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Isolation and identification of *Micrococcus roseus* and *Planococcus* sp. from Schirmacher oasis, Antarctica

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Abstract. Five cultures isolated from soil samples collected in Schirmacher oasis, Antarctica, have been identified as members of the family *Micrococcaceae,* with 3 belonging to the genus *Micrococcus* and two to *Planococcus.* The 3 *Micrococcus* isolates (37R, 45R and 49R) were red-pigmented and h a d ~ 75 mol% G + C in their DNA; they were identified as *Micrococcus roseus.* The two *Planococcus* isolates (30Y and Lz3OR) were yellow and orange in colour, and had 43·5 and 40·9 mol % G + C in their DNA respectively; they were identified as *Planococcus* sp.

Keywords. *Micrococcus; Planococcus;* taxonomy; Schirmacher; Antarctica.

Introduction

Microbiological studies in continental Antarctica are comparatively few and mostly confined to the Victoria dry valley regions and the McMurdo station area (Madden *et al.,* 1979; Johnson and Bellinoff, 1981; Johnson *et al.,* 1981). These studies reveal that the most dominant bacteria in the soils of the dry valleys of Antarctica are *Arthrobacter, Brevibacterium, Corynebacterium* and *Micrococcus.* As yet, there are no reports on the taxonomy of bacteria present in the oasis regions of continental Antarctica. The oasis regions, such as the Schirmacher and Bunger oases, are unique in that they are as cold as the dry valleys but differ from the dry valleys in that they are under ice cover only during the Antarctic winter, and also experience significant precipitation (Walton, 1983). It therefore seemed possible that the terrestrial biology of the oases may vary from that of the dry valley regions. This paper highlights characteristics of a group of 5 Gram-positive nonmotile coccoid bacteria identified as belonging to the genera *Micrococcus* and *Planococcus.*

Materials and methods

Soil samples were collected at random sites around lake Zub, Schirmacher oasis (70°45′12″S and 11°46′E), Antarctica, in the third week of January 1985. The soil temperatures varied from + 6°C to – 6°C.

In all the cases 0·5 cm of the surface layer was cleared with a sterile spatula and the underlying soil collected and plated after serial dilution on preformed plates containing 0·5% peptone, 0·1% yeast extract, 1·5% agar and 5% (v/v) soil extract from Schirmacher oasis. The plates were incubated at 10°C and colony counts were determined after 7 days of incubation. The optimum temperature and pH for growth of the cultures were determined and the cultures were grown under the optimum conditions to determine the generation time. Salt tolerance was tested by

supplementing the plates with appropriate concentrations of NaCl (0·5, 0·1 and 1·5 M).

Cultures in the log phase of growth were observed under the phase contrast microscope for cell shape and size. Motility was determined by direct observation of an overnight culture grown in liquid medium by the hanging drop method and by the piercing of soft agar medium. The presence of flagella was checked by staining the cells by the silver impregnation method (Blenden and Goldberg, 1965).

All tests were performed by growing the cultures at 20°C in the appropriate media. The activities of catalase, oxidase, phosphatase, gelatinase, urease, arginine dihydrolase and ß-galactosidase were determined according to standard methods (Holding and Collee, 1971). Production of indole, utilization of citrate, reduction of nitrate to nitrite, and hydrolysis of starch, Tween 80 and esculin were measured following procedures described earlier (Stainer *et al.,* 1966; Holding and Collee, 1971; Stolp and Gadkari, 1981).

Twenty-six different carbon compounds were used to check the ability of the cultures to utilize a carbon compound, provided as the sole carbon source using minimal A medium without glucose (Miller, 1977) but containing 0·2% (w/v) of the carbon source. The ability to ferment a particular carbohydrate, leading to the formation of acid with or without visible production of gas, was monitored according to Hugh and Leifson (1953).

The sensitivity of the cultures to 17 different antibiotics was carried out using HiMedia antibiotic discs or by supplementing the growth medium with the appropriate concentration of the antibiotic.

DNA was isolated from 1 g (wet weight) of cells according to the procedure of Marmur (1961) and the mol% G + C of the DNA was determined from the melting point (T_m) curves obtained using a Beckman 5260 spectrophotometer. The equation of Schildkraut and Lifson (1965) was used to calculate the mol% G + C of the DNA.

Cell walls were isolated and purified according to the method of Work (1971) and analysed after acid hydrolysis for amino acids in a Beckman analyser.

Results

Bacteria were present in all the soil samples; the bacterial count ranged from $0{\cdot}5\times10^3$ to 15×10^3 cells/g of soil (table 1). From the original plates, about 200 colonies were transferred to fresh plates. Out of these, on the basis of colony

Table 1. Bacterial counts in the soils of Schumacher oasis, Antarctica.

Sample no.	Sample description	Depth of collection (cm)	Colonies x 10^3/g soil	Isolate no.*
37	Soil from lake shore	3	7	37R
45	Soil from lake shore	3	0·53	45R
49	Soil from lake shore	3	1·15	49R
30	Soil from below a lichen bed	2	4·08	30Y
Lz3	Soil from penguin rookery	1	15·1	Lz3OR

*One of the pure colonies established from the particular sample and studied in the present investigation.

morphology, 45 pure cultures of bacteria were established. The pure cultures consisted mostly of rod-shaped or coccoid bacteria; a few appeared either like long filaments or like chains of bacilli.

Morphology

Of the 45 pure cultures, 5 cultures, namely 37R, 45R, 49R, 30Y and Lz3OR, were selected for detailed taxonomic studies (table 1). All the cultures were Gram-positive, nonmotile, coccoid and pigmented. Cultures 37R, 45R and 49R were red, 30Y yellow, and Lz3OR orange in colour. All the colonies were circular and convex and had a smooth margin; their diameter varied from 1–4 mm. Each individual cell was spherical in shape (1–2 μm in diameter) and lacked flagellum; the cells were present as pairs, tetrads or clusters of cocci.

All the cultures exhibited optimum growth at 20°C; at 5°C, 10°C and 25°C, the growth was slower. At 30°C, only 30Y and Lz3OR could grow (table 2). None of the cultures could grow at 37°C. The optimum pH for growth was 6·9; at pH 4, none of the cultures grew. None of the cultures required NaCl for growth. However, they could tolerate up to 0·5 M of NaCl in the growth medium. At concentrations higher than 1 M NaCl, growth was not observed. Under optimum growth conditions, the generation times ranged from 4·5 (30Y) to 20·37 h (45R).

Table 2. Growth characteristics of *M. roseus* and *Planococcus* sp. from Schirmacher oasis, Antarctica.

	M. roseus			*Planococcus* sp.	
Conditions	37R	45R	49R	30Y	Lz3OR
Temperature (°C)*					
5	+	+	+	+	+
15	+ +	+ +	+ +	+ +	+ +
20	+ + +	+ + +	+ + +	+ + +	+ + +
25	+	+	+	+	+
30	–	–	–	+	+
37	–	–	–	–	–
pH*					
4·0	–	–	–	–	–
6·0	+	–	–	–	+
6·9	+ + +	+ + +	+ + +	+ + +	+ + +
9·0	+ +	+ +	+ +	+ +	+ +
[NaCl]* (M)					
0·5	+ + +	+ + +	+ + +	+ + +	+ + +
1·0, 1·5	–	–	–	–	–
Growth on*					
Citrate agar	+ + +	+ + +	+ + +	+ + +	+ + +
Furazolidone agar	+ + +	+ + +	+ + +	–	–
Acid from aerobic					
Glucose or fructose	yes	yes	yes	yes	yes
Lactose or mannose	no	no	no	no	no
Galactose or sucrose	no	no	no	no	no

*The extent of growth was recorded after 5 days of incubation. +, Scanty growth; + +, good growth; + + +, very good growth; –, no growth.

Nutrient requirements

The cultures could grow when L-arabinose, D-xylose, raffinose, glucose, D-fructose, D-mannose, D-galactose, sucrose, D-maltose, mannose, lactose, lactic acid, mannitol, glycerol, myo-inositol, sorbitol, citrate, acetate, pyruvate, pyruvic acid, glutamate, formate, malic acid, dextrin, starch or glucosamine were provided as the sole carbon source. None of the cultures produced gas in the presence of any of the 6 carbohydrates used. However, all the cultures acidified the medium in the presence of certain sugars such as glucose and fructose, but not in the presence of others such as sucrose, galactose, mannose and lactose (table 2).

Biochemical characteristics

The biochemical characteristics of the cultures and their response to 17 different antibiotics is shown in table 3. Amino acid analysis of the purified cell walls indicated the presence of Ala, Glu, Lys, Gly and Asp in all the isolates. In addition, the red isolates 37R, 45R and 49R also showed the presence of Ser and Thr. For the preparation of DNA, the cultures could not be directly lysed with sodium dodecyl sulphate (SDS); hence they were treated with lysozyme (for 2–3 h at 25°C) prior to lysis with SDS. The mol% G + C ranged from 41–80. Batch-to-batch variation in the T_m values of the DNA preparations was ± 2°C.

Table 3. Biochemical characteristics of *M. roseus* and *Planococcus* sp.

	M. roseus			*Planococcus* sp.	
Characteristics	37R	45R	49R	30Y	Lz3OR
Catalase	+	+	+	+	+
Oxidase	–	–	–	–	–
Gelatinase	+	+	+	+	+
Phosphatase	+	+	+	–	–
Urease	–	–	–	–	–
Arginine dihydrolase	–	–	–	–	–
β-Galactosidase	–	–	–	+	+
Indole	–	–	–	–	–
Nitrate reduction	+	+	+	–	–
Hydrolysis of esculin, starch, Tween 80	+	+	+	+	+
Lysozyme susceptibility	+	+	+	+	+
Sensitivity to Kanamycin, streptomycin, erythromycin, novobiocin, neomycin, penicillin G, vancomycin, polymyxin-B, tetracycline, chloramphenicol, ampicillin, nitrofurantoin, gentamycin and rifamycin	S	S	S	S	S
Nystatin and nalidixic acid	R	R	R	R	R
Bacitracin	R	S	S	S	R
Mol% G+C of DNA	73·4	76·6	78·8	43·5	40·9

Discussion

To the best of our knowledge, this is the first report on bacteria from an oasis region of Antarctica. The 5 isolates reported in this paper had all the main features of bacteria belonging to the family *Micrococcaceae* (Schleifer *et al.,* 1981; Schleifer, 1984). This family consists of 4 genera, namely *Micrococcus, Stomatococcus*, *Planococcus* and *Staphylococcus,* which can be differentiated on the basis of their morphology, physiological characteristics, cell wall composition and mol% G + C of DNA (Schleifer, 1984). Based on these criteria, 37R, 45R and 49R, which form irregular clusters in liquid medium, are nonmotile, are capable of growth on furazolidone, do not ferment glucose, and have a mol% G + C of DNA ranging from 73–80%, have been identified as belonging to the genus *Micrococcus* (Schleifer *et al.,* 1981; Kocur, 1984a). The remaining two isolates (30Y and Lz3OR) also formed irregular clusters but differ from the above isolates in that they are incapable of growth on furazolidone agar and have a very low G + C content (41%). Based on these specialized characteristics, isolates 30Y and Lz3OR have been assigned to the genus *Planococcus* (Kocur, 1984b).

A species-level identification of all 5 isolates was attempted based on the characteristics published for the type cultures (Kocur and Schleifer, 1981; Schleifer *et al*., 1981; Kocur, 1984a, b). Isolates 37R, 45R and 49R, which are red in colour, nonmotile, produce acid from glucose, reduce nitrate to nitrite, grow on glutamic acid as carbon, nitrogen and energy source, and have mol% G + C of DNA ranging from 66–75%, have been identified as *M. roseus.* An earlier study by Johnson *et al.* (1981) had identified, in addition to *M. roseus, M. luteus* and *M. freudenreichii* in the soils of the dry valleys of Antarctica. The present isolates resemble *M. roseus* from the dry valleys in having similar maximum temperature (25–30°C) and pH (9–10) for growth, a high mol% G + C of DNA (68–75), and lysine as the diamino acid in the cell wall. Johnson *et al.* (1981) had, however, not studied the other biochemical characteristics of the *Micrococcus* isolates.

Two distinct groups have been identified in *Planococcus:* all strains with 39·5–42·2% G + C in DNA fall into one group, and the remaining, with 47–51 % G + C, into another group. This second group includes two species, *P. citreus* and *P. halophilus.* Our isolates 30Y and Lz3OR which have a low G + C content (41–43%) and are incapable of growing in agar containing 12% NaCl, do not belong to these two species but could be assigned to the other group. Strains belonging to this group (with mol% G + C in the range 39·5–42·2) bear no species name and have been tentatively designated as *Planococcus* sp. (Kocur and Schleifer, 1981; Kocur, 1984b). Further, isolates 30Y and Lz3OR, unlike other species of *Planococcus,* are not motile and do not possess a flagellum. Miller and Leschine (1984) have reported the presence of a *Planococcus* in the dry valley soils of Antarctica that was also nonmotile and did not resemble any of the known species. The present isolates closely resemble this earlier isolate in that they are psychrophilic, halotolerant, yellow to orange in colour, Gram-positive, nonmotile, non-sporulating, strictly aerobic, and oxidase- and phosphatase-negative (Miller and Leschine, 1984).

The medium normally used for enrichment of *Micrococcus* and *Planococcus* is supplemented with 7% and 10% NaCl respectively (Kocur, 1984a,b). If such a medium had been used in the present study, isolates 37R, 45R, 49R, 30Y and Lz3OR would never have been isolated since none of them could grow even in the

presence of 1 M NaCl (5·8%). This is also in agreement with the observation made by Miller and Leschine (1984) that *Planococcus* from the dry valleys of Antarctica show very little growth in the presence of 1·5 M NaCl.

The present isolates of *M. roseus* and *Planococcus* sp. do not identify completely with the respective type strains in that they cannot grow at 37°C or in the presence of 1 M NaCl; they also could hydrolyse starch, esculin and Tween 80. However, at least two other species of *Micrococcus* are capable of hydrolysing esculin, starch and Tween 80 (Schleifer *et al.,* 1981; Kocur, 1984a). These differences between the Antarctic isolates and the mesophilic type strains may reflect the psychrophilic nature of the Antarctic bacteria and their adaptation to the prevailing climatic conditions. Isolates of *Chromobacterium lividium* (Wynn-Williams, 1983) *Halomonas subglaciescola* (Franzmann *et al.,* 1987), *Flectobacillus glomeratus* (McGuire *et al.,* 1987), *Desulfovibrio* sp. (Rees *et al.,* 1986) and *Flavobacterium acquatile* (Tearle and Richard, 1987) from Antarctica have also been shown to have atypical characteristics and do not identify with the type strains. The present study shows, for the first time, the presence of *M. roseus* and *Planococcus* sp. in an oasis region of Antarctica.

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