Psychrophilic pseudomonads from Antarctica: *Pseudomonas antarctica* sp. nov., *Pseudomonas meridiana* sp. nov. and *Pseudomonas proteolytica* sp. nov.

Gundlapalli S. N. Reddy,[1] Genki I. Matsumoto,[2] Peter Schumann,[3] Erko Stackebrandt[3] and Sisinthy Shivaji[1]

Correspondence
Sisinthy Shivaji
shivas@ccmb.res.in

[1]Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad – 500 007, India

[2]Department of Environmental and Information Science, Otsuma Women's University, Tamashi, Tokyo 206, Japan

[3]DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

Thirty-one bacteria that belonged to the genus *Pseudomonas* were isolated from cyanobacterial mat samples that were collected from various water bodies in Antarctica. All 31 isolates were psychrophilic; they could be divided into three groups, based on their protein profiles. Representative strains of each of the three groups, namely CMS 35^T, CMS 38^T and CMS 64^T, were studied in detail. Based on 16S rRNA gene sequence analysis, it was established that the strains were related closely to the *Pseudomonas fluorescens* group. Phenotypic and chemotaxonomic characteristics further confirmed their affiliation to this group. The three strains could also be differentiated from each other and the closely related species *Pseudomonas orientalis*, *Pseudomonas brenneri* and *Pseudomonas migulae*, based on phenotypic and chemotaxonomic characteristics and the level of DNA–DNA hybridization. Therefore, it is proposed that strains CMS 35^T (=MTCC 4992^T=DSM 15318^T), CMS 38^T (=MTCC 4993^T=DSM 15319^T) and CMS 64^T (=MTCC 4994^T=DSM 15321^T) should be assigned to novel species of the genus *Pseudomonas* as *Pseudomonas antarctica* sp. nov., *Pseudomonas meridiana* sp. nov. and *Pseudomonas proteolytica* sp. nov., respectively.

The genus *Pseudomonas* was originally created by Migula (1894). Over the years, the genus has been redefined to differentiate it from other genera (Stanier *et al.*, 1966; Palleroni *et al.*, 1973; De Ley, 1992; Anzai *et al.*, 2000). Moore *et al.* (1996) further delineated the genus *Pseudomonas* into two major intrageneric clusters, namely the *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* clusters. Subsequently, based on phylogenetic analysis of 56 species of *Pseudomonas sensu stricto*, using 1063 bp of the 16S rRNA gene sequence, the genus was categorized into two main clusters (Anzai *et al.*, 2000). The first cluster had six groups within it and these were designated as the *Pseudomonas syringae* group (with 12 species), the *Pseudomonas chlororaphis* group (with five species), the *P. fluorescens* group (with 18 species), the *Pseudomonas putida* group (with six species), the *P. aeruginosa* group (with 11 species) and the *Pseudomonas stutzeri* group (with three species). The second cluster had only one group, the *Pseudomonas pertucinogena* group, which contained two species.

Until now, about 100 species of the genus *Pseudomonas* have been reported from various habitats, including Antarctica. Kriss *et al.* (1976) were the first to report the existence of *Pseudomonas* species in Antarctica. However, they were not identified at species level until 1989, when *Pseudomonas* spp. isolated from Antarctic soil and water samples were identified as psychrophilic strains of *P. aeruginosa*, *P. fluorescens*, *P. putida* and *P. syringae* (Shivaji *et al.*, 1989a). More recently, Maugeri *et al.* (1996) and Bruni *et al.* (1999) isolated bacteria that belonged to the genus *Pseudomonas* from sea water and freshwater samples from Terra Nova Bay and Wanda Lake, Antarctica. However, these were also not characterized at species level. In the present study, attempts were made to identify bacteria that

Published online ahead of print on 14 November 2003 as DOI 10.1099/ijs.0.02827-0.

The GenBank/EMBL/DDBJ accession numbers for the 16S rDNA sequences of strains CMS 35^T, CMS 38^T and CMS 64^T are AJ537601, AJ537602 and AJ537603, respectively.

Tables showing the fatty acid composition of and DNA–DNA relatedness data for the novel pseudomonads are available as supplementary material in IJSEM Online.

belong to the genus *Pseudomonas* that were isolated from cyanobacterial mat samples collected from the McMurdo region, Antarctica.

Source of the organisms, media and growth conditions

Thirty-one bacterial isolates were obtained from cyanobacterial mat that were samples collected from ponds L1 (strains CMS 62–72) and L3 (CMS 33–36) of Wright Valley, Adam's glacier stream 1 (CMS 43–50), Adam's glacier stream 2 (CMS 37, CMS 38^T and CMS 40) and Canada glacier stream (CMS 41–42) of Miers Valley and Lake Canopus (CMS 54, CMS 57 and CMS 60) in Antarctica. Pure cultures of the heterotrophic bacteria were set up as described previously (Reddy *et al.*, 2000). Optimum temperature, pH and salt concentration for growth of cultures were determined by using plates of Antarctic bacterial medium (ABM) that contained 0·5 % (w/v) peptone, 0·2 % (w/v) yeast extract and 1·5 % (w/v) agar (pH 6·9) (Reddy *et al.*, 2002, 2003).

Morphology, motility and biochemical characteristics

Bacterial cultures in the lag, exponential and stationary phases of growth were observed under a phase-contrast microscope (× 1000) to ascertain their shape and motility. All biochemical tests were performed by growing cultures at 22 °C in appropriate medium (Hugh & Leifson, 1953; Stanier *et al.*, 1966; Holding & Collee, 1971; Stolp & Gadkari, 1981). Furthermore, ability of the cultures to utilize a carbon compound as sole carbon source, sensitivity to different antibiotics and DNA G+C contents were determined as described previously (Shivaji *et al.*, 1989b). Total protein profiles of the cultures were determined by SDS-PAGE. For this purpose, cultures were grown in 3 ml ABM broth at 25 °C and harvested at 6000 r.p.m. for 10 min at room temperature; the pellets were resuspended in 100 µl water and 100 µl SDS/sample buffer. The suspension was then boiled for 5 min and centrifuged at 10 000 r.p.m. for 10 min; 50 µl supernatant was loaded onto 12 % SDS/polyacrylamide gel (Laemmli, 1970). Bands were visualized by staining with Coomassie blue.

DNA–DNA hybridization and identification of fatty acids

DNA–DNA hybridization was performed by the membrane filter method (Tourova & Antonov, 1987) as described previously (Shivaji *et al.*, 1992; Reddy *et al.*, 2000). Fatty acids were identified from bacterial cell pellets by comparison with fatty acid standards that were run under similar GC conditions and also by mass spectrometry (Sato & Murata, 1988; Reddy *et al.*, 2003).

Riboprinting

A pure colony of each of strains CMS 35^T, CMS 38^T and CMS 64^T and *Pseudomonas brenneri* was picked up with a sterile toothpick and suspended in a 1·5 ml microfuge tube that contained 200 µl riboprinting buffer (DuPont Qualicon). The tube was then heated to 70 °C for 10 min in a model 480 DNA thermocycler (Perkin Elmer) and the contents were transferred to a sample carrier (DuPont Qualicon). Lysis reagent A and reagent B (5 µl each) were added before inserting the sample carrier into the characterization unit of the Qualicon Riboprinter system, where the samples were processed automatically according to the *Eco*RI standard protocol.

16S rRNA gene sequencing

Amplification of the 16S rRNA gene, purification of the 1·5 kb amplicon and sequencing of the amplicon were carried out by the method of Lane (1991), as described previously (Shivaji *et al.*, 2000).

Phylogenetic analysis

16S rRNA gene sequences of the three bacteria that represented the 31 isolates were aligned with reference sequences of all species in the *P. fluorescens* group (obtained from GenBank/EMBL) by using the multiple sequence alignment program CLUSTAL V (Higgins *et al.*, 1992). The aligned sequences were then checked manually for gaps. The DNADIST program was used to compute pairwise evolutionary distances for the aligned sequences by applying the Kimura two-parameter model (Kimura, 1980). Furthermore, the original sequence dataset was resampled 1000 times by using SEQBOOT and subjected to bootstrap analysis to obtain confidence values for 16S rRNA gene sequence-based genetic affiliations. The multiple distance matrices thus obtained were used to construct phylogenetic trees by using various distance matrix-based clustering algorithms, such as FITCH, KITSCH and UPGMA, as compiled in the Phylogeny Inference Package (PHYLIP; Felsenstein, 1993). Parsimony analysis was also performed for the aligned sequence dataset by 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 7 and ten replications. Majority-rule (50 %) consensus trees were constructed for the topologies by using CONSENSE. All these analyses were done by using the PHYLIP package, version 3.5c (Felsenstein, 1993).

Reference strains

P. brenneri CIP 106646^T, *Pseudomonas orientalis* CIP 105540^T, *Pseudomonas veronii* CIP 104663^T, *Pseudomonas marginalis* ATCC 10844^T, *Pseudomonas rhodesiae* CIP 104664^T, *Pseudomonas tolaasii* ATCC 33618^T, *Pseudomonas migulae* CIP 105470^T and *P. fluorescens* ATCC 13525^T were used as controls in studies that were related to the identification of fatty acids and DNA–DNA hybridization.

Conclusions

Thirty-one individual bacterial colonies were isolated from cyanobacterial mat samples that were collected from various

water bodies in Antarctica. These 31 isolates could be categorized into three groups, based on their protein profiles as analysed by SDS-PAGE (data not shown), namely group I (CMS 33–36 and CMS 44–50), group II (CMS 38^T) and group III (CMS 37, CMS 40–41, CMS 43, CMS 54, CMS 57, CMS 60 and CMS 62–72). Members of the same group exhibited identical protein profiles, indicating that they are probably clonal in origin. Therefore, strains CMS 35^T, CMS 38^T and CMS 64^T were chosen as representative isolates of groups I, II and III, respectively.

These three isolates, namely CMS 35^T, CMS 38^T and CMS 64^T, are aerobic, Gram-negative, rod-shaped and motile, possess a polar flagellum and have $C_{16:0}$, $C_{16:1}\omega7c$, $C_{16:1}\omega9c$ and $C_{18:1}$ as their major fatty acids, indicating their affiliation to the genus *Pseudomonas*. They could all grow at 4–30 °C and did not accumulate polyhydroxybutyric acid. Riboprinting analysis indicated that strains CMS 35^T, CMS 38^T and CMS 64^T are distinctly different from each other (Tables 1 and 2; Supplementary Table A, available in IJSEM Online; Fig. 1).

Phylogenetic analysis of the three isolates, based on 1438 bp of the 16S rRNA gene sequence, indicated a close relationship with species that belong to the *P. fluorescens* group (Anzai *et al.*, 2000) (Fig. 2). Evolutionary distances, as calculated by using the Kimura two-parameter model, indicated that the three isolates are related very closely to each other, with >99 % 16S rRNA gene sequence similarity, and also to other species of the *P. fluorescens* group (Anzai *et al.*, 2000). At the DNA–DNA level, there was 40 % relatedness between strains CMS 35^T and CMS 38^T, 40 % between CMS 35^T and CMS 64^T and 43 % between CMS 38^T and CMS 64^T (Supplementary Table B, available in IJSEM Online).

The topology of the tree indicates that strain CMS 64^T is related phylogenetically to the clade that represents

Table 1. Phenotypic characteristics that differentiate strains CMS 35^T (*P. antarctica*) and CMS 38^T (*P. meridiana*) from each other and from closely related species of the genus *Pseudomonas*

Taxa: 1, CMS 35^T; 2, CMS 38^T; 3, *P. orientalis* [data from Dabboussi *et al.* (1999)]; 4, *P. marginalis* [data from Shinde & Lukezic (1974) and Munsch *et al.* (2002)]; 5, *P. rhodesiae* [data from Coroler *et al.* (1996)]; 6, *P. veronii* [data from Elomari *et al.* (1996)]; 7, *P. extremorientalis* [data from Ivanova *et al.* (2002)]; 8, *P. tolaasii* [data from Ivanova *et al.* (2002)]; 9, *P. costantinii* [data from Munsch *et al.* (2002)]. +, Positive; −, negative; (+), weakly positive; NA, data not available. All taxa are positive for the utilization of trehalose.

Characteristic	1	2	3	4	5	6	7	8	9
Growth characteristics									
Temperature (°C):									
Range	4–30	4–30	4–35	NA	4–36	4–36	4–30	NA	NA
Optimum	22	22	NA	NA	NA	NA	25	NA	NA
Phenotypic characteristics									
Phosphatase	+	−	+	NA	NA	NA	NA	NA	NA
Lipase	(+)	+	+	+	−	−	−	+	+
Urease	+	(+)	+	NA	NA	NA	NA	NA	NA
Gelatinase	−	−	NA	+	−	+	−	+	+
Nitrate to nitrite reduction	+	+	+	+	NA	NA	+	−	−
Production of fluorescent pigment on King's B medium	−	+	+	+	+	+	+	NA	+
Utilization of carbon compounds									
Adonitol	+	+	+	NA	−	−	−	−	+
D-Cellobiose	−	−	−	+	NA	NA	+	NA	−
meso-Erythritol	+	+	−	+	−	+	−	+	+
Fumaric acid	−	−	NA	NA	NA	NA	NA	NA	+
D-Galactose	+	+	+	+	+	+	+	−	+
D-Glucose	+	+	+	NA	+	+	−	+	+
Glycerol	+	+	NA	NA	NA	+	+	−	+
meso-Inositol	+	+	+	+	+	+	−	+	+
Lactose	−	−	−	NA	NA	−	−	+	−
D-Maltose	−	−	−	NA	NA	+	+	+	−
D-Mannitol	+	+	+	−	+	+	+	−	+
D-Melibiose	−	−	−	NA	NA	−	−	+	−
L-Rhamnose	−	−	NA	NA	−	−	−	+	−
Sucrose	−	−	NA	+	+	+	+	+	+
D-Xylose	−	−	+	+	+	+	+	+	+

Table 2. Phenotypic differences between strain CMS 64^T (*P. proteolytica*), *P. brenneri* and *P. migulae*

Taxa: 1, CMS 64^T; 2, *P. brenneri* [data from Baïda *et al.* (2001)]; 3, *P. migulae* [data from Verhille *et al.* (1999)]. +, Positive; −, negative; NA, data not available.

Characteristic	1	2	3
Growth characteristics			
Temperature range for growth (°C)	4–30	4–37	4–35
Optimum growth temperature (°C)	22	25	30
Phenotypic characteristics			
Phosphatase	−	+	NA
Lipase	+	+	−
Gelatinase	+	+	−
Levan formation on sucrose	−	+	+
Utilization of carbon compounds			
Adonitol	+	+	−
L-Arabinose	−	−	+
Erythritol	+	+	−
Fumaric acid	−	−	+
meso-Inositol	+	+	−
L-Rhamnose	−	+	−
Sorbitol	+	−	−
D-Xylose	−	−	+
L-Aspartic acid	−	+	+
L-Proline	−	+	+
L-Tryptophan	+	−	−

P. brenneri (Baïda *et al.*, 2001) and *P. migulae* (Verhille *et al.*, 1999) (Fig. 2), with a bootstrap value of 86 %. The other two Antarctic isolates, CMS 35^T and CMS 38^T (Fig. 2), appear to be related more closely to *P. orientalis* (Dabboussi *et al.*, 1999).

Identification of strain CMS 35^T as *Pseudomonas antarctica* sp. nov.

Strain CMS 35^T can be differentiated from strains CMS 38^T and CMS 64^T with respect to its protein profile, riboprint, phenotypic characteristics and low (40 %) DNA–DNA relatedness (Tables 1 and 2; Supplementary Table B, available in IJSEM Online; Fig. 1). Strain CMS 35^T can also be differentiated easily from the closely related species *P. orientalis* (Dabboussi *et al.*, 1999), *P. marginalis*, *P. rhodesiae* and *P. veronii*, based on phenotypic characteristics (Table 1) and the fact that it shows <60 % relatedness at the DNA–DNA level with these species. Therefore, strain CMS 35^T is proposed as the type strain of a novel species of the genus *Pseudomonas*, to which the name *Pseudomonas antarctica* sp. nov. is assigned.

Identification of strain CMS 38^T as *Pseudomonas meridiana* sp. nov.

Strain CMS 38^T, which is different from CMS 35^T (Table 1), shares 99·46 % 16S rRNA gene sequence similarity with *P. orientalis* (Fig. 2); however, it differs from *P. orientalis*

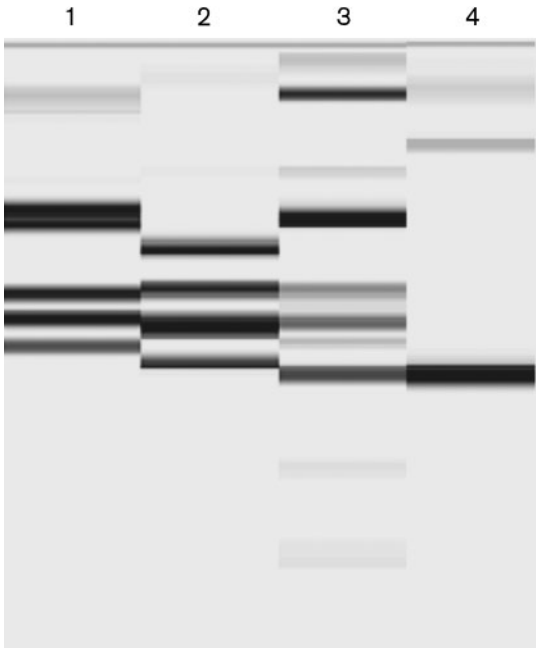

Fig. 1. Riboprinting of strains: 1, CMS 38^T (*Pseudomonas meridiana*); 2, CMS 35^T (*Pseudomonas antarctica*); 3, CMS 64^T (*Pseudomonas proteolytica*); 4, *P. brenneri* CIP 106646^T.

(Dabboussi *et al.*, 1999) in a number of phenotypic traits (Table 1) and its protein profile and exhibits 64 % relatedness at the DNA–DNA level (Supplementary Table B, available in IJSEM Online). In addition, strain CMS 38^T exhibits 52, 58 and 49 % DNA–DNA relatedness (Supplementary Table B, available in IJSEM Online) respectively with *P. marginalis*, *P. rhodesiae* and *P. veronii*, which are part of the main cluster of the phylogenetic tree (Fig. 2). DNA–DNA hybridization was not performed between strain CMS 38^T and *Pseudomonas extremorientalis* (Ivanova *et al.*, 2002), *P. tolaasii* (Bradbury, 1987) or *Pseudomonas costantinii* (Munsch *et al.*, 2002), but they can be differentiated easily, based on phenotypic characteristics (Table 1). Thus, based on the above differences between strains CMS 38^T, CMS 35^T and *P. orientalis*, CMS 38^T is proposed as the type strain of a novel species of the genus *Pseudomonas*, to which the name *Pseudomonas meridiana* sp. nov. is assigned.

Identification of strain CMS 64^T as *Pseudomonas proteolytica* sp. nov.

Strain CMS 64^T can be differentiated from strains CMS 35^T and CMS 38^T by phenotypic characteristics (Tables 1 and 2). In addition, these isolates differ in their protein profiles, riboprints and DNA–DNA relatedness (Supplementary Table B, available in IJSEM Online; Fig. 1). At the 16S rRNA gene sequence level, strain CMS 64^T is related closely to *P. brenneri* (99·73 %) and *P. migulae* (99·75 %) (Fig. 2). Characteristics that differentiate strain CMS 64^T from *P. migulae* (Verhille *et al.*, 1999) and *P. brenneri* (Baïda *et al.*, 2001) are listed in Table 2. Furthermore, strain CMS

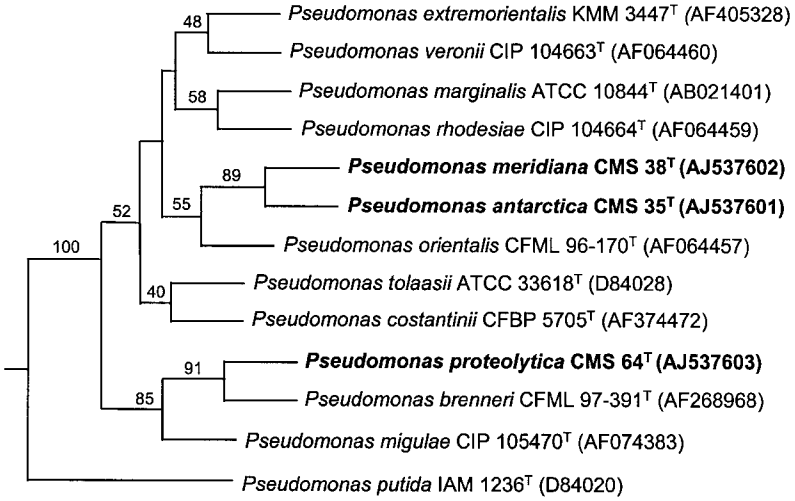

Fig. 2. UPGMA phenogram showing the phylogenetic relationship between the psychrophilic species *P. antarctica* (CMS 35^T), *P. proteolytica* (CMS 64^T) and *P. meridiana* (CMS 38^T) and other related species of the genus *Pseudomonas*, based on 16S rDNA sequence analysis. Bootstrap values are given at nodes. Branch-lengths in the phenogram are not to scale.

64^T exhibits differences in protein profile and shows only 55 % relatedness with *P. migulae* and 68 % with *P. brenneri* at the DNA–DNA level (Supplementary Table B, available in IJSEM Online). The riboprint of strain CMS 64^T is also different from that of *P. brenneri* (Fig. 1). Thus, based on phenotypic, genotypic and phylogenetic characteristics, strain CMS 64^T is proposed as the type strain of a novel species of the genus *Pseudomonas*, to which the name *Pseudomonas proteolytica* sp. nov. is assigned.

Description of *Pseudomonas antarctica* sp. nov.

Pseudomonas antarctica (an.tarc′ti.ca. N.L. fem. adj. *antarctica* pertaining to Antarctica).

Forms circular, convex, smooth and translucent colonies with a diameter of 1–2 mm. Cells are Gram-negative, motile with a polar flagellum, rod-shaped and psychrophilic (grow at 4–30 °C), can tolerate 3 % NaCl (w/v) and grow at an optimum pH of 7. Positive for catalase, oxidase, urease and phosphatase and weakly positive for lipase; reduces nitrate to nitrite; negative for β-galactosidase, gelatinase, arginine dihydrolase, arginine decarboxylase, lysine decarboxylase, indole production, methyl red and Voges–Proskauer test. Does not hydrolyse aesculin, starch or cellulose. Acid is produced from D-fructose, D-galactose, D-glucose, D-mannose and D-mannitol, but not from L-arabinose, lactose, L-rhamnose, sucrose or D-xylose. Utilizes acetate, adonitol, citrate, *meso*-erythritol, D-fructose, D-galactose, D-glucose, glycerol, *meso*-inositol, lactic acid, D-mannose, D-mannitol, pyruvate, D-ribose, sorbitol, trehalose, L-alanine, L-arginine, L-glutamic acid, L-glutamine, L-lysine, L-serine, L-tyrosine and L-tryptophan as sole carbon sources, but not L-arabinose, D-cellobiose, cellulose, dextran, dulcitol, fumaric acid, inulin, lactose, D-maltose, D-melibiose, melezitose, D-raffinose, L-rhamnose, D-sorbose, sucrose, succinic acid, thioglycollate, D-xylose, L-aspartic acid, L-aspargine, L-cysteine, L-glycine, L-histidine, L-leucine, L-isoleucine, L-methionine, L-proline, L-threonine or L-valine. Sensitive to the antibiotics ampicillin, amoxycillin, bacitracin, carbenicillin, chloramphenicol, chlortetracycline, colistin, cotrimoxazole, erythromycin, kanamycin, gentamicin, lincomycin, nitrofurazone, nitrofurantoin, nystatin, oxytetracyclin, penicillin, polymyxin B, rifampicin, tetracycline and tobramycin, but resistant to furazolidone, furoxone and trimethoprim. DNA G+C content is 60·7 mol%.

The type strain is CMS 35^T (=MTCC 4992^T=DSM 15318^T).

Description of *Pseudomonas meridiana* sp. nov.

Pseudomonas meridiana (me.ri.di.a′na. L. fem. adj. *meridiana* of or belonging to the south or south side, southern, southerly, meridional; pertaining to the South Pole).

Forms circular, convex, smooth and translucent colonies with a diameter of 1–2 mm. Cells are Gram-negative, motile with a polar flagellum, rod-shaped and psychrophilic (grow at 4–30 °C), can tolerate 3 % NaCl (w/v) and grow at an optimum pH of 7. Positive for catalase, oxidase and lipase and weakly positive for urease; reduces nitrate to nitrite; negative for phosphatase, β-galactosidase, gelatinase, arginine dihydrolase, arginine decarboxylase, lysine decarboxylase, indole production, methyl red and Voges–Proskauer test. Does not hydrolyse aesculin, starch or cellulose. Acid is produced from D-fructose, D-galactose, D-glucose, D-mannose and D-mannitol, but not from L-arabinose, lactose, L-rhamnose, sucrose or D-xylose. Utilizes acetate, adonitol, citrate, *meso*-erythritol, D-fructose, D-galactose, D-glucose, L-arabinose, glycerol, *meso*-inositol, lactic acid, D-mannose, D-mannitol, pyruvate, D-ribose, sorbitol, trehalose, L-alanine, L-arginine, L-glutamic acid, L-glutamine, L-lysine, L-serine, L-tyrosine and L-tryptophan as sole carbon sources, but not D-cellobiose, cellulose, dextran, dulcitol, fumaric acid, inulin, lactose, D-maltose, D-melibiose, melezitose, D-raffinose, L-rhamnose, D-sorbose, sucrose, succinic acid, thioglycollate, D-xylose, L-aspartic acid, L-aspargine, L-cysteine, L-glycine, L-histidine, L-leucine, L-isoleucine, L-methionine, L-proline, L-threonine

or L-valine. Resistant to ampicillin, amoxycillin, bacitracin, carbenicillin, chloramphenicol, colistin, cotrimoxazole, erythromycin, furazolidone, furoxone, gentamicin, lincomycin, nitrofurantoin, nystatin, penicillin, polymyxin B and trimethoprim, but sensitive to chlortetracycline, kanamycin, nitrofurazone, oxytetracycline, rifampicin, tetracycline and tobramycin. DNA G+C content is 63·2 mol%.

The type strain is CMS 38^T (=MTCC 4993^T=DSM 15319^T).

Description of *Pseudomonas proteolytica* sp. nov.

Pseudomonas proteolytica (pro.te.o.ly′ti.ca. N.L. fem. adj. *proteolytica* proteolytic).

Forms circular, convex, smooth and translucent colonies with a diameter of 1–2 mm. Cells are Gram-negative, motile with a polar flagellum, rod-shaped and psychrophilic (grow at 4–30 °C), can tolerate 3 % NaCl (w/v) and grow at an optimum pH of 7. Positive for catalase, oxidase, lipase and gelatinase; reduces nitrate to nitrite; negative for phosphatase, urease, β-galactosidase, arginine dihydrolase, arginine decarboxylase, lysine decarboxylase, indole production, methyl red and Voges–Proskauer test. Does not hydrolyse aesculin, starch or cellulose. Acid is produced from D-fructose, D-galactose, D-glucose, D-mannose and D-mannitol, but not from L-arabinose, lactose, L-rhamnose, sucrose or D-xylose. Utilizes acetate, adonitol, citrate, *meso*-erythritol, D-fructose, D-galactose, D-glucose, glycerol, *meso*-inositol, lactic acid, D-mannose, D-mannitol, pyruvate, D-ribose, sorbitol, trehalose, L-alanine, L-arginine, L-glutamic acid, L-glutamine, L-lysine, L-serine, L-methionine, L-phenylalanine, L-tyrosine and L-tryptophan as sole carbon sources, but not D-cellobiose, cellulose, dextran, dulcitol, fumaric acid, inulin, lactose, D-maltose, D-melibiose, melezitose, D-raffinose, L-rhamnose, D-sorbose, sucrose, succinic acid, thioglycollate, D-xylose, L-aspartic acid, L-aspargine, L-cysteine, L-glycine, L-histidine, L-leucine, L-isoleucine, L-proline, L-threonine or L-valine. Resistant to ampicillin, amoxycillin, bacitracin, carbenicillin, chloramphenicol, cotrimoxazole, erythromycin, furazolidone, furoxone, lincomycin, nitrofurazone, nitrofurantoin, nystatin, penicillin and trimethoprim, but sensitive to chlortetracycline, colistin, gentamicin, kanamycin, oxytetracycline, polymyxin B, rifampicin, tetracycline and tobramycin. DNA G+C content is 58·3 mol%.

The type strain is CMS 64^T (=MTCC 4994^T=DSM 15321^T).

Acknowledgements

This work was supported by a grant from the Department of Biotechnology, Government of India, New Delhi, India. The riboprinting work was carried out at DSMZ, Germany, through the UNESCO–IUMS–MIRCENS–SGM short-term fellowship awarded to G. S. N. R. by the International Union of Microbiological Societies (IUMS).

References

Anzai, Y., Kim, H., Park, J. Y., Wakabayashi, H. & Oyaizu, H. (2000). Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol* **50**, 1563–1589.

Baïda, N., Yazourh, A., Singer, E. & Izard, D. (2001). *Pseudomonas brenneri* sp. nov., a new species isolated from natural mineral waters. *Res Microbiol* **152**, 493–502.

Bradbury, J. F. (1987). *Pseudomonas tolaasi*. In *CMI Descriptions of Pathogenic Fungi and Bacteria*, no. 894. Kew, UK: CAB International Mycological Institute.

Bruni, V., Gugliandolo, C., Maugeri, T. & Allegra, A. (1999). Psychrotrophic bacteria from a coastal station in the Ross sea (Terra Nova Bay, Antarctica). *New Microbiol* **22**, 357–363.

Coroler, L., Elomari, M., Hoste, B., Gillis, M., Izard, D. & Leclerc, H. (1996). *Pseudomonas rhodesiae* sp. nov., a new species isolated from natural mineral waters. *Syst Appl Microbiol* **19**, 600–607.

Dabboussi, F., Hamze, M., Elomari, M., Verhille, S., Baida, N., Izard, D. & Leclerc, H. (1999). Taxonomic study of bacteria isolated from Lebanese spring waters: proposal for *Pseudomonas cedrella* sp. nov. and *P. orientalis* sp. nov. *Res Microbiol* **150**, 303–316.

De Ley, J. (1992). The Proteobacteria: ribosomal RNA cistron similarities and bacterial taxonomy. In *The Prokaryotes*, 2nd edn, pp. 2111–2140. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.

Elomari, M., Coroler, L., Hoste, B., Gillis, M., Izard, D. & Leclerc, H. (1996). DNA relatedness among *Pseudomonas* strains isolated from natural mineral waters and proposal of *Pseudomonas veronii* sp. nov. *Int J Syst Bacteriol* **46**, 1138–1144.

Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Department of Genetics, University of Washington, Seattle, USA.

Higgins, D. G., Bleasby, A. T. & Fuchs, R. (1992). CLUSTAL V: improved software for multiple sequence alignment. *Comput Appl Biosci* **8**, 189–191.

Holding, A. J. & Collee, J. G. (1971). Routine biochemical tests. *Methods Microbiol* **6A**, 2–32.

Hugh, R. & Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *J Bacteriol* **66**, 24–26.

Ivanova, E. P., Gorshkova, N. M., Sawabe, T. & 8 other authors (2002). *Pseudomonas extremorientalis* sp. nov., isolated from a drinking water reservoir. *Int J Syst Evol Microbiol* **52**, 2113–2120.

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.

Kriss, A. E., Mitskevich, I. N., Rozanova, E. P. & Osnitskaia, L. K. (1976). Microbiological studies of the Wanda Lake (Antarctica). *Mikrobiologiya* **45**, 1075–1081 (in Russian).

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Maugeri, T. L., Gugliandolo, C. & Bruni, V. (1996). Heterotrophic bacteria in the Ross Sea (Terra Nova Bay, Antarctica). *New Microbiol* **19**, 67–76.

Migula, W. (1984). Über ein neues System der Bakterien. *Arb Bakteriol Inst Karlsruhe* **1**, 235–238 (in German).

Moore, E. R. B., Mau, M., Arnscheidt, A., Böttger, E. C., Hutson, R. A., Collins, M. D., Van De Peer, Y., De Wachter, R. & Timmis, K. N.

(1996). The determination and comparison of the 16S rRNA gene sequences of species of the genus *Pseudomonas* (sensu stricto) and estimation of the natural intrageneric relationships. *Syst Appl Microbiol* **19**, 478–492.

Munsch, P., Alatossava, T., Marttinen, N., Meyer, J.-M., Christen, R. & Gardan, L. (2002). *Pseudomonas costantinii* sp. nov., another causal agent of brown blotch disease, isolated from cultivated mushroom sporophores in Finland. *Int J Syst Evol Microbiol* **52**, 1973–1983.

Palleroni, N. J., Kunisawa, R., Contopolou, R. & Doudoroff, M. (1973). Nucleic acid homologies in the genus *Pseudomonas*. *Int J Syst Bacteriol* **23**, 333–339.

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., Matsumoto, G. I., Stackebrandt, E. & Shivaji, S. (2002). *Arthrobacter roseus* sp. nov., a psychrophilic bacterium isolated from an Antarctic cyanobacterial mat sample. *Int J Syst Evol Microbiol* **52**, 1017–1021.

Reddy, G. S. N., Prakash, J. S. S., Prabahar, V., Matsumoto, G. I., Stackebrandt, E. & Shivaji, S. (2003). *Kocuria polaris* sp. nov., an orange-pigmented psychrophilic bacterium isolated from an Antarctic cyanobacterial mat sample. *Int J Syst Evol Microbiol* **53**, 183–187.

Sato, N. & Murata, N. (1988). Membrane lipids. *Methods Enzymol* **167**, 251–259.

Shinde, P. A. & Lukezic, F. L. (1974). Isolation, pathogenicity and characterization of fluorescent pseudomonads associated with discoloured alfalfa roots. *Phytopathology* **64**, 865–871.

Shivaji, S., Rao, N. S., Saisree, L., Sheth, V., Reddy, G. S. N. & Bhargava, P. M. (1989a). Isolation and identification of *Pseudomonas* spp. from Schirmacher Oasis, Antarctica. *Appl Environ Microbiol* **55**, 767–770.

Shivaji, S., Rao, N. S., Saisree, L., Reddy, G. S. N., Seshu Kumar, G. & Bhargava, P. M. (1989b). Isolates of *Arthrobacter* from the soils of Schirmacher Oasis, Antarctica. *Polar Biol* **10**, 225–229.

Shivaji, S., Ray, M. K., Rao, N. S., Saisree, L., Jagannadham, M. V., Seshu Kumar, G., 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.

Shivaji, S., Vijaya Bhanu, N. & Aggarwal, R. K. (2000). Identification of *Yersinia pestis* as the causative organism of plague in India as determined by 16S rDNA sequencing and RAPD-based genomic fingerprinting. *FEMS Microbiol Lett* **189**, 247–252.

Stanier, R. Y., Palleroni, N. J. & Doudoroff, M. (1966). The aerobic pseudomonads: a taxonomic study. *J Gen Microbiol* **43**, 159–271.

Stolp, H. & Gadkari, D. (1981). Nonpathogenic members of the genus *Pseudomonas*. In *The Prokaryotes*, vol. 1, pp. 719–741. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.

Tourova, T. P. & Antonov, A. S. (1987). Identification of micro-organisms by rapid DNA-DNA hybridisation. *Methods Microbiol* **19**, 333–355.

Verhille, S., Baïda, N., Dabboussi, F., Hamze, M., Izard, D. & Leclerc, H. (1999). *Pseudomonas gessardii* sp. nov. and *Pseudomonas migulae* sp. nov., two new species isolated from natural mineral waters. *Int J Syst Bacteriol* **49**, 1559–1572.