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Air Microflora of Antarctica

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Abstract

The bacteria and fungal population of Antarctica atmosphere has been monitored. The bacterial population was dominated by Gram positive rods and the fungal population by *Penicillium* species. Most of the bacteria could grow at less than 20°C. No coliform was detected in MacConkey plates. Qualitative screening of bacteria showed the presence of proteolytic, lipolytic and amylolytic populations. *Bacillus* species could reduce COD of SNS by twenty one per cent at 10°C.

Introduction

A vast proportion of the earth's surface is cold (less than 5°C). The polar regions, including the continent of Antarctica and permanent cold areas surrounding the Arctic circle represent approximately 14 % of the earth's surface. Though some work has been carried out on the microflora of soil, alga, moss and water of Antarctica (Warwick, 1988) however, very little work has been done on the incidence of air microflora of Antarctica continent (Kushner, 1978). Psychrophiles have been investigated less perhaps because of their slow growth and the difficulty in handling them. However, the potential will be increased if psychrophiles would be studied thoroughly (William, 1975).

The microorganisms present in the atmosphere originate from the soil, water arid other materials and as such they do not have any opportunity for growth (Frazier and Westhoff, 1981). The ultimate fate of air microflora is governed by complex set of circumstances such as type of contaminations, amount of movement, sunshine, humidity, temperature and amount of suspended ivoshein, 1980).

In this study we have isolated bacteria and fungi from the atmosphere of Antarctica near Maitri station, Schirmacher Oasis, Antarctica. The attempt has been made to screen the bacterial isolates for potential to biodegrade night soil

at low temperature which is a burning problem in high altitude of India as well as Antarctica.

Material and Methods

Isolation: MacConkey agar, potato dextrose agar, nutrient agar and brain heart, infusion agar (Himedia, India) were suspended in required amount of water and autoclaved at 121°C for 20 minutes. Aseptically media were poured in sterilized petri dishes and plates were exposed to the atmosphere near Maitri station for a period of 30 minutes to 3 hours. The plates were incubated at 30°C for 3 days.

Maintenance of culture: Individual colonies of bacteria and fungi were purified by repeated subculturing on respective media and stored in agar slant at 4°C, The slants were transported to the laboratory for further studies.

Morphology and identification: Freshly grown culture of the bacteria were subjected to Gram staining and their staining characteristics and morphology were observed. The fungal cultures were got identified at Indian Agricultural Research Institute, N. Delhi, India.

Screening of bacteria for hydrolytic enzymes: Freshly grown bacterial isolates in nutrient broth were used for their ability to hydrolyze protein, lipid, starch and cellulose. The plates of skim milk agar, tributyrin agar, starch agar (Nutrient agar + 0.2 % soluble starch) and carboxy methyl cellulose agar media (g/L $NaNO_3$, 2.0 ; $MgSO_4$, 0.5; K_2HPO_4, 0.05; $FeSO_4$, 0.01; $CaCl_2$, 0.02; $MnSO_4$ 0.002; CMC, 1.0 and Agar 20.0) *p*H 7.0 were inoculated for assigning the characteristic of proteolysis, lipolysis, amylolysis and cellulolysis, respectively. The plates were incubated at 10°C for 5 days and zone of hydrolysis was observed.

Biodegradation studies: The bacterial strains were inoculated from slant cultures into 10 ml of nutrient broth. After incubation at 10°C for 5 days the broth culture were used as inoculum. Experiments were carried out in 500 ml Erlenmeyer flasks with 200 ml sterile synthetic night soil, (SNS) (Susumu *et al.,* 1982). Ten ml of the inoculum was added to the SNS and incubation was done at 10°C for 10 days with continuous shaking (100 rpm/min). After incubation the cultures were centrifuged at 5000 rpm and the reduction of chemical oxygen demand were estimated by the methods of APHA (1985).

Results and Discussion

Plates of agar exposed for 5 to 30 minutes to atmosphere at three different locations around Maitri station, Schirmacher Oasis, Antarctica showed hardly any colony after incubation at 30°C. Most of the investigators who sampled air for microorganisms used incubation temperatures exceeding 20°C (Sieburth, 1965). The exposure for longer time (1 to 3 hours) showed 5 to 15 colonies of bacteria and 2 to 5 colonies of fungi. It shows the presence of very little air microflora in the atmosphere of Antarctica compared to India when such counts are observed by exposure of plates only for few minutes. Surprisingly no bacterial colony was detected on MacConkey agar which depicts absence of faecal contamination in the environment of frozen continent. In agreement with our findings Ekelof (1908) isolated from over 50 % of the plates exposed to Antarctic air, and it was estimated that there was a settling rate of one bacterium per two hours. The bacteria and fungi have been reported from soil of Schirmacher Oasis, Antarctica by other workers (Shivaji, *et al.,* 1989a, Shivaji *etal,* 1989b).

The bacterial population was comprised of mainly Gram positive bacteria consisting of 52 % rods and 20% cocci. Gram negative rods accounted only 28 % of the total bacterial load. Thirty eight per cent of bacterial isolates showed their ability to grow even at 5°C and 44% at 15°C. About one-fifth population showed growth only at the temperature of more than 20 °C. It shows the dominance of psychrotrophic population in the atmosphere because inspite of their growth at low temperature the optimum temperature has been either/or more than 25°C. Elis Evans (1985) has also reported majority of Antarctica bacteria as psychrotrophs.

The fungal population was dominated by *Penicillium* species and was consisted of P. *olivicolor, P. corylophilum, P. viridicatum, P. chrysogenum, P. waksmanii, P.camemberti* besides few colonies of *Fusarium oxysporum* and *Paecilomyces variotii* were also detected. The optimum temperature for fungi has been found around 30°C.

Hydrolytic enzymes: Fifty three bacterial isolates were subjected to qualitative screening for proteolytic, lipolytic, amylolytic and cellulolytic activity. Fourteen bacterial isolates showed zone of hydrolysis on milk agar, tributyrin agar and starch agar and cellulolytic activity was not observed by any isolate. The results of hydrolysis has been shown in Table-1. The lipolytic activity was shown by maximum number of isolates (10 isolates) followed by proteolytic activity (6 isolates) and amylolytic activity (3 isolates). Isolate no. AW1 showed hydrolysis of protein, lipid and starch simultaneously whereas isolate no. AK1 hydrolyzed only lipid and starch. Both isolates were Gram positive rods in

Table 1: Qualitative estimation of hydrolytic activity of psychrotrophic bacterial isolates of Antarctica air (incubation temperature 10°C for 5 days)

Bacterial Isolates	Morphology	Proteolytic Activity	Lipolytic Activity	Amylolytic Activity
AO 2	+rods	-	-	+
AO 3	+ cocci	+	-	-
AO 4	+ rods	-	++	-
AO 6	+ rods	-	++	-
AK 1	+ rods	-	+++	++
AW 1	+ rods	+++	++	++
AU 2	+ rods	+	-	-
AV 3	+ rods	+	+	-
AT 1	+ rods	-	++	-
AT 2	+ cocobac	+	+	-
AN 2	- rods	+	-	-
AN 3	+ cocci	-	+	-
AN 5	+ cocci	-	+	-
AX 4	+ rods	-	++	-

chains and belong to *Bacillus* species. The simultaneous hydrolysis of protein and lipid was also observed by isolate no. AV3 and AT2 however the zone of hydrolysis has been very poor all other isolates showed hydrolysis of only single carbon source.

Biodegradation: The bacterial isolates were screened for their ability to degrade synthetic night soil (SNS). SNS contained soluble chemical oxygen demand (COD) 14800 MgO_2/L.

Isolate no. AW1 reduced the maximum COD to the extent of 21 %followed by AK1 which caused reduction of 17 % at 10 °C in a period of 10 days of incubation (Table- 2). Both of these isolates were further tested for biodegradation of night soil (1:9 dilution). Isolate no. AW1 caused 23% COD reduction of night soil at 20°C and 21.8 % and 15.6 % at 10 and 5°C respectively at the end of 10 days of incubation. From the studies conducted it is observed that the microflora of Antarctica has better growth at lower temperature and have potential of biodegradation in cold climatic environments. There is evidence that bacterial are important in the production of ice-nucleated particles, particularly those associated with decaying tree leaves (Schnell and Vali, 1972,1973). However the biodegradation ability of isolates of air is comparatively lesser than the bacterial isolates of soil from the same continent. Our earlier work with soil bacteria have shown the biodegradation potential ranging from 6.25 % to 47.91 % (Agarwal and Singh, 1993). Further studies are required on characterization of hydrolytic enzymes specially with the isolates showing hydrolysis

Table 2 : COD reduction of night soil by psychrotrophic bacterial isolates of Antarctica (incubation temperature 10°C for 10 days)

Bacterial Isolates	COD (MgO_2/L)	COD Reduction (%)
AO 2	14105	4.7
AO 3	13172	11.0
AO 4	13202	10.8
AO 6	13365	9.7
AK 1	12284	17.0
AW 1	11692	21.0
AU 2	13202	10.8
AV 3	13232	10.6
AT 1	13646	7.8
AT 2	13143	11.2
AN 2	13690	7.5
AN 3	14267	3.6
AN 5	14030	5.2
AX 4	13438	9.2

Initial COD of SNS (MgO_2/L): 14800

of protein, lipid and starch simultaneously and whether these bacteria can contribute supporting role for biodegradation of night soil by *Arthrobacter* at low temperature.

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