construct phylogenetic trees using various distance matrix-based clustering algorithms such as FITCH, KITCH, and UPGMA (unweighted pair group method with arithmetic mean), as compiled in the Phylogeny Inference Package (Felsenstein 1993). Parsimony analysis was also performed for the aligned sequence data set using DNAPARS. In all cases, the input order of species added to the topology being constructed was randomized by using the jumble option with a random seed of seven and ten replications. Majority rule (50%) consensus trees were constructed for the topologies found by each method by using CONSENSE. All these analyses were done using the PHYLIP software package, version 3.5c (Felsenstein 1993).

Reference strains

P. mcmeekinii (ATCC 700539^T) and *P. okeanokoites* (ATCC 33414^T) were used as controls in some of the studies related

to morphology, motility, biochemical tests, identification of fatty acids, and DNA–DNA hybridization.

Random amplified polymorphic DNA analysis

Random amplified polymorphic DNA (RAPD) analysis was carried out as described previously (Shivaji et al. 2000) by using the primers OPA-02 (5′-TGC CGA GCT-3′) and OPA-03 (5′-AGT CAG CCAC-3′). Genomic DNA was amplified as reported recently (Reddy et al. 2000; Shivaji et al. 2000). In each PCR cycle, the DNA was denatured for 5 s at 94°C, annealed for 10 s at 34°C, and extended for 30 s at 72°C. PCR was carried out for 35 cycles, after which a final extension was performed for 5 min at 72°C. Subsequently, the amplified products were resolved following electrophoresis on a 1.5% agarose gel, and the bands were visualized after staining with ethidium bromide to determine polymorphism, if any.

Results and discussion

The four cyanobacterial mat samples used in the present study to isolate the associated heterotrophic bacteria varied with respect to their site of collection and also with respect to the cyanobacteria, green algae, and diatoms associated with them (Matsumoto et al. 1993). The cyanobacterium *Aphanothece castagnei* was abundant in Balham Lake and SF1 mat samples, whereas *Phormidium laminosum* was abundant in all samples except that from SF1, in which *Phormidium sp*. was very dominant. Diatoms (*Hantzschia amphioxys* and *Navicula multicopsis*) were not very common but could be detected in SF1 and Lake Canopus. Further, except for the mat sample from SF1, all the samples contained coccoid green algae (Matsumoto et al. 1993).

The number of bacterial colonies per gram of cyanobacterial mat sample following incubation at 5°C for 15 days was about 1.4×10^6, 1.6×10^5, 1.5×10^6, and 2.5×10^5 in the mat samples from Balham Lake, E4, Lake Canopus, and SF1, respectively. The colonies in the mat sample from Balham Lake were all orange in color, whereas in the mat samples from the remaining three lakes, the colonies were orange or white in color, with the pigmented colonies constituting 60%–70% of the total colonies. Since the pigmented bacteria were the predominant bacteria associated with the cyanobacterial mats, attempts were made to establish pure colonies of these bacteria by streaking a single isolated colony from one plate onto another media plate a number of times. By this method, 13 pure colonies of orange-pigmented bacteria were established (CMS 2or, CMS 3or, CMS 20or to 26or, CMS 53or^T, CMS 82or, CMS 84or, and CMS 88or), and they were studied in detail to establish their taxonomic identity and phylogenetic position.

On the basis of their morphology, growth characteristics, biochemical and chemotaxonomic characteristics, 16S rRNA gene sequence analysis, and RAPD analysis, the 13 isolates of orange-pigmented bacteria could be classified into four groups: Group I (CMS 2or and CMS 3or), Group II (CMS 20or to 26or and 82or), Group III (CMS 53or^T), and Group IV (CMS 84or and CMS 88or). In fact, from the RAPD analysis (data not shown), it appears that members of the same group were probably clonal in origin because they showed the identical number of DNA bands with identical electrophoretic mobility, and this similarity was observed with both the RAPD primers used in the present study. That members of the same group were also isolated from the same cyanobacterial mat further strengthens their clonal origin. Therefore, although all 13 isolates were studied in detail with respect to the characteristics listed above, here we present data only for CMS 3or (MTCC 3856; DSM 14504), CMS 26or^T (MTCC 3854; DSM 14505), CMS 53or^T (MTCC 3812; DSM 14507), and CMS 84or (MTCC 3855; DSM 14506) as representative strains of each of the groups (I to IV), respectively.

Morphology and growth characteristics

The 13 orange-pigmented colonies were about 1–2 mm in diameter, circular, convex, and smooth, and they exhibited optimum growth at 22°C and pH 7. However, they could grow between 2° and 30°C and pH 6 and 12, and either in the absence or presence of NaCl, and they could tolerate up to 12% NaCl. Further, cells of CMS 3or, CMS 26or^T, and CMS 84or were all Gram positive, they occurred singly, in pairs, or in groups of three to four cells (except CMS 53or^T, which was rod shaped) (Table 1), and they were all motile and nonsporulating.

Biochemical and chemotaxonomic characteristics

From the above morphological features and the biochemical and chemotaxonomic characteristics that follow, it is apparent that CMS 3or, CMS 26or^T, CMS 53or^T, and CMS 84or are members of the genus *Planococcus* (Kocur 1986; Claus et al. 1992). Like all the reported species of *Planococcus,* the four isolates from Antarctica are orange pigmented, halotolerant (up to 12% NaCl), and catalase positive, and they hydrolyze gelatin, do not hydrolyze starch, do not reduce nitrate to nitrite, and do not require growth factors. They were observed to be negative for phosphatase, indole production, the methyl red test, the Voges-Proskauer reaction, and levan formation (Kocur 1986; Nakagawa et al. 1996; Junge et al. 1998; Engelhardt et al. 2001). None of the four bacteria produced acid from lactose, cellobiose, sodium glutamate, or thioglycollate, and they failed to produce gas from these four compounds or even from glucose and sucrose. MK-7 and MK-8 were the major menaquinones; phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine were the polar lipids; and L-Lys-D-Glu was the peptidoglycan common to the four isolates. Further, the pigments from these isolates were insoluble in water but could be extracted with methanol, and they exhibited a fine structure in their absorption spectrum (with absorption maxima at 440, 465, and 487.5 nm), a characteristic feature of carotenoids. But they differed from

the reported species and among themselves with respect to many characteristics such as colony color, cell morphology, Gram-staining reaction, growth temperature, NaCl requirement, halotolerance, oxidase, phosphatase, and nitrate reduction reactions, ability to hydrolyze Tween 80, starch, and esculin, mol% G+C of their DNA, ability to produce acid from glucose and sucrose, utilization of carbon sources, and fatty acid composition (Tables 1, 2). Further details related to pigment characteristics, utilization of various carbon compounds as the sole carbon source, sensitivity to antibiotics, and other phenotypic characteristics are included below in the description of the species.

Phylogenetic analysis.

To establish the phylogenetic position of CMC 3or, CMS 26or^T, CMS 53or^T, and CMS 84or, the 16S rDNA sequence consisting of 1,450 base pairs was compared with other sequences of closely related species of *Planococcus* and related genera retrieved from the EMBL database. From the topology of the phylogenetic tree (Fig. 1), it is clear that CMS 3or, CMS 26or^T, and CMS 84or are closely related to one another (99%–99.8% similarity) and form one compact clade that differs from CMS 53or^T by 2.5% (Table 3). In fact, these three also differ by more than 2.5% from all reported species at the 16S rDNA sequence level. For instance, all three differed from *P. okeanokoites* by 2.6% to 2.7%, from *P. mcmeekinii* by 2.8% to 3.0%, from *P. alkanoclasticus* by 2.6% to 2.9%, from *P. citreus* by 3.1% to 3.6%, and from *P. kocurii* by 5% to 5.3%, clearly suggesting that the three closely related *Planococcus* isolates from Antarctica probably belong to a new species. Earlier studies have shown that bacteria that differ by more than 2.5% at the 16S rRNA gene sequence level are unlikely to belong to the same species, and such bacteria are unlikely to exhibit >60% to 70% similarity at the genomic DNA level (Stackebrandt and Goebel 1994). On this basis, it could be inferred that CMS 3or, CMS 26or^T, and CMS 84or most likely represent a distinct species different from all reported species of *Planococcus*. However, on the basis of the 16S rRNA sequence data alone, it may not be correct to conclude that the present isolates represent a single new species, because 16S rRNA gene sequence identity among different species of *Planococcus*, such as *P. kocurii*, *P. citreus*, and *P. okeanokoites*, is very high (99%) (Nakagawa et al. 1996). Nevertheless, these have been classified as distinct species on the basis of differences in phenotypic and chemotaxonomic characteristics and low DNA–DNA reassociation values (15%–27%) (Nakagawa et al. 1996). DNA–DNA hybridization values between CMS 3or, CMS 26or^T, and CMS 84or with *P. mcmeekinii* and *P. okeanokoites* were in the range of 30%–35% and 26%–29%, respectively (Table 4), thus indicating that they are distinct species. This is further strengthened by the fact that the three isolates could be easily differentiated from *P. okeanokoites*, *P. mcmeekinii*, *P. alkanoclasticus*, *P. citreus*, and *P. kocurii* on the basis of phenotypic and chemotaxonomic characteristics (Table 1). For instance, unlike *P. mcmeekinii*, *P. okeanokoites*, and *P. alkanoclasticus*, which are rod shaped and capable of growth at 37°C or above (Junge et al. 1998; Nakagawa et al. 1996; Engelhardt et al. 2001), none of the *Planococcus* isolates that were coccoid in shape could grow above 30°C. However, they could utilize succinate, glutamate, and glucose as the sole carbon source and hydrolyze esculin and Tween 80, and they differed from the

Table 2. Comparison of the fatty acid composition (%) of CMS 3or, CMS 26or^T, CMS 84or, and CMS 53or^T with the other species of *Planococcus*

Fatty acid	CMS 3or	CMS 26or^T	CMS 84or	*P. okeanokoites*[a]	*P. mcmeekinii*[b]	*P. alkanoclasticus*[c]	*P. citreus*[c]	*P. kocurii*[c]	CMS 53or^T
Iso-$C_{14:0}$	3.5	1.0	4.5	33.9	10.0	6.0	3.3	16.4	3.2
$C_{14:1}$	0	traces	0	traces	0	0	0	0	0
Iso-$C_{15:0}$	2.1	1.3	2.7	2.9	6.0	7.5	0	4.9	5.6
Anteiso-$C_{15:0}$	44.8	43.2	28.2	14.0	38.0	45.5	61.7	41.6	41.3
$C_{15:0}$	6.6	14.2	2.1	traces	0	0	3.7	11.3	0
$C_{15:1}$	0.8	9.8	1.1	0	0	0	0	0	0
Iso-$C_{16:0}$	9.6	4.0	11.6	28.1	6.0	17.1	6.0	11.2	8.1
$C_{16:0}$	2.2	4.2	2.0	4.7	0	0	4.1	0	4.4
$C_{16:1}$	2.0	3.0	3.4	2.8	23.0	6.4	2.5	4.3	3.2
Iso-$C_{16:1}$	3.7	1.2	5.3	11.7	0	0	0	0	7.2
Iso-$C_{17:0}$	1.4	0.3	7.6	traces	3.0	7.4	0	0	0
Anteiso-$C_{17:0}$	9.8	9.5	9.3	traces	3.0	10.2	13.9	3.6	7.3
$C_{17:0}$	4.1	1.0	2.0	0	0	0	0	0	6.1
$C_{17:1}$	5.8	0	5.5	0	4.0	0	0	0	0
Iso+anteiso-$C_{17:1}$	2.5	4.2	17.3	0	5.0	9.3	0	0	11.3
Iso-$C_{18:0}$	1.1	0	0.3	traces	0	1.2	0	0	0
$C_{18:0}$	0	0.3	0	1.8	0	0	0	0	1.6
$C_{18:1}$	0	1.0	1.0	0	0	2.7	0	0	1.0

In CMS 3or, CMS 26or^T, and CMS 84or only iso-$C_{17:1}$ was present
[a]Nakagawa et al. (1996)
[b]Junge et al. (1998)
[c]Engelhardt et al. (2001)

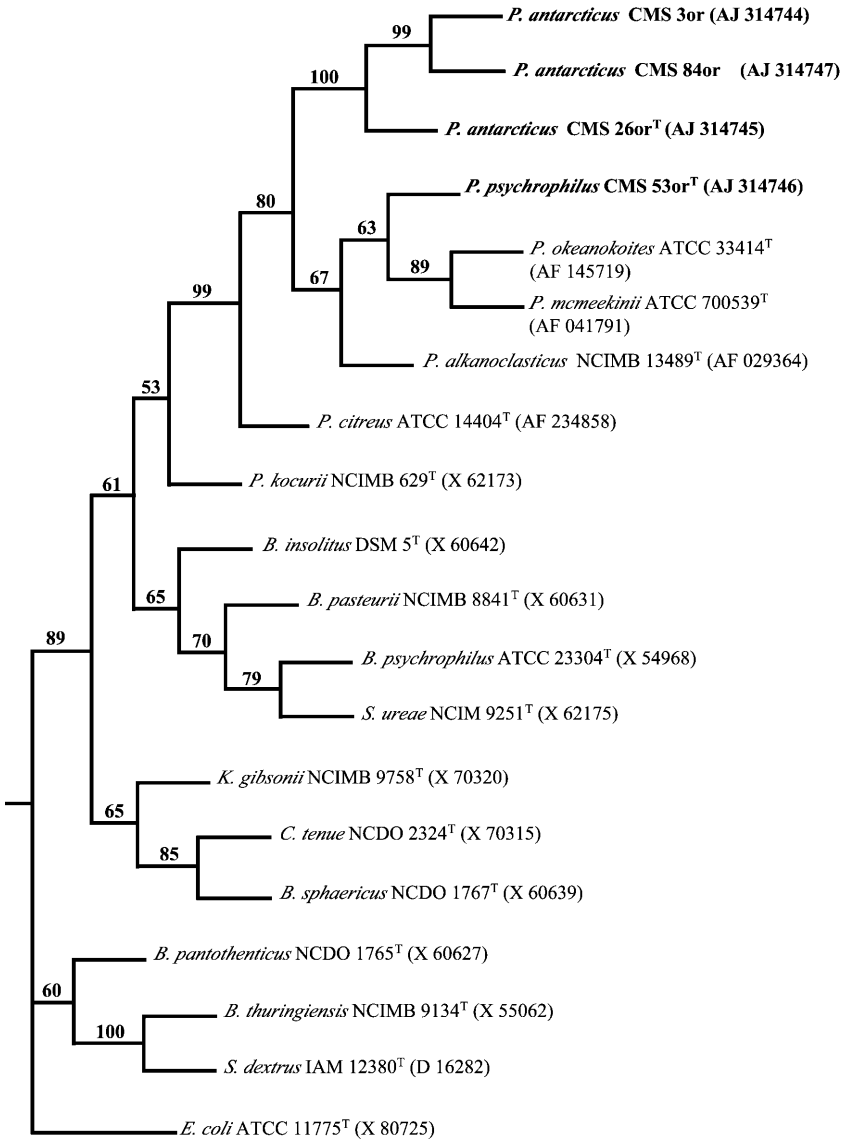

Fig. 1. Unweighted pair group method with arithmetic mean (UPGMA) phenogram showing the phylogenetic relationship between *P. antarcticus* (CMS 3or, CMS26or^T, and CMS 84or) and *P. psychrophilus* (CMS 53or^T) and other species of *Planococcus* and related reference microorganisms based on the 16S rDNA sequence analysis. Bootstrap values are given at the nodes. The branch lengths in the phenogram are not to scale. Genus names: *P.*, *Planococcus*; *B.*, *Bacillus*; *S. ureae*, *Sporosarcina*; *K.*, *Kurthia*; *C.*, *Caryophanon*; *S. dextrus*, *Sporolactobacillus*

Table 3. Similarity (%) between CMS 26or^T and CMS 53or^T and other related species of *Planococcus* as determined by 16S rRNA gene sequence analysis

Strain	*P. okeanokoites*	*P. mcmeekinii*	*P. alkanoclasticus*	*P. citreus*	*P. kocurii*	CMS 53or^T	CMS 26 or^T
CMS 26or^T	97.5	97.1	97.4	96.9	95.0	97.5	100
CMS 53or^T	98.1	98.0	97.5	96.7	94.0	100	97.4

reported species in their mol% G+C content of DNA (Table 1). Further, the fatty acid composition was different in these psychrotrophic species of *Planococcus* (Table 2). Like all reported species of *Planococcus*, anteiso-$C_{15:0}$ was the predominant fatty acid (28% to 45%) in CMS 3or, CMS 26or^T, and CMS 84or (Nakagawa et al. 1996; Junge et al. 1998; Engelhardt et al. 2001). However, they could be differentiated from all reported isolates of *Planococcus* because they contained significant amounts of $C_{15:1}$ (1% to 10%), iso-$C_{16:1}$ (1.2% to 5.3% in CMS 26or^T and CMS 84or), and $C_{17:0}$ (1% to 4%), and no anteiso-$C_{17:1}$. Thus, these three isolates probably represent strains of a new species of *Planococcus*, for which the name *Planococcus antarcticus* sp. nov. is proposed, and CMS 26or^T is designated as the type strain.

The 16S rDNA-based phylogenetic analysis clearly indicated that CMS 53or^T is a member of the Genus *Planococcus* and forms a robust clade with *P. mcmeekinii* and *P. okeanokoites*. But CMS 53or^T differed from the above species by 2% and 1.9%, respectively, at the 16S rRNA gene sequence level (Table 3). Further, it differed from the three remaining reported species, *P. alkanoclasti-*

Table 4. DNA–DNA relatedness (%) of CMS 3or, CMS 26or^T, CMS 53or^T, and CMS 84or with *P. mcmeekinii*, *P. okeanokoites*, and CMS 53or^T

S. No.	Bacterium	DNA–DNA hybridization (%)		
		P. mcmeekinii	*P. okeanokoites*	CMS 53or^T
1.	CMS 3or	30	28	16
2.	CMS 26or^T	35	26	25
3.	CMS 53or^T	35	36	100
4.	CMS 84or	33	29	15

cus, *P. citreus*, and *P. kocurii*, and the present new species, *P. antarcticus*, by 2.5%, 3.3%, 6.1%, and 2.6%, respectively. Because of its close similarity with *P. mcmeekinii*, *P. okeanokoites*, and *P. antarcticus*, DNA–DNA hybridization tests are essential to establish that CMS 53or^T is a new species of *Planococcus*. In fact, DNA–DNA hybridization studies have shown that CMS 53or^T has approximately 35%, 36%, and 25% similarity with *P. mcmeekinii*, *P. okeanokoites*, and *P. antarcticus*, respectively, clearly suggesting that it is a new species. The low DNA–DNA hybridization values between CMS 53or^T and *P. mcmeekinii*, *P. okeanokoites*, and *P. antarcticus*, despite their close relationship at the 16S rRNA sequence level, is in accordance with the earlier observations of Nakagawa et al. (1996), who demonstrated that although *P. kocurii*, *P. citreus*, and *P. okeanokoites* exhibit high sequence identity (99%), they can be identified as distinct species on the basis of their phenotypic differences and low DNA–DNA reassociation values (15%–27%). For instance, CMS 530r and *P. mcmeekinii* are similar only with respect to their orange pigment, Gram staining, and glucose utilization (Junge et al. 1998), and they differ with respect to the other ten phenotypic characteristics listed in Table 1. At the phenotypic level, CMS 530r appears to be more closely related to *P. okeanokoites*, but it differs in that it is psychrotrophic, can hydrolyze both esculin and Tween 80, and has a distinctly different fatty acid composition (Table 2) (Nakagawa et al. 1996). In CMS 53or^T (unlike *P. okeanokoites*), the predominant fatty acid present was anteiso-$C_{15:0}$ (41.3%), whereas in *P. okeanokoites*, not anteiso-$C_{15:0}$ (14%) but iso-$C_{14:0}$ was the predominant fatty acid (33.9%). Further, in CMS 53or^T, significant amounts of anteiso-$C_{17:0}$, $C_{17:0}$, iso-$C_{17:1}$, and $C_{18:1}$ were also present, but they were not detectable in *P. okeanokoites*. CMS 53or^T phenotypically appeared to have many characteristics similar to those of *P. antarcticus* (Table 2), but it was distinct in that it was rod shaped and oxidase positive, could not utilize glucose provided as the only carbon source, could not acidify glucose, and also varied in its mol% G+C content of DNA, sensitivity to antibiotics, fatty acid composition, and utilization of various carbon compounds. CMS 53or^T also exhibited distinct differences from *P. alkanoclasticus*, another rod-shaped *Planococcus*, in that, unlike this species, which is a mesophile and capable of growth between 15° and 41°C and which requires NaCl for growth, CMS 530r is psychrotrophic and halotolerant, not halophilic (Engelhardt et al. 2001). It also showed differences with respect to many other phenotypic traits (Table 1). Further it differed from other species (namely, *P. citreus* and *P. Kocurii*) by more than 2.5% at the 16S rRNA gene sequence level and with respect to a number of the characteristics (Kocur 1986; Claus et al. 1992) listed in Table 1, and with respect to sensitivity to antibiotics, ability to utilize various carbon compounds, and fatty acid composition (Table 2). Thus, on the basis of these differences, it would appear that CMS 53or^T is yet another new species of *Planococcus*, for which the name *P. psychrophilus* is proposed. The type strain is CMS 53or^T.

Description of *Planococcus antarcticus* sp. nov.

Planococcus antarcticus (antarcticus, N.L. pertaining to the Antarctic). Cells are coccoid, and they occur singly, in pairs, in groups of three, or as tetrads and are motile. They are Gram positive, aerobic, and lack endospores. Colonies on peptone–yeast extract medium were orange, smooth, convex, circular, uniform-edged, and 1–2.0 mm in diameter. Pigment is insoluble in water but soluble in methanol and exhibits a fine structure in its absorption spectrum with absorption maxima at 440, 465, and 487.5 nm. Pigment production is not dependent on any specific growth conditions or on the composition of the medium. It is psychrophilic and exhibits optimum growth at 20°C, but is able to grow between 2° and 30°C and pH 6 and 12, and it tolerates up to 12% NaCl. It is catalase, lipase, gelatinase, β-galactosidase, and arginine dihydrolase positive, but negative with respect to oxidase, urease, and phosphatase, esculin hydrolysis, starch hydrolysis, nitrate to nitrite reduction, the indole, methyl red, and Voges-Proskauer tests, and levan formation. It can utilize glucose, rhamnose, melibiose, lysine, fructose, xylose, glycerol, acetate, succinate, inositol, glutamic acid, and pyruvate, but it cannot utilize mannose, galactose, maltose, sorbose, glucosamine, glutamine, ribose, raffinose, trehalose, cellulose, dulcitol, cellobiose, sorbitol, mannitol, melezitol, adonitol, sucrose, lactose, lactic acid, inulin, β-hydroxybutyric acid, dextrin, glycine, alanine, polyethylene glycol, lysine, phenylalanine, serine, arginine, arabinose, cellulose, methionine, tyrosine, myristic acid, creatinine, potassium hydrogen phthalate, or glycogen, when these are provided as the only carbon source. It produces acid from glucose and sucrose but not from lactose, cellobiose, sodium glutamate, or thioglycollate. Gas is not produced from any of the above six carbon sources tested for acid production. The cell-wall peptidoglycan is L-lys-D-glu, and MK-7 and MK-8 are the major menaquinones. Iso-$C_{14:0}$, $C_{14:1}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{15:0}$, iso-$C_{15:1}$, iso-$C_{16:0}$, $C_{16:1}$, iso- $C_{16:1}$, iso-$C_{17:0}$, $C_{17:0}$, anteiso-$C_{17:0}$, $C_{17:0}$, iso-$C_{17:1}$, $C_{17:0}$, $C_{18:0}$, and $C_{18:1}$ are the cellular fatty acids. The predominant polar lipids are phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine. The mol% G+C content of the DNA is 41.5 mol%, and this species is phylogenetically related to *P. mcmeekinii* and *P. okeanokoites*, on the basis of 16S rDNA analysis. It is resistant to carbenicillin, tobramycin, nitrofurazone, furazolidone, colistin, kanamycin, nystatin, ampicillin, and amoxicillin, but sensitive to chlortetracycline, polymyxin, oxytetracycline, nitrofurantoin, penicillin, baci-

tracin, gentamicin, lincomycin, rifampicin, cotrimoxazole, chloramphenicol, tetracycline, trimethoprim, nalidixic acid, neomycin, streptomycin, novobiocin, and erythromycin. *P. antarcticus* sp. nov. was isolated from a cyanobacterial mat sample from McMurdo Dry Valleys, Antarctica. The type strain is CMS 26or^T (MTCC 3854; DSM 14505). The EMBL accession number for the 16S rDNA sequence of CMS 26or^T is AJ314745.

Description of *Planococcus psychrophilus* sp. nov.

Planococcus psychrophilus (Psychrophilus adj. derived from Latin meaning cold loving) Cells are rod shaped, single, Gram positive, non-spore-forming, and motile. Colonies on peptone–yeast extract medium are orange, smooth, convex, circular, and 1–2 mm in diameter. Pigment is insoluble in water but soluble in methanol and exhibits absorption maxima at 440, 465, and 487.5 nm. Pigment synthesis is not dependent on the growth phase or the growth conditions. Cultures can grow from 2° to 30°C and pH 6 to 12, and they tolerate up to 12% NaCl. Optimum growth was observed at 22°C and pH 7. It is catalase, oxidase, lipase, gelatinase, β-galactosidase, and arginine dihydrolase positive, but negative with respect to urease and phosphatase, nitrate to nitrite reduction, indole production, the methyl red and Voges-Proskauer tests, and levan formation. It can hydrolyze esculin and Tween 80 but not starch.

It can utilize rhamnose, melibiose, trehalose, xylose, glycerol, lysine, sodium acetate, sodium succinate, inositol, glutamic acid, and pyruvate, but not glucose, lactose, sorbose, arabinose, cellobiose, sucrose, fructose, mannose, mannitol, raffinose, ribose, lactose, lactic acid, adonitol, maltose, glucosamine, sorbitol, melizitol, β-hydroxybutyric acid, dulcitol, dextran, PEG, glycine, sodium citrate, cellulose, inulin, alanine, phenylalanine, methionine, glutamine, arginine, serine, potassium hydrogen phthalate, myristic acid, creatinine, tyrosine, or glycogen as the sole carbon source. Further, it does not produce acid or gas from glucose, sucrose, cellobiose, lactose, sodium glutamate, or sodium thioglycollate. The cell-wall peptidoglycan is L-lys-D-glu, and the major menaquinones are MK-7 and MK-8. The cellular fatty acids are iso-$C_{14:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{16:0}$, $C_{16:0}$, $C_{16:1}$, iso-$C_{16:1}$, anteiso-$C_{17:0}$, $C_{17:0}$, iso-$C_{17:1}$, anteiso-$C_{17:1}$, $C_{18:0}$, and $C_{18:1}$. The polar lipids present are phosphatidylglycerol, diphosphatidylglycerol, and phosphatidylethanolamine. The DNA base composition is 44.5 mol% G+C. It is closely related phylogenetically to *P. mcmeekinii*, *P. alkanoclasticus*, *P. antarcticus*, and *P. okeanokoites,* as determined by 16S rDNA analysis, but it exhibits very low DNA–DNA similarity. It is sensitive to penicillin, chlortetracycline, chloramphenicol, neomycin, streptomycin, novobiocin, tetracycline, bacitracin, furazolidone, colistin, kanamycin, lincomycin, cotrimoxazole, ampicillin, amoxicillin, trimethoprim, erythromycin, nalidixic acid, nystatin, gentamicin, and polymyxin B, but resistant to carbenicillin, tobramycin, oxytetracycline, nitrofurazone, and nitrofurantoin. It was isolated from a cyanobacterial mat sample from McMurdo Dry Valleys, Antarctica. The type strain is CMS 53or^T (MTCC 3812; DSM 14507).

The EMBL accession number for the 16S rDNA sequence of CMS 53or^T is AJ 314746.

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