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Isolation and Identification of *Pseudomonas* spp. from Schirmacher Oasis, Antarctica

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Ten cultures of *Pseudomonas* spp. were established from soil samples collected in and around a lake in Antarctica. Based on their morphology, biochemical and physiological characteristics, and moles percent G+C of their DNA, they were identified as *P. fluorescens*, *P. putida*, and *P. syringae*. This is the first report on the identification of *Pseudomonas* spp. from continental Antarctica.

Microbiological studies in Antarctica have so far been confined to maritime Antarctica (4, 5, 7, 8, 10, 13, 34) and specific regions of continental Antarctica such as the Victoria dry valleys and the McMurdo station area (16, 17, 19, 22). There have been no reports on the taxonomy of bacteria present in the oasis regions of continental Antarctica. The oasis regions, such as Schirmacher and Bunger, are unique in that they are as cold as the dry valleys, but they differ from the dry valleys in that they are under ice cover only during the Antarctic winter and experience significant precipitation (37). The present investigation was undertaken to study the bacteria present in the Schirmacher Oasis, Antarctica, to establish their taxonomy and to compare the predominant genera of bacteria with those reported from the other regions of Antarctica.

The Schirmacher Oasis, Queen Maud Land (referred to as Dakshin Gangotri Hill Ranges by India), is situated on the coast of Antarctica between the inland and shelf ice and occupies an area of 35 km^2. The geographical coordinates of the sampling area are 70°45′12″ S and 11°46′ E. A prominent feature of Schirmacher Oasis is the presence of a number of small ponds and about twenty lakes (37). The largest, Lake Zub, has an area of 0.5 km^2 and a maximum depth of 5 m. The supply of melt water to the lakes from the inland and shelf glaciers begins in November and ends in February (29), after which the lakes freeze. Since all the lakes in the Schirmacher Oasis are 80 to 100 km from the sea, these lakes, in contrast to the saline lakes of the coastal oases, show a very low level of mineralization.

Sediment and soil were collected at random in and around Lake Zub and five other unnamed lakes in the vicinity. During the collection period (that is, the third week of January and of February 1985), these lakes and the surrounding areas were free of ice, and the soil samples were sandy. The soil temperature varied from +6 to −6°C. In areas immediately bordering the lakes, luxuriant growth of algae, mosses, and lichens was observed. Skua was the only bird species residing in this area.

For collection of the samples, about 1 cm of the surface soil was cleared with a sterile spatula and the underlying soil was collected and transferred to sterile polythene bags, which were then sealed. The samples were plated either directly at the site of collection on prepoured media plates (0.5% peptone, 0.1% yeast extract, 1.5% agar, 5% [vol/vol] soil extract from the Schirmacher Oasis) or after serial dilution in the laboratory at the Dakshin Gangotri base camp. The colony counts were determined after 2 weeks of incubation at 10°C.

All the isolates described in the present study could be maintained in the above-described medium in the absence of soil extract. Morphology, motility, and optimum conditions for growth were determined by conventional methods. Flagellum staining was by the silver impregnation method (3). The growth of the cultures on YDC agar (1% yeast extract, 2% glucose, 2% calcium carbonate, 1.5% agar) and NY medium (0.5% peptone, 0.5% yeast extract, 0.3% beef extract, 0.8% sodium chloride) (33) in the presence of different carbon compounds and 18 antibiotics (HiMedia disks) was also checked. All the biochemical tests listed in Table 1 and sensitivity tests were performed by growing the cultures at 20°C in the appropriate media and following procedures described earlier (14, 15, 26, 32, 33). DNA was isolated by the procedures of Marmur (20) and Beji et al. (2), and the moles percent G+C of the DNA was determined from the melting curves (T_m) obtained with a Beckman 5260 spectrophotometer and the equation of Schildkraut and Lifson (30). Type cultures of *Pseudomonas aeruginosa* (NCTC 6751), *P. putida* (NCIM 2102), and *P. fluorescens* (ATCC 8251) were used as positive controls. Cultures of all the Antarctica organisms have been deposited with the National Collection of Industrial Microorganisms, National Chemical Laboratory, Poona 411 008, India.

The bacteria of Antarctica studied earlier have shown a great degree of variation in size and shape (12, 37). The soils of Schirmacher Oasis also contain a heterogeneous population of bacteria consisting of rods, cocci, clumps of cocci, chains of rods, and single long, filamentous cells. The bacterial count varied from 2×10^3 to 1.2×10^5 cells per g of soil (Table 2); it is known that the abundance of bacteria in the soils of Antarctica is dependent on the local climatic conditions, the richness of the soil, and the nutrient supply (1, 12, 31). From the original medium plates, about 200 colonies were transferred to fresh medium plates. Of these, on the basis of colony morphology—that is, the shape, size, and color of a colony—45 pure cultures of bacteria were obtained, of which 10 were gram-negative motile rods, 3 were gram-negative nonmotile rods, 5 were gram-positive cocci, and the remaining 27 were gram-positive nonsporulating rods. At this stage, we normally would have selected for *Pseudomonas* spp. by using specific enrichment media but did not do so because many of the bacteria isolated earlier from Antarctica appear to possess atypical character-

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TABLE 1. Characteristics of *Pseudomonas* spp. from Antarctica[a]

Isolate no. or strain	No. of polar flagella	Presence of:							Generation time (h)	mol% G+C	Acid from:				
		Oxidase	Phosphatase	Gelatinase	Urease	Arginine dihydrolase	Denitrification	Levan formation			Fructose or lactose	Glucose	Galactose	Mannose	Sucrose
1W	1	+	+	+	+	+	−	−	1.2	62.4	−	+	−	−	−
9AW	2	+	−	+	+	+	−	+	1.1	63.2	−	−	−	+	−
9BW	3	+	−	−	−	+	−	+	1.3	63.5	−	+	−	+	+
10AW	2	+	−	+	+	+	+	+	1.1	63.2	−	+	+	+	−
10CW	2	+	−	+	+	+	−	−	1.0	63.5	−	−	+	+	−
29W	1	+	−	+	+	+	−	−	1.4	58.3	−	−	+	+	−
39W	5	+	+	+	+	+	−	+	1.1	68.0	−	+	+	+	−
44W	1	+	+	+	+	+	+	−	1.1	61.1	−	−	+	+	−
51W	1	+	−	+	+	+	−	−	1.0	57.1	−	+	+	+	−
Lz4W	1	−	+	+	+	−	−	−	1.1	64.4	−	+	−	+	+
P. fluorescens[b]	3	+	+	+	+	+	+	+	ND	63.2	−	+	+	ND	ND
P. putida[b]	2	+	+	−	+	+	−	−	ND	61.5	−	+	+	ND	ND
P. aeruginosa[b]	1	+	+	+	+	+	+	−	ND	67.0	−	+	+	ND	ND

[a] All the isolates were positive for catalase and lipase and were capable of growing on citrate agar and cetrimide agar; they were negative for β-galactosidase, indole production, methyl red test, polyhydroxybutyrate accumulation, starch hydrolysis, and esculin hydrolysis. ND, Not determined.
[b] Type strains. For details, see the text.

istics; therefore, the procedures used for enrichment and isolation of mesophilic strains are not successful for isolating similar bacteria from Antarctica (24, 28, 38). We selected 10 gram-negative motile rods for detailed studies.

To the best of our knowledge, this is the first report on bacteria from an oasis region of Antarctica. The 10 isolates reported in this paper had the following common features: they were gram-negative straight rods (length, 0.8 to 2 μm; diameter, 0.5 to 0.8 μm), motile by one or more polar flagella, aerobic, did not grow under acidic conditions (pH 4), did not require organic growth factors, grew on acetate as the sole carbon source, were oxidase (except Lz4W) and catalase positive but indole negative, did not produce gas from sugars, and had 57 to 68 mol% G+C in their DNA. Because these characteristics are the main features of bacteria belonging to the genus *Pseudomonas* (6, 27), it was concluded that the 10 isolates belong to the genus *Pseudomonas*. All the isolates were white, but on YDC and NY agar they appeared cream colored.

All the isolates were psychrotrophs in that they could grow at 4, 10, 20, and 30°C, with an optimum temperature around 20°C. Ellis-Evans (9) has already indicated that a majority of the terrestrial bacteria of Antarctica are psychrotrophic. None of the cultures grew at pH 4, but growth occurred between pHs 6 and 9, with an optimum around pH 6.9. Except for isolates 1W, 9BW, and 39W, all the isolates could tolerate 5.8% salt in the medium. In 11.6% salt, growth was not observed in any of the cultures. The generation time of the cultures when determined under optimum conditions of growth ranged from 1 to 1.4 h (Table 1).

Like the mesophilic *Pseudomonas* spp., the present psychrotrophic isolates also could utilize a number of carbon compounds when they were provided as the sole carbon source in minimal A medium without citrate and glucose (23). They could grow on L-arabinose, D-xylose, L-rhamnose, D-ribose, D-fructose, D-mannose, D-galactose, sucrose, raffinose, erythritol, mannitol, glycerol, *m*-inositol, sorbitol, succinate, citrate, acetate, pyruvate, lactate, glutamate, formate, salicylate, β-hydroxybutyrate, L-malate, valine, L-alanine, L-arginine, L-methionine, L-lysine, or glutamine provided as the sole carbon source. Isolates 9BW and Lz4W failed to grow on L-histidine, aspargine, and *n*-propanol, but the remaining cultures could utilize these compounds. Unlike the other cultures, the culture of Lz4W also did not grow on glycine, L-serine, ethanol, phenylalanine, L-tyrosine, and polyethylene glycol. None of the cultures could grow when maltose, lactose, inulin, cellulose, melibiose, dextrin, glycogen, phthalate, butanol, or starch was provided as the sole carbon source. None of the cultures produced gas in the presence of any of the six carbohydrates used (Table 1). However, all the cultures acidified the medium. Most of the cultures showed a preference for galactose and mannose over other carbon sources (Table 1).

All the cultures were sensitive to kanamycin (amount per disk, 40 μg), gentamicin (10 μg), tobramycin (15 μg), polymyxin B (300 U), tetracycline (30 μg), rifamycin (10 μg), colistin (10 μg), streptomycin (10 μg), and nalidixic acid (30 μg) but were resistant to erythromycin (15 μg), penicillin (10 U), bacitracin (10 U), carbenicillin (50 μg), trimethoprim (10 μg), vancomycin (30 μg), ampicillin (10 μg), and nystatin

TABLE 2. Bacterial counts in soils of Schirmacher Oasis, Antarctica

Sample no.	Source	Depth of collection	CFU/g of soil (10^3)	Isolate no.[a]
1	Lake sediment	5 m	30	1W
9	Soil from below an algal felt	2 cm	4.4	9AW, 9BW
10	Soil from below a moss bed	2 cm	20	10AW, 10CW
29	Soil from below a moss bed	2 cm	28	29W
39	Soil from a water puddle	5 cm	4	39W
44	Soil from the lake shore	2 cm	7	44W
51	Soil from below an algal felt	2 cm	2	51W
Lz4	Soil from a penguin rookery with algae	2 cm	120	Lz4W

[a] The pure colonies established from sources listed and studied in the present investigation.

(100 μg). Sensitivity to chloramphenicol (30 μg) varied; isolates 1W, 9AW, 10AW, 10 CW, and 29W were resistant, while 9BW, 39W, 44W, 51W, and Lz4W were sensitive.

The results of all the biochemical tests are summarized in Table 1. The moles percent G+C ranged from 57 to 68 (Table 1). Batch-to-batch variation in the T_m values of the DNA preparations was ±1°C.

A species-level identification of the isolates was attempted with the keys published by Palleroni (27) and Stolp and Gadkari (33). A majority of the isolates from Schirmacher Oasis, viz., 1W, 9AW, 10AW, 10CW, 29W, 39W, 44W, and 51W, appeared very similar to *P. fluorescens* in that they produced a fluorescent green pigment only on King B medium, did not produce pyocyanin on King A medium (18), produced cream-colored colonies on YDC agar and NY agar, and were capable of growing on cetrimide agar and on media containing ammonium as the sole nitrogen source and glucose as the sole carbon source. Further, like *P. fluorescens*, all these isolates were positive for catalase, oxidase, lipase, arginine dihydrolase, gelatinase, and urease and did not hydrolyze starch. They could grow at 4°C but not at 41°C (33). Their sensitivity to antibiotics, as well as their nutrient requirements, also matched those reported for *P. fluorescens* (27, 33). The moles percent G+C of 1W, 9AW, 10AW, 10CW, 29W, 39W, 44W, and 51W varied between 57.1 and 68.0 (Table 1); in the reference strains, it varied between 59.4 and 63.6 (27, 33).

P. fluorescens has been differentiated into biotypes A, B, C, F, and G (6, 27, 33) on the basis of levan formation, denitrification, and pigment production. When these criteria were used, 44W was assigned to biotype C; 9AW and 39W were assigned to biotype A; 1W, 10CW, 29W, and 51W were assigned to biotype G; and 10AW was assigned to biotype F of *P. fluorescens*.

Isolate 9BW produced fluorescent pyoverdin, was gelatinase and denitrification negative, and had all the other key characteristics of *P. putida* (27, 33), including various enzymatic activities and nutrient requirements, except that it failed to grow in the presence of L-histidine and butanol. The moles percent G+C was 63.5, which is comparable to the 62.5% reported for *P. putida*. Since this isolate was capable of assimilating L-arabinose and D-galactose and was also capable of growing at 4°C, it probably belongs to biotype B of *P. putida* (33). However, it differed from *P. putida* in that it was sensitive to the antibiotic chloramphenicol.

Biovars of *P. syringae* are mainly foliar pathogens. However, they could also survive as free-living microorganisms in soil, utilizing the residues of diseased plants (25). Isolate Lz4W, which is oxidase negative, arginine dihydrolase negative, white to cream in color, and lipase positive and does not utilize D-(−)-tartarate, seems to be *P. syringae*. The nutrient requirements of Lz4W were found to be identical to that reported for the type strain of *P. syringae* in that it did not utilize glycine, serine, *n*-propanol, ethanol, butanol, phenylalanine, L-tyrosine, and polyethylene glycol but utilized all the other compounds tested. The moles percent G+C of DNA was 64.4, compared with 59 to 61 mol% reported in the literature for *P. syringae* (27).

The terrestrial microflora of greater Antarctica, peninsula of Antarctica, and maritime Antarctica is dominated by *Pseudomonas*, *Micrococcus*, *Achromobacter*, *Alcaligenes*, *Chromobacterium*, *Flavobacterium*, *Aeromonas*, and *Planococcus* species (24, 35, 36, 38). Of these bacteria, the only gram-negative bacteria which have been identified to the species level belong to the genera *Chromobacterium*, *Flavobacterium*, *Aeromonas*, and *Pseudomonas* (36, 38); these were all isolated from the soils of Signy Island in maritime Antarctic. In a recent study by Tearle and Richard (36), 10 of the 14 gram-negative isolates were identified as *Pseudomonas* spp.; 7 of the 10 were identified as *P. fluorescens*, and 3 were identified as *P. paucimobilis* (36), clearly indicating that *P. fluorescens* is the dominant *Pseudomonas* species in the soils of Signy Island. Detailed studies on *Pseudomonas* spp. from other regions of Antarctica are not available.

The present study clearly establishes for the first time the presence of *Pseudomonas* spp. in an oasis region of Antarctica. It also indicates that in the oasis regions, as in maritime Antarctica, a *Pseudomonas* spp. may dominate the gram-negative bacterial population, the predominant species being *P. fluorescens*.

The 10 isolates of *Pseudomonas* spp. from Schirmacher Oasis possess certain unique characteristics by which they differ from the corresponding mesophilic *Pseudomonas* spp. They could all grow at low temperatures (4°C) and were sensitive to gentamicin, kanamycin, streptomycin, and tetracycline, unlike the mesophilic *Pseudomonas* spp. This is particularly interesting since resistance to these antibiotics in *P. fluorescens* and *P. putida* is chromosomal. These characteristics probably reflect the adaptation of these microorganisms to the prevailing climatic and biotic conditions in their immediate environment. In fact, earlier isolates of *Halomonas subglaciescola* (11), *Flectobacillus glomeratus* (21), *Desulfovibrio* sp. (28), *Chromobacterium* spp. (38), *Planococcus* spp. (24), and *Flavobacterium aquatile* (36) from Antarctica have been shown to have atypical characteristics and not be identical with the type strains.

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