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Studies on the Effect of Antarctic Environment on Some Saprophytic Tropical Fungi

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Abstract

Ten strains of saprophytic tropical fungi were exposed to the Antarctic environment for 14 months, beginning with Fifth Indian Expedition to Antarctica, with a view to study the effect of extreme climate variations on their survival, rate of growth and virulence. The data obtained suggested slight changes in the activity of a few types. No major variations were observed in any of the exposed strains.

Introduction

Antarctica is a desolate isolated plateau measuring approximately 5 million sq. miles in area with an average altitude of 8000 ft. It is considered to be the coldest region on the globe (McGraw Hill, 1982). The lowest temperature recorded is -88°C whereas the normal range is +5°C to -50°C. However, life existed there from times immemorial in the form of the most primitive living beings the microbes, alongwith some other specialised types of flora and fauna (Tubaki, 1961.) The microbes included are lichens, algae, yeasts, bacteria and, some psychrophilic fungi mostly belonging to basidiomycetes and ascomycetes groups. According to some workers (Singer, 1954) the dominance of these groups of fungi is due to very much reduced activity of soil bacteria and fungi of the imperfecti group in breaking down of the organic matter. The presence of this microbial population is restricted to small areas free from permanent coverings of ice and snow and having a mean temperature around 0°C. The most likely areas are rocks sheltered from wind and covered with bird droppings which provide good so'urce of food for the microbes. Growth of fungi at snow-banks at altitudes between 3000 ft. to 8000 ft. has also been reported (Cooke, 1955).

The detection of some strains of fungi of the Imperfecti groups, *e.g. Penicillium* sp., *Scopulariopsis brevicaulis, Botrytis* sp. and *Hormicium* sp. in Antarctica has been reported long back (Dodge & Baker, 1938). However, no study on the metabolic activity of these saprophytes exposed to Antarctic climate has so far been reported. One such incidence was also observed in our

own country during 1977-78 when fungal deterioration of Tent Arctic exposed at subzero temperature in the snow bound Himalayas was observed (DMSRDE). It clearly indicated that some saprophytes whose normal temperature range of activity is between 25°C to 35°C gave an indication that they can maintain their metabolic activity at a much lower temperature. With a view to confirm the above observations, 10 strains of tropical saprophytes, isolated from deteriorated Defence stores were exposed to weather in specially designed wooden boxes in the Antarctic environment for 14 months. Comparative studies of the exposed strains vis-a-vis their counterparts (control set) maintained at Kanpur were carried out with regard to their survival and possible changes in rates of growth and virulence.

Materials and Methods

A. Exposure of fungi

Ten strains of saprophytic fungi from the DMSRDE Culture Collection, with different substrates of isolation and varying growth rates and sporulation were selected for the studies. An effort was made to restrict the selection to the most common types found in varying climates universally. These are listed in Table I.

Table I. Fungi exposed at Antarctica indicating their original substrates.

Sl. No.	Fungus	Strain No.	Substrate of isolation	Remarks
1.	*Chaetomium globosum*	6	Jute fabric	Optimum growth temp. 20°C.
2.	*Stemphylium Sp.*	704	Dosooti Cotton	Isolate from Low temperature (Hilly region)
3.	*Curvularia lunata*	851	Lingo cellulose	—
4.	*Memnoniella echinata*	970	Outer fly tent	Copper tolerant
5.	*Aureobasidium pullulans*	981	Painted surface	—
6.	*Aspergillus flavus*	1017	Field Gun furniture fittings	Surface grower
7.	*Aspergillus niger*	1019	Timber	—
8.	*Paecilomyces varioti*	1103	Synthetic rubber	—
9.	*Penicillium funiculosum*	1109	PVC	—
10.	*Cladosporium sp.*	1085	Aviation fuel	Prolific consumer of fuel

Three sets of the above fungi were subcultured on Potato Dextrose Agar (PDA) (Fred and Walksman, 1928) slants and incubated at 30°C ± 1°C for a period of 7 days. Due to variations in their growth rates all the culture did not attain optimum growth and sporulation at the time of their despatch. Two of these sets were packed in specially designed wooden boxes with perforated sides to permit exchange of the air and keep the cultures very close to environmental temperatures. The third set (control) was kept at room temperature at Kanpur. Out of the two boxes carried to Antarctica during the fifth expedition one was marked 'Summer' while the other was marked 'Winter'. Both these boxes were fixed on wooden poles erected vertically in the snow outside Dakshin Gangotri Station (DGS). The poles were very firmly dug into the snow so as to withstand the blizzards ranging between 40 to 100 knots at very low temperatures (—12°C and below). The box marked 'Summer' was taken out after 45 days only owing to return of the summer team, while the box marked 'Winter' was left behind for continued exposure through the long winter. It was brought back in March after 14 months by the Third Wintering Team.

B. Laboratory examination of the Sets after Exposure

I. Summer *set*

All the tubes were visually and microscopically examined after unpacking. Except for *Cladosporium* sp. (Culture No. 1085) the remaining 9 fungi were found to be viable on transfer to fresh media. The non-viable strain No. 1085 showed very slight germination without fresh mycelial ramifications. The remaining 9 strains showed drying up of the Agar base medium up to 20% with condensate deposited on the inner walls of the tubes. The colonies on the slants looked normal without any apparant drying up or disruption of the spore chains. Considering the age of the strains (6 months) maintenance, viability and the brief period (45 days) of exposure in the climate of Antarctica no change of any type was anticipated in the summer set.

II. *Winter set*

At the time of examination the age of the strains was 17 months, out of which they remained exposed to Antarctica environment for approximatly 14 months.

(a) *Visual and microscopic examination*

On visual examination the 9 fungi [excluding the nonviable strain of *Cladosporium* sp. (1085)] exhibited peculiar characters. Strains of *M. echinata* (970), *Aureobasidium pullulans* (981), *Aspergillus niger* (1019) and *Penicillium funiculosum* (1109) presented a wet, shining and convoluted surface of the colony. The culture of *Paecilomyces varioti* (1103) looked dusty due to breaking up and dispersal of spore chains on account of loss of moisture. The remaining 4 strains viz. *Chaetomium*

globosum (6), *C. lunata* (851), *Stemphylium* (704) and *Aspergillus flavus* (1017) looked shrivelled up due to loss of moisture but were otherwise normal. All the 9 fungi were found viable on subculturing.

(b) *Comparative rates of growth*

For evaluation of the comparative rates of growth of the strains exposed at Antarctica and the control maintained at Kanpur two techniques were used, *viz.* measuring the radial growth and linear growth of both the sets subcultured and incubated under identical condition.

(b-1) *Radial growth*

Radial growth measurement studies were carried out in 10 cm diameter petri plates each with 10 ml of PDA media. Seven days old cultures of the fungi were used for inoculations. A block of one cubic mm was cut for a thickly sporulating region and carefully transferred to the centre of the plate with the help of an inoculating needle. Five replicates were taken from each culture for both the sets. All the plates were incubated at 30°C ± 2°C. The diameter of the growing colony was measured crosswise in two directions at 48 hourly intervals. The average of these two readings was taken as diameter of the colony. The diameter of each fungal colony was determined on the basis of the 5 replicates (Table II).

(b-2) *Measurement of linear growth*

The linear growth measurements were done by growing these cultures in specially designed 40 cm long and 1.4 cm dia glass tubes. Approx. 5 cm length of these tubes at both the ends were bent upwards at 130°C leaving a 30 cm long central portion, the open ends of which were plugged with non-absorbent cotton (Fig. 1). In each tube 10 ml of the PDA media was pipetted from one end and the tubes were

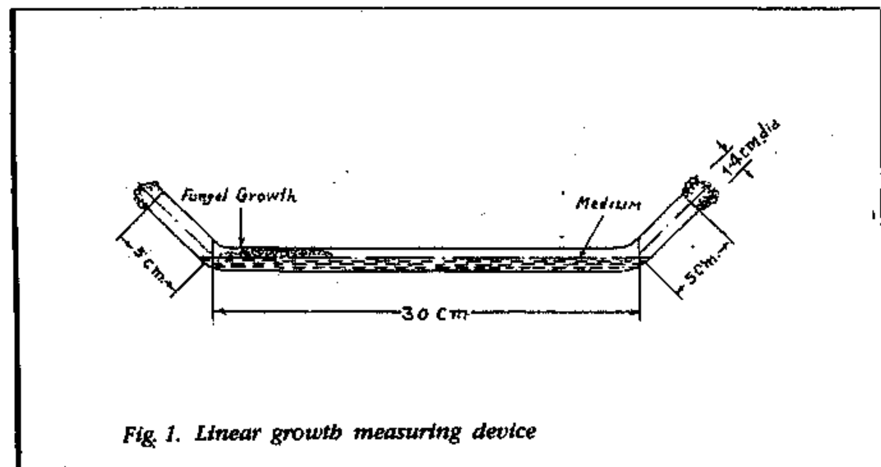

Fig. 1. Linear growth measuring device

sterilised in an autoclave in a horizontal position with both bent ends facing upwards. After setting up of the agar base media, inoculations were made at one end using 7 days old cultures. One cubic mm of inoculum picked up from the sporulating region was used as inoculum. Five replicates for each culture were prepared from each set and all the tubes were incubated at 30°C ± 2°C for 14 days. Since the fungus was allowed to grow in only one direction, the growth could be measured in a linear direction after 48 hourly intervals. The average of the 5 replicates was taken as mean rate of growth for each culture from both the sets (Table II).

(c) *Determination of virulence*

The degree of virulence of the experimental fungi of both the sets was measured by their material degrading capacity. Since majority of these strains were cellulose destroyers in nature it was considered desirable to use fine pure cellulosic fabric (cambric) as a substrate material for these tests. Cambric test pieces 25 cm X 6.5 cm in size were cut in the warp direction of the

Table II. Comparative rate of growth of unexposed and fungi exposed at Antarctica.

Sl. No.	Organisms	Culture Number	Type of set	Average radial growth (mm) in hours			Average linear growth (mm) in hours		
				48hrs	192 hrs	336 hrs	48 hrs	192 hrs	336 hrs
1.	*Chaetomium globosum*	6	Control	64	90	90	35	90	158
			Ant. exposed	66	90	90	33	90	167
2.	*Stemphylium sp.*	704	Control	18	57	90	20	45	75
			Ant. exposed	22	53	90	20	50	82
3.	*Curvularia lunata*	851	Control	30	50	95	35	60	95
			Ant. exposed	27	55	95	35	75	95
4.	*Memnoniella echinata*	970	Control	12	22	40	12	28	40
			Ant. exposed	15	28	50	15	35	50
5.	*Aureobasidium pullulans*	981	Control	21	35	60	21	35	60
			Ant. exposed	22	40	65	22	40	65
6.	*Aspergillus flavus*	1017	Control	59	95	130	59	95	193
			Ant. exposed	50	87	120	50	87	145
7.	*Aspergillus niger*	1019	Control	35	80	120	50	80	120
			Ant. exposed	30	75	120	30	75	120
8.	*Paecilomyces varioti*	1103	Control	43	102	200	43	102	190
			Ant. exposed	46	110	200	40	110	200
9.	*Penicillium funiculosum*	1109	Control	16	48	88	18	39	60
			Ant. exposed	16	42	90	45	60	90

fabric. Ten replicates were prepared for each fungus (5 for each set). Besides these, 5 control pieces were prepared for each batch. With the help of 15 cm long glass rods each of the test strips was put into a 500 ml Erlenmeyers flask separately in such a way that both the ends of the strip touched the bottoms of the flasks while the central part of the strip was held up with one end of the rod (Fig. 2). Fifty ml of Greathouse mineral salts solution

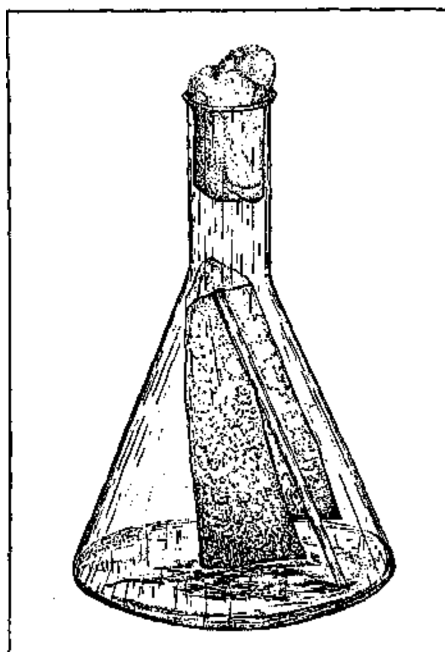

Fig. 2. Method for determining the cellulolytic capacity of fungi

(Fred & Walksman, 1928) was also put in each of the above flasks in such a way that the ends of the test strips were dipped into the solution. All the flasks were sterilised at 15 lbs pressure for 20 minutes. The test strips were inoculated with the pure culture spore suspensions of the experimental fungi prepared separately as per standard techniques (Nigam, 1966). The five replicates of test strips for each culture were inoculated by pipetting one ml of the spores suspension at top of the glassrod holding the central part of the test strip in the flask. The suspension was absorbed by the strip. The mineral salts which had risen through the capillary action in the fine fabric provided nutrition for the germination and growth of fungus spores in the inoculated central region of the strips. The inoculated flasks alongwith the uninoculated control flasks were incubated at 30° ± 2°C for a period of 14 days.

After 14 days, the degree of fungal growth on each strip was recorded and all the strips were washed under running tap water to remove the growth and salts from the fabric. The test strips alongwith control were then dried in shade and subjected to conditioning for 24 hours at 25°C and 68% RH (ISI, 1959). Subsequently the tensile strength of each of the test strips was determined on a Good brand tensile strength testing machine. The average data of 5 replicates for each fungus indicated the loss in strength of the fabric caused by each fungus. The percentage loss caused by each fungus was calculated vis-a-vis the average strength loss in the uninoculated control strips as per IS: 1389 - 1959. The comparative data of the exposed cultures and the unexposed set of cultures is presented in Table III.

Table III. Comparative Virulence Determination of the exposed fungi vis-a-vis the unexposed set of fungi

SI. No.	Fungus	Culture No.	DMSRDE Av.B.S. in Lbs	Set loss	Antarctica Av.B.S. in Lbs	set loss	Remarks
1.	Uninoculated control samples.	—	236	No loss	236	No loss	—
2.	*C. globosum*	6	Nil	100%	18	92%	—
3.	*Stemphyllium*	704	15	94%	9	96%	—
4.	*C. lunata*	851	214	5%	172	27%	Increase in virulence
5.	*M. echinata*	970	Nil	100%	Nil	100%	—
6.	*A. pullulans*	981	236	No loss	226	4%	—
7.	*A, flavus*	1017	193	17%	226	4%	Decrease in virulence
8.	*A. niger*	1019	193	17%	233	1%	Decrease in virulence
9.	P. *varioti*	1103	211	11%	236	No loss	—
10.	P. *funiculosum*	1109	Nil	100%	126	44%	—

Results and Discussion

Out of the 10 fungi sent for exposures one fungus *Cladosporium* sp. (1085) did not grow in any of the 3 sets presumably becoming non-viable and hence was discarded from further studies. During 45 days of summer exposure there were only 7 blizzards lasting from about 5 hours to more than 24 hours with lowest temperature touching down to — 14°C and a wind speed of about 40 knots. Except these short time variations in temperature and wind velocity the normal temperature in the region ranged betwen + 1°C to — 5°C. This temperature range is in close proximity of the normal range of storage temperature in the laboratory and hence was not supposed to produce any physiological changes in the cultures. Except for about 20% drying in the medium, the fungi grew and sporulated in the normal way and hence no further investigations were considered necessary for the summer set.

The winter set of fungi got an exposure of 14 months during which it faced very low temperature (upto -52°C) and wind speeds of 60-80 knots for very long periods. As a consequence the liquid within the cells and the medium froze. Presumably this resulted in the disruption of mycelial network as well as surface of the media. On being brought back to the laboratory room temperature, the molten ice in the form of water got deposited at the bottom of the test tubes. In a few cases the fungi developed a wet shiny and convoluted surface due to initial drying up and then retention of water droplets on the dry and brittle surface. A perusal of Table IT indicates the comparative growth rates of all the 9 fungi exposed in the Antarctic environment vis-a-vis the rates of growth of the control set. Except *M. echinata,* which after exposure showed a faster growth by both the techniques of measurement, the other fungi did not indicate any major variation. Identical results were obtained in repeat experiments and this confirmed that this strain of *M. echinata* developed some change due to freeze drying under natural conditions. No other fungus developed variation in respect of rate of growth.

In case of virulence measured by the increase and decrease in the cellulolytic activity of each fungus, it is clear from Table II that there has been a definite decrease in the virulence of 4 fungi viz. *A. flavus, A. niger, P. varioti* and *P. funiculosum.* On the other hand *Curvularia lunata* has indicated an increase in virulence. The remaining 4 fungi remained unaltered. These observation go to prove that except slight physiological changes the exposed fungi maintained their metabolic activity without any major change.

Conclusion

Some strains of tropical saprophytic fungi were exposed to Antarctica environment for 14 months to study the effect of environmental variations of that region on the viability, growth rate and virulance of these fungi. No major variation in their activity were indicated after the exposures. This study suggests that biodegradable materials and equipment held at Antarctica can get damaged during summer when the temperature rises to around 10°C. Such materials need protective measures for their proper maintenance and use for longer periods.

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