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BACTERIA AND YEASTS OF SCHIRMACHER OASIS, ANTARCTICA: TAXONOMY, BIOCHEMISTRY AND MOLECULAR BIOLOGY

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Abstract: Water and soil samples from Schirmacher Oasis, Antarctica were found to contain a heterogenous group of psychrotrophic bacteria and yeasts. The bacteria belong to the genera *Pseudomonas, Sphingobacterium, Micrococcus, Planococcus* and *Arthrobacter*. The yeasts belong to the genera *Rhodotorula, Bullera* and *Candida*. Biochemical studies indicate that these psychrotrophs are capable of both translation and transcription even at 0°C and have enzymes which are cold-active, heat-labile and freeze-thaw resistant. Carotenoid pigments which interact with the bacterial membranes have been implicated in cold adaptation. Plasmids with antibiotic resistant genes have also been detected in some of the bacteria. All the results are discussed with a view to obtain possible insight into the biological basis of adaptation of microorganisms to low temperatures.

1. Introduction

Despite the extreme climatic conditions that persist in the icy Antarctic continent, microorganisms have been detected in all the distinctive habitats (such as lakes, ponds, rivers, streams, rocks and Antarctic soils) which differ from one another with respect to the range of temperature, availability of water, richness of the nutrients, etc. Since these factors influence the survival and growth of microorganisms, the microbial flora vary from habitat to habitat. In fact, the bacteria present in the cold deserts of Antarctica are bound to be different from those present in the coastal fringe which is warmer. Microbiological studies in Antarctica have so far been confined to maritime regions and specific regions of continental Antarctica such as McMurdo Oasis, which is an ice free region in Antarctica. SIMONOV (1971) described 16 ice-free areas around the coastal regions of the continent. The McMurdo Dry Valleys (also known as the McMurdo Oasis or Ross Desert) is a typical and the largest oasis in Antarctica (MATSUMOTO *et al.*, 1992) and consists of a number of lakes and ponds. However, there have been very few reports on the taxonomy of bacteria and yeasts present in oasis regions of Antarctica (GOTO *et al.*, 1969). It is with this in view that attempts have been made to identify the bacteria and yeasts of Schirmacher Oasis, Antarctica and also to use them as model systems to understand the biological basis of cold adaptation. This article reviews our work on bacteria and yeasts of Schirmacher Oasis, Antarctica with reference to their taxonomy, biochemistry

and molecular biology.

2. Taxonomy of the Bacteria and Yeasts

The Schirmacher Oasis is a rocky region in Queen Maud Land (70°45′12″S and 11°46′E) studded with a number of lakes, ponds, streams, hills and exposed rocks with extensive ice free regions during the summer months. During the collection period (January and February of 1985) the soil temperature varied from +6°C to −6°C. Soil samples were collected and analyzed under aseptic conditions using conventional and standard microbiological methods to identify the bacteria and yeasts.

An analysis of 118 samples of soils derived from various sites in Schirmacher Oasis, indicated that the viable bacterial count was in the range 1×10^3 to 1×10^6 colony forming units (CFU) per gram of soil (SHIVAJI, 1987). However, yeasts were detected only in a few of the soil samples; the number was 0.5×10^3 to 3×10^3 CFU/g soil (RAY *et al.*, 1989). Taxonomic studies have indicated that the bacteria and yeasts belong to different genera (Table 1). These studies, apart from confirming the presence of *Arthrobacter, Micrococcus* and *Planococcus* in Antarctic soils (SHIVAJI *et al.*, 1988, 1989a), have indicated for the first time the presence of *Pseudomonas* (SHIVAJI *et al.*, 1989b) and *Sphingobacterium* (SHIVAJI *et al.*, 1992) in Antarctica. Yeasts belonging to the genera *Rhodotorula, Bullera* and *Candida* were also identified in the soils of the oasis (RAY *et al.*, 1989; Table 1). All the microbes from Schirmacher Oasis were psychrotrophic and were capable of growth between 2°C and 30°C.

Our studies have indicated the presence of many atypical Antarctic bacteria. For instance, the Antarctic isolates of *Pseudomonas* were sensitive to gentamycin, kanamycin, streptomycin and tetracycline, unlike the mesophilic *Pseudomonas* (SHIVAJI *et al.*, 1989a). The Antarctic *Arthrobacter* isolates contained glucose in the cell wall instead of galactose and some of them had LL-diaminopimelic acid instead of lysine (SHIVAJI *et al.*, 1989b; SIEBERT and HIRSCH, 1988). The Antarctic *Micrococcus roseus* could not survive in the presence of 1 M salt and the *Planococcus* isolates were non-motile (SHIVAJI *et al.*, 1988; MILLER and LESCHINE, 1984).

As yet, it has not been possible to account for the presence of atypical characteristics in Antarctic bacteria. The ability of Antarctic bacteria to utilize carbon compounds such as glutamate, mannitol, ribitol, glycolate and arabitol, in contrast to the corresponding mesophilic species, may be a consequence of the preferential availability of these compounds in the soil as exudates of algae, fungi, moss or lichens (SIEBERT and HIRSCH, 1988; TEARLE and RICHARD, 1987). The preference of Antarctic strains of *Halomonas subglaciescola* for amino acids as sources of carbon and energy has been attributed to the release of these compounds from the degradation of penguin feathers (FRANZMANN *et al.*, 1987). However, certain other characteristics of Antarctic bacteria which distinguish them from their corresponding mesophilic organisms, such as the cell wall composition (JOHNSON and BELLINOFF, 1981; JOHNSON *et al.*, 1981; SHIVAJI *et al.*,

Table 1. Bacteria and yeasts from the Schirmacher Oasis, Antarctica.*

Organisms	Source of soil(s)	Microbial activity (cfu**/g of wet soil)
Bacteria		
1 *Pseudomonas fluorescens*	Lake sediment and soil from below an algal mat and moss bed	$2-30\times10^3$
2 *P. putida*	Soil from below an algal mat	4.4×10^3
3 *P. syringae*	Soil from a penguin rookery	120×10^3
4 *Arthrobacter globiformis*	Lake sediment and soil from below an algal mat and lichen bed	$2-7\times10^3$
5 *A. pascens*	Lake sediment and soil from lake shore and from below an algal mat	$1-4\times10^3$
6 *A. protophormiae*	Decaying moss and soil from a penguin rookery	$7-120\times10^3$
7 *Micrococcus roseus*	Soil from lake shore	$0.5-1.15\times10^3$
8 *Planococcus* spp.	Soil from a lichen bed and a penguin rookery	$4-15\times10^3$
9 *Sphingobacterium antarcticus*	Soil from a penguin rookery	$4-15\times10^3$
Yeasts		
1 *Rhodotorula rubra*	Lake sediment and soil from under a rock	$0.6-3\times10^3$
2 *Bullera alba*	Soil from a moss-bed	1×10^3
3 *Candida humicola*	Soil from the shore of a lake	1.5×10^3
4 *C. famata*	Soil from a Skua's nest	3×10^3
5 *C. ingeniosa*	Lake sediment	3×10^3
6 *C. auriculariae*	Soil from a moss-bed	3×10^3

* For details of taxonomy please refer to SHIVAJI (1987), SHIVAJI *et al.* (1988, 1989a, b, 1992) and RAY *et al.* (1989).

**cfu: colony forming unit.

1989b), mole% GC in DNA, pattern of antibiotic sensitivity, absence of pigmentation and a flagellum (MILLER and LESCHINE, 1984; SHIVAJI *et al.*, 1988, 1989a; TEARLE and RICHARD, 1987; WYNN-WILLIAMS, 1983), have not yet been explained; they tempt one to assign them to a new species. DNA-DNA hybridization studies and comparison of 16S rRNA sequences may help in arriving at a firmer decision in this regard.

3. Biochemical Adaptation to Cold

3.1. Role of enzymes

Psychrotrophic (and psychrophilic) microorganisms which are capable of growing at low temperatures are unable to grow at higher temperatures, probably due

to the thermolabile nature of certain essential cellular components such as enzymes, regulatory factors and (or) membrane components (GOUNOT, 1986). Studies carried out on characterization of enzymes from psychrotrophic microorganisms have indicated that the enzymes are cold-active, exhibit optimal activity toward lower temperatures (10 to 25°C) and are heat-labile (KOBORI *et al.*, 1989; FELLER *et al.*, 1990). However, it has also been observed that in certain psychrotrophic microorganisms the enzymes are cold-active and heat-labile but exhibit optimal activity around 35°C (FELLER *et al.*, 1992), a temperature at which enzymes from mesophilic microorganisms exhibit optimal activity.

3.1.1. Cold-active heat-labile alkaline phosphatase

Our own studies on the enzyme alkaline phosphatase (APase) in eight psychrotrophic terrestrial bacteria belonging to four different genera have indicated that the enzyme is active even at 5°C and exhibits optimum activity around 37 to 45°C

Table 2. Specific activity of alkaline phosphatase in Antarctic bacteria.

Bacteria	Specific acticity at different temperatures (°C)						
	5	15	23	30	37	45	65
	(n moles of paranitrophenol produced min^{-1} mg^{-1} $x10^{-1}$)						
Micrococcus roseus	1.0	4.5	7.0	9.4	10.4	1.7	0.5
*M. roseus**	0.9	3.8	6.5	8.1	9.2	1.1	0.4
Arthrobacter protophormiae	0.3	2.0	3.5	4.8	7.0	2.6	2.0
A. globiformis	0.1	0.7	1.0	1.5	1.7	2.4	1.2
*Escherichia coli**	3.1	3.7	6.1	7.9	11.2	13.4	12.3
	(μmoles of paranitrophenol produced min^{-1} mg^{-1} $x10^{-3}$)						
Pseudomonas fluorescens	1.8	2.8	3.8	5.2	9.0	21.6	8.0
P. putida	1.2	2.1	2.9	4.1	7.2	11.8	6.8
	(μmoles of paranitrophenol produced min^{-1} μg^{-1} $x10^{-1}$)						
Sphingobacterium antarcticus	3.4	6.4	7.3	9.9	10.2	14.1	5.9
*S. multivorum**	2.1	3.7	5.7	7.7	10.9	12.6	5.0
*Flavobacterium odoratum**	0.6	0.9	1.3	1.4	2.1	3.0	4.0

* *M. roseus* (MTCC 678), *E. coli* (W3110), *S. multivorum* (MTCC 498) and *F. odoratum* (MTCC 489) were mesophilic bacteria. The remaining eight isolates are psychrotrophic bacteria from Antarctica.

(Table 2). In mesophilic bacteria such as *Escherichia coli, Sphingobacterium multivorum* and *Flavobacterium odoratum*, the enzyme was active at 5°C and exhibited optimum activity at 45°C. But, unlike in mesophiles, the enzyme from the psychrotrophs was heat labile and was totally inactivated at 62°C in 10 minutes. Further, the production of the enzyme was repressed by inorganic phosphate. The enzyme has been partially purified (20 fold) from *Sphingobacterium antarcticus* following ammonium sulphate precipitation and Fast protein liquid chromatography (FPLC) on a Mono Q anion-exchange column.

3.1.2. Cold-active heat-labile ribonuclease

A cold-active heat-labile ribonuclease (RNase-HL) was purified to homogeneity from a psychrotrophic *Pseudomonas fluorescens* (Fig. 1). The molecular weight of the RNase-HL was 16000, and it exhibited optimum activity at pH 7 and 30°C. It could hydrolyze polyuridylic acid, polycytidylic acid and polyadenylic acid, but not polyguanylic acid. The unique features of RNase-HL are that unlike pancreatic ribonuclease A it is irreversibly denatured at 65°C, and it differs from pancreatic RNase A in its substrate specificity.

3.1.3. A cold-active freeze thaw resistant protease

The psychrotrophic, dimorphic yeast *Candida humicola*, isolated from Antarctic soil, secretes an acidic protease into the medium (Ray *et al.*, 1992). The secretion of this protease by *C. humicola* was found to be dependent on the composition of the medium. In YPD (yeast extract 1% ; peptone 1% ; dextrose 2%) or yeast nitrogen base medium containing either amino acids or ammonium sulphate as the nitrogen source, the activity of the protease in the medium was low (basal level). However, when yeast nitrogen base medium was depleted of amino acids or ammonium sulfate

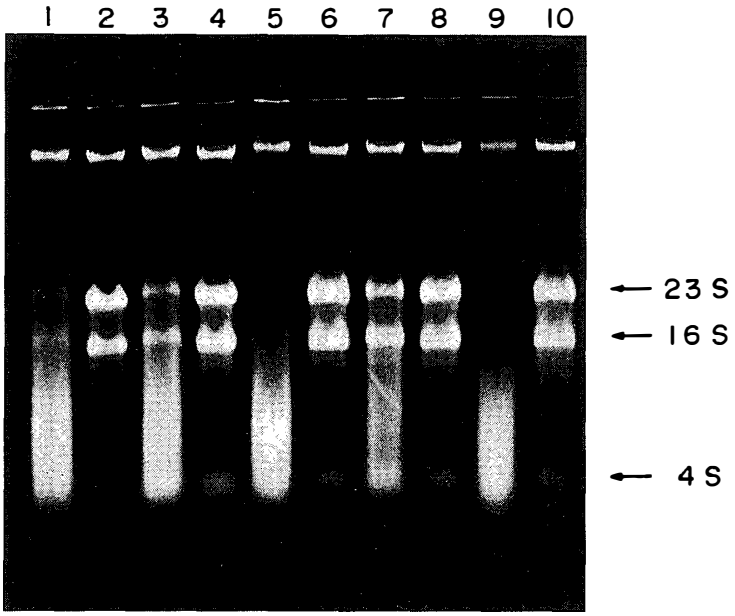

Fig. 1. Activity of the cold-active Ribonuclease (RNase) from psychrotrophic Pseudomonas fluorescens. Lanes 1, 3, 5, 7 and 9 indicate activity of the RNase at 5, 15, 30, 65 and 50°C respectively. Lanes 2, 4, 6, 8 and 10 indicate the unhydrolyzed RNA which was used as the control at the above temperatures respectively.

and supplemented with proteins, the activity of the enzyme increased. The secretion of the enzyme was greater during exponential growth at low temperatures than during growth at higher temperatures. The purified protease had a molecular weight of 36000 daltons and was inhibited by pepstatin, iodoacetamide and sodium dodecyl sulfate (Table 3). Despite the prevalent cold temperatures in Antarctica, this extracellular protease of the psychrotrophic yeast *C. humicola* was active at temperatures ranging from 0 to 45°C, with an optimum activity at 37°C and was resistant to 20 cycles of repeated freeze thaw. The enzyme was heat labile and was totally inactivated within 30 min at 56°C (Fig. 2).

3.2. Changes in the translational machinery

Psychrotrophic bacteria from Antarctica were capable of synthesising proteins even at 0°C (unlike mesophilic bacteria) and exhibited 20% of the maximum protein synthesis which occurred at 37°C. Thus, psychrotrophs have apparently acquired the ability to carry out translation at low temperatures and also retained the capacity to

Table 3. Effect of inhibitors on the activity of the acid protease secreted by Candida humicola.

Inhibitor	Concentration	Protease activity (% of control)*
Phenylmethylsulfonyl fluoride(PMSF)	1 mM	98
	10 mM	82
Ethylene diamine tetra acetate (EDTA)	1mM	96
	10 mM	93
Pepstatin	0.001 mM	34.5
	0.025 mM	0
N-α-p-tosyl-L-lyse chloromethyl ketone (TLCK)	1 mM	71
	5 mM	52
Iodoacetamide	5 mM	73
	10 mM	6
Sodium dodecyl sulfate (SDS)	0.05%(wt/vol)	0
Soybean trypsin inhibitor	5μg/ml	100
None	–	100

* The control had 35 U of protease activity.

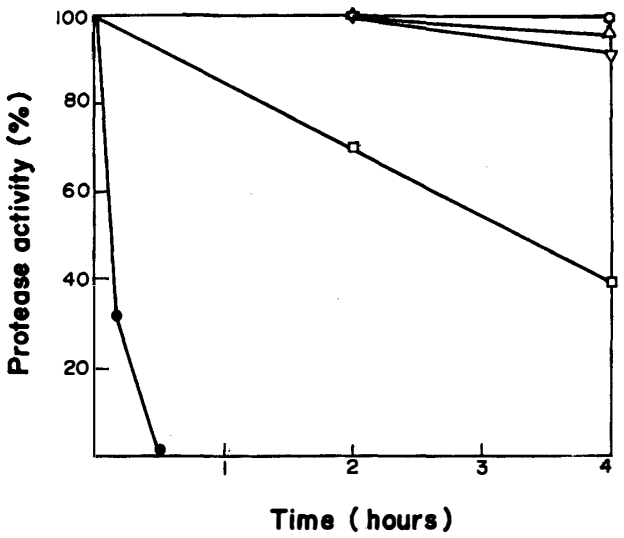

Fig. 2. Heat inactivation of the acidic extracellular protease from a psychrotrophic yeast, Candida humicola. *The enzyme was preincubated at 15°C (O), 22°C (△), 37°C (▽), 45°C (□) or 56°C (●) for varying periods of time (10 minutes to 4 hours) prior to assaying the activity at 37°C.*

function optimally at 37°C. *In vitro* studies using a cell-free system (prepared from a few psychrotrophic species of *Pseudomonas*) which was capable of polyuridylic acid directed poly phenylalanine synthesis indicated that the translational machinery was most efficient at 37°C as observed for mesophilic bacteria, but, unlike mesophilic bacteria, incorporation of ^{14}C phenyl alanine into polypeptide was also observed at 0°C. A similar result was observed for another psychrotrophic bacterium, *Pseudomonas* sp. 412 by SZER (1970). Further, the heat inactivation studies indicated that the cell-free translation system from psychrotrophic *Pseudomonas syringae* was totally inactivated within 10 minutes at 45°C whereas in the mesophilic *E. coli* and *P. fluorescens* under similar conditions (45°C) the translational machinery was unaffected (Table 4).

Table 4. Heat inactivation of the cell-free translation system in psychrotrophic P. syringae *and mesophilic* E. coli *and* P. fluorescens.

Microorganism	% translation activity following preincubation at 45°C for		
	10 min	30 min	60 min
Pseudomonas syringae	6	5	0
Escherichia coli	100	82	58
Pseudomonas fluorescens	100	25	5

3.3. Changes in the transcriptional machinery

Psychrotrophic *P. syringae* from Antarctica incorporated [^3H] uridine into RNA at 0°C but showed maximum incorporation at 30°C. The incorporation at 0°C was 5%

of that observed at 30°C; 85% of the synthesis was sensitive to rifampicin indicating that initiation of new RNA chains was also occurring. Mesophilic *E. coli* and *P. fluorescens*, on the other hand, did not transcribe at 0°C and transcribed efficiently at 30 to 37°C. Studies with a partially purified cell-free extract from *P. syringae* which was enriched in RNA polymerase was also capable of transcriptional activity between 0 and 37°C, and was also found to be more resistant to freeze-thaw, unlike a similar preparation from *E. coli*. These results suggested that the transcriptional machinery of Antarctic bacteria is probably better adapted to the Antarctic conditions. Preliminary studies on the purification of RNA polymerase from cold *Pseudomonas syringae* (Lz4W) indicated that it has an altered α-subunit and sigma factor.

3.4. Role of carotenoid pigments

The predominance of pigmented psychrotrophic bacteria in Antarctic soil may hint at a useful role for the pigment in cold adaptation. Further, little is known whether pigments are identical or different in bacteria that belong to the same species but differ in that one is a mesophile or a psychrophile. The major carotenoid pigment of a psychrotrophic *Micrococcus roseus* was purified to homogeneity from methanol extracts of dried cells by reverse phase liquid chromatography and designated as P-3. On the basis of the UV-visible, infrared, mass and ^{1}H nuclear magnetic resonance spectra of P-3, it was identified as bisdehydro-β-carotene-2-carboxylic acid (Fig. 3; JAGANNADHAM *et al.*, 1991). The pigment interacted with synthetic membranes of phosphatidylcholine and dimyristoyl phosphatidylcholine, and stabilized the membranes (Fig. 4). These results also indicate that P-3 is different from canthaxanthin, the major carotenoid pigment from mesophilic *M. roseus* (COONEY *et al.*, 1966). Earlier studies had also indicated that carotenoid-rich membranes are less fluid than carotenoid-poor membranes, but the physiological significance of this observation is still unknown. Further, it is also not known whether carotenoids, by their ability to rigidify membranes, influence the survival of microorganisms at low temperatures.

To further investigate, the role of carotenoids in cold adaptation pigmentation in *Sphingobacterium antarcticus* was studied with respect to its growth at different temperatures. *S. antarcticus* exhibited enhanced pigmentation with increasing temperatures of incubation. This behavior was opposite to that in mesophilic flavobacteria/*Sphingobacterium* in which pigmentation is reduced upon raising temperatures (HOLMES *et al.*, 1984). Use of diphenylamine (DPA), a standard biochemical blocker of carotenoid biosynthesis resulted in reduced synthesis of the pigment and also reduced growth of broth cultures when grown at extremes of the optimum temperature, probably indicating that pigment is required for growth. But mutants defective in pigmentation (like the wild type *S. antarcticus*) were capable of growing between 1–32°C suggesting that pigmentation does not play any role in adapting the bacterium to the psychrotrophic growth temperature. These results may indicate that DPA, which is known to block desaturation reactions in the carotenoid biosynthesis pathway, may be affecting other desaturation reactions within the bacterium, thereby causing reduced growth at extreme temperatures, since at least a few of these reactions involving unsaturation of fatty acids are important in maintaining membrane fluidity during thermal adaptation.

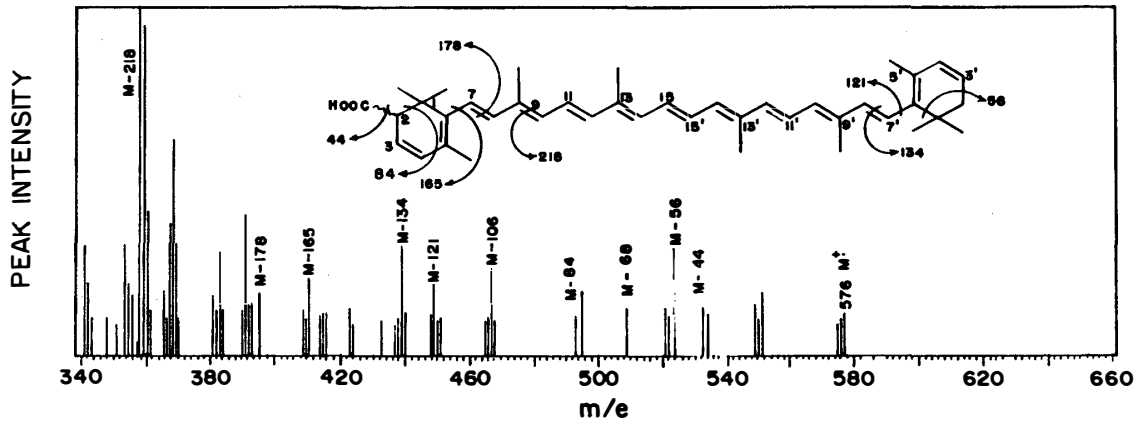

Fig. 3. Mass spectrum of the major carotenoid pigment bisdehydro-β-carotene-2-carboxylic acid of psychrotrophic Micrococcus roseus.

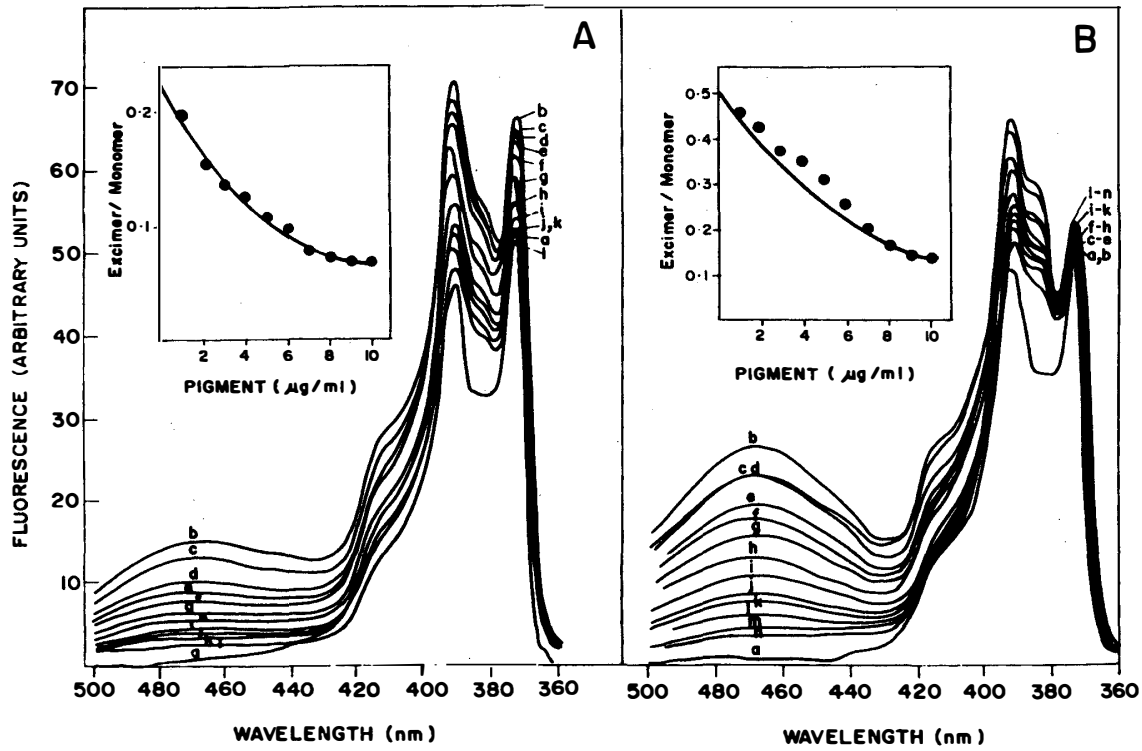

Fig. 4. Fluorescence emission spectra of pyrene (4 µM) incorporated into phosphatidylcholine (A) and dimyristoyl phosphotidyl choline (B) vesicles in the presence and absence of P-3 of M. roseus. *(excitation) 333 nm; lipid concentration 150 µM. (A) a, free pyrene; b, a plus 150 µM phosphotidyl choline vesicles; c to l, successive additions of 1 µg of P-3 to b. (B) a, free pyrene; b, a plus 150 µM dimyristoyl phosphotidyl choline; c to m, successive additions of 1 µg of P-3 to b. Insets in panels A and B depict the decrease in the pyrene excimer/monomer (372 nm/470 nm) intensity ratio in the presence of P-3 indicative of decrease in fluidity of the membranes.*

4. Plasmids from the Soil Bacteria

Ecological studies on the incidence of plasmids in natural populations of terrestrial bacteria have revealed that the frequency of plasmids in bacteria varies from site to site depending on the extent of pollution. For instance, the incidence of plasmids in bacteria was found to be greater in polluted compared to cleaner sites (HADA and SIZEMORE, 1981). Hence, in Antarctica, especially in those regions free of human intervention, the frequency of plasmids should be low. Our studies detected plasmids in ten out of thirty-one bacterial isolates, belonging to the genera *Pseudomonas, Arthrobacter, Sphingobacterium, Planococcus* and *Micrococcus*, from the Schirmacher Oasis (Table 5; RAY *et al.*, 1991; SHIVAJI, 1991). The plasmids were either small (about 4 kilobase pairs) or large (about 85 kilobase pairs). Plasmid-curing experiments in isolates of *Sphingobacterium* indicated that the plasmids contained antibiotic-resistant markers. DNA hybridization studies and restriction analysis showed that the plasmids from the two isolates of *S. antarcticus* were identical.

Table 5. Molecular size of plasmids isolated from Antarctic bacteria.

Bacteria	No. of isolates	Isolates with plasmid	Size (kilobase pairs)
Pseudomonas fluorescens	9	29W	~85
		30W	~4
P. putida	1	None	–
P. syringae	1	Lz4W	~4.3
Sphingobacterium antarcticus	2	4B1Y & 6B1Y	~85
Micrococcus roseus	3	None	–
Planococcus sp	2	30Y & Lz30R	~85
Arthrobacter globiformis	3	4B2W**	~45
A. pascens	3	None	–
A. protophormiae	7	7Y**	~4.5
		Lz4Y	~85

**These two isolates of *Arthrobacter* contained multiple froms of low molecular weight plasmids.

5. Conclusions

Taxonomic studies of the terrestrial samples of Schirmacher Oasis, Antarctica indicate the presence of a heterogenous group of bacteria and yeasts. To adapt to the prevailing cold conditions in Antarctica, these microorganisms seem to have acquired enzymes which are cold-active, heat-labile and freeze-thaw resistant. Further, they efficiently carry out both transcription and translation at low temperatures and also have carotenoids which could modulate the fluidity of their membranes. But, as yet, the exact mechanism by which these microorganisms sense low temperatures and

adapt to these conditions is unknown. Identification of gene(s) responsible for growth in cold and their regulation would ultimately unveil the mechanism of cold adaptation.

References

COONEY, J. J., MARKS, H. W. and SMITH, M. A. (1966): Isolation and identification of canthaxanthin from *Micrococcus roseus*. J. Bacteriol., **92**, 342–345.

FELLER, G., THIRY, M., ARPIGNY, J.L., MERGEAY, M. and GERDAY, C. (1990): Lipase from psychrotrophic antarctic bacteria. FEMS Microbiol. Lett., **66**, 239–244.

FELLER, G., LONHIENNE, T. DEROANNE, E., LIBIOULLE, C., BEEUMEN, J.V. and GERDAY, C. (1992): Purification characterization and nucleotide sequence of the thermolabile α-amylase from the Antarctic psychrotroph *Alteromonas haloplanctis* A23. J. Biol. Chem., **267**, 5217–5221.

FRANZMANN, P. D., BURTON, H. R. and MCMEEKIN, T. A. (1987): *Halomonas subglaciescola*, a new species of halotolerant bacteria isolated from Antarctica. Int. J. Syst. Bacteriol., **37**, 27–34.

GOTO, S., SUGIYAMA, J. and IIZUKA, H. (1969): Taxonomic study of Antarctic yeasts. Mycologia, **61**, 748–774.

GOUNOT, A. M. (1986): Psychrophilic and psychrotrophic microorganisms. Experientia, **42**, 1192–1197.

HADA, H. S. and SIZEMORE, R. K. (1981): Incidence of plasmids in marine *Vibrio* spp. isolated from an oil field in the north-western Gulf of Mexico. Appl. Environ. Microbiol., **41**, 199–202.

HOLMES, B., OWEN, R. J. and MCMEEKIN, T. A. (1984): Genus *Flavobacterium*. Bergey's Manual of Systematic Bacteriology, Vol. 1, ed. by N. R. KRIEG and J. G. HOLT. Baltimore, The Williams & Wilkins, 353–361.

JAGANNADHAM, M. V., RAO, V. J. and SHIVAJI, S. (1991): The major carotenoid pigment of a psychrotrophic *Micrococcus roseus*: Purification, structure and interaction of the pigment with synthetic membranes. J. Bacteriol., **173**, 7911–7917.

JOHNSON, R. M. and BELLINOFF, R. B. (1981): A taxonomic study of a dominant coryneform bacterial type found in Antarctic soils. Terrestrial Biology III, ed. by B. C. PARKER. Washington, D. C., Am. Geophys. Union, 169–184 (Antarct. Res. Ser., Vol. 30).

JOHNSON, R. M., INAI, M. and MCCARTHY, S. (1981): Characteristics of cold desert Antarctic coryneform bacteria. J. Ariz-Nev. Acad. Sci., **16**, 51–60.

KOBORI, H., SULLIVAN, C. W. and SHIZUYA, H. (1989): Heat-labile alkaline phosphatase from Antarctic bacteria : Rapid 5' end labelling of nucleic acids. Proc. Natl. Acad. Sci. U. S. A., **81**, 6691–6695.

MATSUMOTO, G. I., NAKAYA, S., MURAYAMA, H., MASUDA, N., KAWANO, T., WATANUKI, K. and TORII, T. (1992): Geochemical characteristics of Antarctic lakes and ponds. Proc. NIPR Symp. Polar Biol., **5**, 125–145.

MILLER, K. J. and LESCHINE, S. B. (1984): A halotolerant Planococcus from Antarctic dry valley soil. Curr. Microbiol., **11**, 205–210.

RAY, M. K., SHIVAJI, S., RAO, N. S. and BHARGAVA, P.M. (1989): Yeast strains from the Schirmacher Oasis, Antarctica. Polar Biol., **9**, 305–309.

RAY, M. K., KUMAR, G. S. and SHIVAJI, S. (1991): Plasmids from the soil bacteria of Schirmacher Oasis, Antarctica. Microbioscience, **67**, 151–157.

RAY, M. K., DEVI, K. U., KUMAR, G. S. and SHIVAJI, S. (1992): Extracellular protease from the Antarctic yeast *Candida humicola*. Appl. Environ. Microbiol., **58**, 1918–1923.

SHIVAJI, S. (1987): A preliminary note on bacteria and yeasts of Antarctica. Department of Ocean Development, India, Tech. Publ., **4**, 155–157.

SHIVAJI, S. (1991): Plasmids in Antarctic bacteria: Detection, isolation and characterization. Biotas Manual of Methods, ed. by D. D. WYNN-WILLIAMS. Cambridge, Scientific Committee on Antarctic Research, 1–26.

SHIVAJI, S., RAO, N. S., SAISREE, L., SHETH, V., REDDY, G. S. N. and BHARGAVA, P.M. (1988): Isolation and identification of *Micrococcus roseus* and *Planococcus* sp. from Schirmacher

Oasis, Antarctica. J. Biosci., **13**, 409–414.

SHIVAJI, S., RAO, N. S., SAISREE, L., SHETH, V., REDDY, G. S. N. and BHARGAVA, P. M. (1989a): Isolation and identification of *Pseudomonas* species from Schirmacher Oasis, Antarctica. Appl. Environ. Microbiol., **55**, 767–771.

SHIVAJI, S., RAO, N. S., SAISREE, L., REDDY, G. S. N., KUMAR, G. S. and BHARGAVA, P. M. (1989b): Isolates of arthrobacter from the soils of Schirmacher Oasis, Antarctica. Polar Biol., **10**, 225–229.

SHIVAJI, S., RAY, M. K., SAISREE, L., JAGANNADHAM, M. V., KUMAR, G. S., REDDY, G. S. N. and BHARGAVA, P. M. (1992): *Sphingobacterium antarcticus* sp. nov, a psychrotrophic halotolerant bacteria from the soils of Schirmacher Oasis, Antarctica. Int. J. Syst. Bacteriol., **42**, 102–116.

SIEBERT, J. and HIRSCH, P. (1988): Characterization of 15 selected coccal bacteria isolated from Antarctic rock and soil samples from the McMurds Valley (South Victoria lands). Polar Biol., 9, 37-44.

SIMONOV, I. M. (1971): Oazsy Bostochnoy Antarktidy (The Oases of Eastern Antarctica). Leningrad, Gidromet. Izd., 176 p.

SZER, W. (1970): Cell-free protein synthesis at 0°C. An activating factor from ribosomes of a pyshrophillic microorganisms. Biochim. Biophys. Acta, **213**, 159–120.

TEARLE, P. V. and RICHARD, K. J. (1987): Ecophysiological grouping of Antarctic environmental bacteria by API ZONE fatty acid finger prints. J. Appl. Bacteriol., **63**, 497–503.

WYNN-WILLIAMS, D. D. (1983): Distribution and characteristics of *Chromobacterium* in the maritime and sub-Antarctic. Polar Biol., **2**, 101–108.

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