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Recording Aerobic Culturable Microbial Load of Soil Core Samples from Larsemann Hills, East Antarctica

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ABSTRACT

Larsemann Hills is an ice-free area of around 40 square km, located in Princess Elizabeth Land, East Antarctica. In this study, five soil cores were collected from five different locations in Larsemann Hills. A great variation of four major types of bacterial colonies, based on their size and colour, was found and recorded; when the soil suspension was plated both onto Nutrient Agar and Yeast Extract Agar media. These microbes were grown in different media and different temperatures. Their morphology was examined by Scanning Electron Microscopic and growth pattern at different temperature ranging from 4°C to 37°C was also recorded. The samples showed a great variability, although collected from almost same geographical region.

Keywords: Aerobic, Bacteria, Yeast, Larsemann Hills, Antarctica.

1.0 INTRODUCTION

Antarctica is one of the most pristine and extreme places on Earth. This is because it is not so easily accessible and there is no native human population there. Exploitation of natural resources is prohibited, which makes it uninteresting for industries, as there is no immediate economic benefit. The whole continent is used for scientific study. Larsemann Hills is an ice-free area of around 40 square km, located approximately in the middle of the Vestfold Hills and the Amery Ice Shelf on the southeastern coast of Prydz Bay, Princess Elizabeth Land, East Antarctica[1]. The dominant rock types in the Larsemann Hills are metapelitic cordierite and Fe-Ti oxide-rich gneisses and various leucogneisses[2].

Near-zero and sub-zero environments on the surface of our earth have been lucratively colonized by plentiful organisms, exacting algae[3], fungi[4], lichens[5] and bacteria[6]. Fundamental conditions for life are its ability to self-replicate and to catalyze chemical reactions efficiently and selectively. As these organisms do not have any temperature regulation, their inner

temperature is close to the temperature of immediate environment. Despite the strong depressing effect of low temperature on biochemical reactions, these organisms grow and breed at rates comparable to those achieved by related species living in mild environments. It is obvious from the information, dating as early as 1990, that densities of bacterial cells as high as 10^7 ml^{-1} have been established in the Antarctic Ocean[7]. In the present investigation, an attempt has been taken to enumerate the bacterial and yeast load of five soil samples of Larsemann Hills of East Antarctica, which can be cultured on microbial growth media.

2.0 METHODOLOGY

In this study, five soil cores were collected from five different locations in Larsemann Hills. For collection, sterilized steel borers were used, with a diameter of 1 cm and length of 8 cm. Hammer was used to push the borer into the soil. Steel piston of compatible diameter was used for pushing the soil out of the borer into sterilized cryovials of 5 ml volume. The borer and piston were washed and burned every time, before collecting soil from a new location, to eliminate any chance of contamination. In every location air temperature, soil temperature, and the coordinates were recorded. After collection, the samples were stored in –24°C in the refrigerator. The list of collected samples is given in a tabular form in **Table-1.**

Table 1– Description of Antarctic Soil Sample

Sample No.	Location		Temperature (in ^{O}C)		Remark
	Latitude	Longitude	Soil	Air	
L.H.-01	69.41333 S	76.18916 E	-1.2	-4.7	Soil from snow covered area.
L.H.-02	69.40555 S	76.19166 E	-0.6	-1.1	Soil from the moss-bed near lake.
L.H.-03	69.40583 S	76.19305 E	-0.8	-1.3	Sand from between the cracks of the rock.
L.H.-04	69.37388 S	76.13861 E	-2.2	-5.5	Soil from under the rocks.
L.H.-05	69.40583 S	76.19444 E	-3.2	-7.6	Soil from between the cracks of rocks.
L.H.=Larsemann Hills					

Sterile glycerol 10% was used (1ml 10% glycerol for 1gm of soil) as a cryopreservent to minimize the detrimental effect of the freeze-thaw cycle, which was quite impossible to avoid while working with it.

One gram of soil was suspended in 5 ml of water. The suspension was subjected to vigorous vortexing to take the bacterial cells into suspension, detached from soil particles. It was diluted according to the need. Then 100µl of that suspension was spread on different culture media and kept for incubation. After the appearance of visible colonies, they were counted and calculated to correspond to per gram of soil. This same procedure is applied for all the five samples in triplicates. Different culture media used were: Nutrient Agar (Peptic digest of animal tissue 5gm/L, Beef Extract 3gm/L and Agar 15gm/L) and Yeast Extract Agar (Peptic digest of animal tissue 5gm/L, Yeast extract 3gm/L and Agar 15gm/L). Both of these media are commonly used for enumeration of bacteria in water, fecal matter, food and from other natural sources.

3.0 RESULTS AND DISCUSSION

Five different soil samples from the core area of Larsemann Hills were collected. These were from subzero soil temperature and showed a variation from: snow-covered area, below-moss bed, soil under the rocks (**Table 1**). A great variation of numbers of four major types of bacterial colonies, based on their size and colour, was found when the soil suspension was plated both onto Nutrient Agar and Yeast Extract Agar media. The colonies were either large or small in size and white or yellow in colours.

All the types of colonies were isolated originally at 4°C. All of them were tested for their ability to grow at different temperatures, at 4°C, 15°C, 28°C and 37°C. The response of one of the sample isolate is given in **Fig. 1.** Most of the isolates grew best at 15°C, indicating their

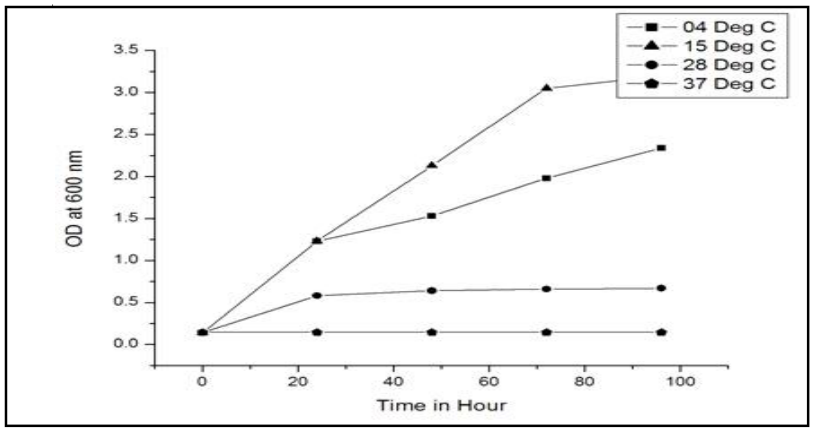


Fig 1. Response of one isolate to different temperature.

psychrophilic to psychrotolerant nature; which is quite understandable for their environment and conferred by natural selection over a long time. Although few of these isolates were able to show some growth at 28°C during the first few hours, but after 24 hours they stopped growing. All isolates showed no growth at all at 37°C. Details of the aerobic culturable microbial load is given in **Table- 2** and **Table- 3**. The soil sample LH5 that was collected from cracks of rocks showed maximum numbers of white bacterial colonies of both large and small types; and LH4 that was collected from below the rock contained minimum number of bacterial colonies.

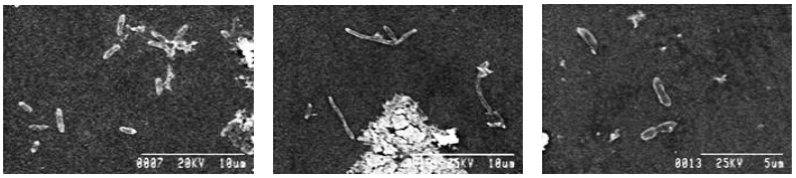
Table 2– Number of Microbial Population from Different Soil Samples on Nutrient Agar Medium at 15°C

Antarctic soil Sample No.	No. of colony forming unit bacteria (CFU) / gm of soil. All the values are approximated to the closest value of their mean.				No. of Fungal (Yeast) Colonies / gm of soil	Total Microbial count (CFU/gram soil.)
	Small		Large			
	White	Yellow	White	Yellow		
L.H.-1	150	0	50	0	0	$2x10^2$
L.H.-2	4650	0	2900	150	0	$7.7x10^3$
L.H.-3	7450	0	5100	0	0	$1.25x10^4$
L.H.-4	50	0	200	0	2450	$2.7X10^3$
L.H.-5	21150	0	3000	0	0	$2.41x10^4$

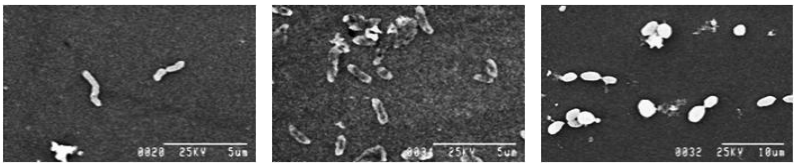
Table 3– Number of Microbial Population from Different Soil Samples on Yeast Extract Agar Medium at 15°C

Antarctic soil Sample No.	No. of colony forming unit bacteria (CFU) / gm of soil. All the values are approximated to the closest value of their mean.				No. of Fungal (Yeast) Colonies / gm of soil	Total Microbial count (CFU/gram soil.)
	Small		Large			
	White	Yellow	White	Yellow		
L.H.-1	200	0	0	50	0	$2.5x10^2$
L.H.-2	4350	600	2800	450	0	$8.2x10^3$
L.H.-3	6850	150	5600	0	0	$1.26x10^4$
L.H.-4	100	50	0	150	1850	$2.15x10^3$
L.H.-5	21550	0	2900	0	0	$2.44x10^4$

Surprisingly one of these soil samples showed quite a good number of yeast colonies. The number of both large and small white colonies did not vary much in Nutrient Agar and Yeast Extract Agar. Whereas the appearance of yellow colonies significantly increased in Yeast Extract Agar medium. All the colonies were smooth, slimy and had entire margins. As it is a preliminary report on the total microbial load, further characterization of biochemical and molecular studies are needed before comments on their generic taxonomical categories. Scanning Electron Microscopy of some of the members is given in the photographs (**Fig. 2, A to F**). Most of the bacteria are found to be gram-negative compared to the number of gram-positive ones; details are given in the **Table- 4**. Highest colony forming unit count in Nutrient Agar was found to be 2.41×10^4 per gram of soil and for Yeast Extract Agar it was found to be 2.44×10^4.



A. (LH-1) B. (LH-2) C. (LH-3)



D. (LH-4) E. (LH-5) F. (LH-4)

Fig. 2: Scanning Electron Microscopic photographs of bacterial samples (A to E) and Yeast (F) found in different soil samples of Larsemann Hills (Sample numbers are given in the parenthesis)

Table 4– Characteristic Feature of the Types of Isolated Microorganisms

Colony type	Gram Character	Morphology
Bacteria		
White large	+	Rod of varying size, may be right after division.
White small	-	Rod, some chain of rods is also noticed.
Yellow large	+	Rod of varying size.
Yellow small	+	Short rod.
Fungi/Yeast		
Yeast like colony	Yeast cells with budding ovoid structures was found.	

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