A Preliminary Note on Bacteria and Yeasts of Antarctica

S. Shivaji

Centre for Celluar and Molecular Biology (CCMB), Hyderabad-500007.

ABSTRACT

Pure cultures of bacteria and yeast have been set up using water, soil and lake sediment samples collected at the Schirmacher Oasis, Antarctica. Conditions for the growth of these microorganisms have been optimised. Majority of the microorganisms were observed to be psychrophiles and could tolerate 1M salt in the medium. Preliminary studies on the pigments isolated from the chromogenic bacteria are presented. Plasmids have been detected in two out of the twenty bacteria checked. The G+C content of the DNA of some of the bacteria has been determined.

INTRODUCTION

Microorganisms of Antarctica are likely to be unusual, being adapted to extreme cold conditions. Hence, it would be of interest to investigate the molecular biology of these microorganisms with a view to understand the molecular mechanism of such adaptation. Further, though the thermophillic anaerobes are considered to be the most primitive, microorganisms of Antarctica are likely to be less advanced than the mesophilic bacteria. Hence their study and analysis may shed some light on the early evolution. With this in view, the microorganisms were collected during the Fourth Indian Scientific Expedition to Antarctica (December 1984-March 1985). During the last nine months, pure cultures of bacteria and yeast have been set up, growth conditions have been established and morphological and biochemical studies have been carried out in an attempt to identify these microorganisms.

MATERIAL AND METHODS

Water, soil and lake sediment samples were collected from the Schirmacher Oasis (Queen Maud Land, also referred to as Dakshin Gangotri Hill Ranges by India) situated along the coast of the Antarctic continent. The geographical coordinates of the oasis are 70°46'S and 11°49'E. The average annual temperature is -11°C; during the sampling period (January to February, 1985) the average temperature was -2°C. A dominant feature of the Schirmacher Oasis is the presence of twenty lakes, all of which are naturally-created glacial troughs bordered by land surface and rocky mountains. These lakes are separated from the sea by an enormous shelf glacier; the depth of the lakes varies considerably, from a few metres to tens of metres.

About 150 samples of water, soil and lake sediment were collected at random sites under sterile conditions from seven lakes in the Schirmacher Oasis. The samples were plated for the detection of bacteria and yeasts, on suitable culture media plates, either directly at the site or after serial dilution in the laboratory at the Dakshin Gangotri base camp (the permanent manned station of India), situated 70 km from the Oasis. The plates were incubated at temperatures ranging from 0-10°C and colony counts were determined after three to seven days of incubation. The bacterial medium consisted of peptone (0.5%) and yeast extract (0.2%) whereas yeast was cultured in a medium containing yeast extract (1%) and glucose (3%). Both the media were supplemented with 20% soil extract. The soil extract was prepared from the soil collected around the lakes in Schirmacher Oasis.

The morphology, motility, gram staining properties, indole production, catalase reaction, oxidase reaction and phosphatase reaction by the psychrophiles was determined. For all these tests the cultures were incubated at 20°C.

For the isolation of total DNA and plasmids, cultures were grown in 2 litres of media and the cells pelleted at late log phase. Cells were lysed and DNA isolated according to the method of Marmur (1961). The G+C content of the isolated pure DNA was determined following a standard procedure (Skidmore and Duggan, 1966). The presence of plasmids in the total cell lysates was detected using a rapid procedure employing agarose electrophoresis (Meyers et al., 1976).

RESULTS

All the samples which were plated showed the presence of bacteria and the bacterial counts varied from $1X10^3$ to $1X10^6$ cells/gm of soil or per ml of water. More than 200 isolates of bacterial colonies were picked up and replated onto fresh plates. Subsequently, on the basis of colony morphology, i.e., shape, size and colour, it was possible to isolate and establish forty four pure cultures of bacteria. Preliminary morphological studies indicated that the cultures included predominantly rod shaped and coccoid bacteria with a few appearing either like long filaments or like chain of bacilli. The rod shaped bacteria varied in length from short to long and some of them were curved. The cocci were present either as single cells, as pairs or in clumps of three or more. The bacilli were all gram negative but the cocci were gram positive or gram negative. All the rods were motile but the long filaments, chains of bacilli and cocci were not motile.

Bacteria were grown at four different temperatures (10,20,30 and 37°C) so as to determine the optimum temperature for growth. It was observed that more than 75% of the bacteria were psychrophiles in that they had an optimum temperature of 20°C for growth. They are slow growing at 5°C and the growth was severely retarded at temperatures above 25°C and the growth was completely absent at 37°C.

Many of the bacterial cultures were pigmented. Out of forty four cultures, eleven were yellow, three were orange, three were red, one was purple, one was mustard and the remaining were white. Extraction of the pigments with methanol and spectral studies indicated that the red pigment showed absorption maxima at the following wavelengths: 523,491,466,385,368,316,305,263 and 244 nm; the orange pigment at 474, 448, 424, 333, 249 and 261 nm; and the yellow pigment at 467, 438, 413, 393 and 261.

Out of the twenty pure cultures checked so far, all of them were positive for catalase, 50% were positive for oxidase and 50% were positive for phosphatase. None of them produced indole from the medium containing tryptophan.

Isolation of DNA from the pigmented bacteria has posed a number of problems since these could not be lysed easily using detergents like SDS, Triton X 100 and Brij-35. Hence enzymatic methods using lysozyme, glusulase and proteinase had to be standardised. However, the nonpigmented bacteria lysed easily. So far DNA has been isolated from twenty different microorganisms. The G+C content of the DNA varied from 43 to 60%.

For the detection of plasmids in bacteria, twenty different cultures were lysed with 1% SDS and the lysates were electrophoresed on 0.89% agarose gels. Only two out of the twenty cultures tested showed the presence of 2 bands which were slightly larger than PBR 322.

Yeast colonies were detected in 10% of the soil samples which were plated. Out of about fifteen different isolates, about 8 pure cultures were set up. The cultures could be easily distinguished on the basis of their colour which was either red, yellow, creamish or white. Microscopic observations revealed that they were all nonfilamentous yeast and the individual pear to round-shaped cells exhibited budding. Growth curve studies at four different temperatures (8, 15, 22 and 30°C)

indicated that all of them exhibit optimum growth at 15-22°C. At 8°C all the cultures showed a retarded rate of growth. However, at 30°C only six out of the eight cultures showed appreciable growth.

The growth of the eight different yeasts was also checked using twenty different sugars, glycerol, ethanol or methanol as the only carbon source. It was observed that the preference for a particular sugar varied from yeast to yeast as reflected in the difference in the extent of their growth. However, all of them could grow in glucose, fructose, arabinose, xylose, galactose, sucrose, melibiose, sorbitol, lactose, maltose, mannitol, raffinose and mannose as the only carbon source. However, when glycerol, ethanol, methanol, succinate, citrate, glucosamine and starch were used as the only carbon source none of the yeast could grow.

DISCUSSION

The results clearly indicate that bacteria and yeast are predominantly present in the soil samples of Antarctica. Most of the bacteria are true psychrophiles and can also grow in the presence of 1 M salt. The yeasts also can grow in the presence of 1 M salt and have an optimum temperature around 18°C. Plasmids have also been detected in bacteria. The preliminary morphological studies and biochemical tests including the determination of the G + C content of the DNA are not sufficient to even make a tentative identification of these microorganisms. Hence, our efforts are directed at the moment to carry out more such tests which may ultimately aid in the identification of these microorganisms.

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