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RESEARCH ARTICLE

A PRELIMINARY STUDY OF COLONY FORMING UNITS OF BACTERIA FROM THE SOILS OF YUSMARG FOREST, KASHMIR VALLEY INDIA

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ABSTRACT

The present study was carried out to get an idea about the bacterial load (density and diversity) and to identify and isolate the bacteria from the soils of Yusmarg forest. A series of dilutions were made from soil samples, from the dilutions, 0.1ml inoculum was poured onto Nutrient agar(NA)and incubated at 28±2°C for 24 hours to study the growth of Bacteria.Comparative analysis of different types of colonies found at the four sites during the study indicates that the bacterial load was dominant in the month of November. The Individual Colony counts of the different types of isolates at the four sites shows that the most dominant isolate type was B_8 with a maximum of 73 colonies followed by B_{25} with 65. The total colony count was maximum at site III (256) followed by site II (198), site I (174) and site IV (144). The total bacterial population was maximum at site III with a cfu/g of 1.8×10^4 and minimum at site I with a cfu/g of 0.7×10^4 in the month of November and in December the maximum bacterial population was found at site 1 with cfu/g of 1.0×10^4 and minimum at site IV with a cfu/g of 0.4×10^4. During the study it was found that isolates B_1, B_2, B_4, B_6 and B_9 were present only in November while as isolates B_{15}, B_{21}, B_{25}, B_{27}, B_{28}, B_{29}, B_{30} and B_{31} were present only in December. However, isolates B_7 and B_8 were present both in the month of November and December. Among the different isolates obtained from site I B_{21}, B_{25} and B_{31} were having maximum cfu/g of 1.5×10^3 and B_4 was having a minimum cfu/g of 0.7×10^3 and among the different colonies of site II B_{11} was having maximum cfu/g of 1.6×10^3 and B_{26} was having a minimum cfu/g of 0.2×10^3, similarly among the different colonies of site III B_{18}, B_{21}, B_{22} and B_{23} were having a maximum cfu/g of 2×10^3 and B_{30} and B_{33} were having a minimum cfu/g of 0.7×10^3, while as among the different colonies obtained at site IV B_{16} and B_{26} were having a maximum cfu/g of 2×10^3 and B_8, B_{21} and B_{31}were having a minimum cfu/g of 0.3×10^3.

INTRODUCTION

The soil ecosystem is tremendously varied more so than many above-ground plant and animal food webs. Each species has slightly different requirements. Many organisms can digest simple sugars, while only a few species have the enzymes to digest lignin, a major component of woody tissue. At the microscopic level, soil conditions can change drastically from one point to the next, so a variety of organisms may be present in a single soil sample. Healthy soil is a jungle of rapacious organisms devouring everything in sight (including each other), processing their prey or food through their innards, and then excreting it. The value of these creatures to farmer's lies in, cycling nutrients, enhancing soil structure, which improves water and air movement, controlling disease and enhancing plant growth. Soil is a natural body consisting of layers (soil horizons) of mineral constituents of variable thicknesses, which differ from the parent materials in their morphological, physical, chemical and mineralogical characteristics. Soil may also be defined as the thin layer of earth's crust which serves as a natural medium for the growth of plants and micro-organisms. Soil microbial population is the key element in the bio-geochemical cycling of nutrients in nature (Pelczar *et al.,* 1993). More recently microbial diversity (community structure) has also been recommended as biological indicator of soil quality, micro-organisms are thus a source of nutrients at the base of all ecological food chains and webs. The number and kind of bacteria found in different types of ecosystems vary and are influenced by the ecosystem processes maintaining plant primary productivity (Griffiths *et al.,* 2003). Soil has a complex nutritional availability and is the natural habitat for highly diverse microbial flora. Torsvik *et al.,* (1990) found that above 4,000 differently sized microbial genomes are present per gram of soil representing roughly 13,000 different species. The major factors that influence the microbial community are: Moisture, *P*h, Temperature, Gases Organic and inorganic chemical (fertilizers), Organic matter, types of vegetation and its growing stages, Ploughing and particle size. Micro-organisms are essential to our very existence. They are ubiquitous found in common environments such as soil, water and air. It is estimated only about one percent of all of the microbial species on earth have been studied. Bacteria inhabit an extra ordinary array of habitats, from those that offer ideal conditions for most living creatures to those too extreme to

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support most life forms. Thus Soil, one of the greatest gifts of nature is a vital factor for life. Survival and development of living organisms including man depends on the presence and quality of soil, it is the best medium for the growth of micro-organisms

MATERIAL AND METHODS

Study Area and Study Sites

Yusmarg-situated at an altitude of about 2743m asl, lying in the Budgam District of Jammu and Kashmir state, is a small idyllic meadow set in the heart of mountains to the South West of Srinagar. It is situated at a distance of 47 kms from Srinagar city. It is an emerging tourist destination which is completely raw, pristine and still unspoiled, bandied by lush green grasslands, rivers and the backdrop of snow capped mountains(Fig 1).

Site I (Fenced Area)

It was that area which was not affected by the human and animal activities, the grazing rate in this area was negligible (almost zero). It is renowned for its green pasture and lies between the geographical co-ordinates of 74°40′ 1.653″ E and 33° 50′ 0.665″ N, having an elevation of 2418 m.

Site II (Grazing Area)

This area was under high grazing pressure was highly influenced by the human and animal activities and lies between the geographical coordinates of 74° 39′ 57.555″ E and 33°50 ′ 1.768″ N**,** having an elevation of 2411 m.

Site III (Deforested Area)

This site was close to main forest and was marked by deforestation. It lies between the geographical coordinates of 74° 39′ 57.506″ E and 33° 50′ 0.034″ N having an elevation of 2446 m.

Site IV (Forested Area)

This area was a dense forest of conifers, it lies between geographical coordinates of 74° 39′ 56.262″ E and 33° 49′ 55.747″ N, having an elevation of 2451 m and there was a large volume of litter and humus at this site.

Laboratory Analysis

Collection of Samples

Composite samples of soil from the four sites were collected during the study period, from a depth of 5 inches. Samples were collected in sterile polythene bags and carried to laboratory for bacteriological analysis. The samples were processed using the soil plate method (Warcup, 1950) and Soil dilution plate Method (Waksman, 1922).

Soil plate method

About 1g of soil was scattered on the bottom of a sterile Petri dish and molten cooled (40-45oC) agar medium (NA) was added, which was then rotated gently to disperse the soil particles in the medium. The plates were then incubated at 28±2°C for 24 hours.

Soil dilution plate method

The soil samples were mixed with sterile distilled water and a series of dilutions were made. From the dilutions, 0.1mlinoculum was poured onto Nutrient agar and incubated at28±2°C for 24 hours. The number of colonies counted was expressed as cfu/g and were calculated by using the formula.

$$Cfu/g = n \times d$$

Where n= number of colonies; d = dilution factor = 1/dilution

RESULTS AND DISCUSSION

Different types of colonies were obtained during the study period. Some colonies were circular in shape and some irregular, some rhizoid and some filamentous. A total of 36 colonies were obtained during the study and were assigned the names from B_1 to B_{36} (Table 1). Among the different isolates obtained from site I B_{21}, B_{25} and B_{31} were having maximum cfu/g of 1.5×10^3 and B_4 was having a minimum cfu/g of 0.7×10^3 (Table 3) and among the different colonies of site II B_{11} was having maximum cfu/g of 1.6×10^3 and B_{26} was having a minimum cfu/g of 0.2×10^3(Table 4), similarly among the different colonies of site III B_{18}, B_{21}, B_{22} and B_{23} were having a maximum cfu/g of 2×10^3 and B_{30} and B_{33} were having a minimum cfu/g of 0.7×10^3(Table 5) while as among the different colonies obtained at site IV B_{16} and B_{26} were having a maximum cfu/g of 2×10^3 and B_8, B_{21} and B_{31}were having a minimum cfu/g of 0.3×10^3(Table 6).

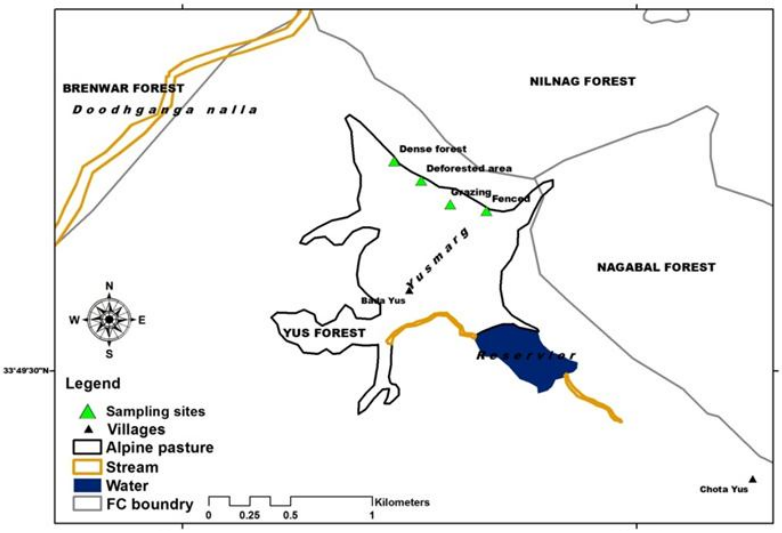

Fig 1: Map showing the study areas

Table 1. Individual colony counts of different types of colonies found at the 4 sites in the months of Nov. and Dec. 2010.

Isolate number	Site I		Site II		Site III		Site IV		Total
	Nov.	Dec.	Nov.	Dec.	Nov.	Dec.	Nov.	Dec.	
B_1	9	0	8	0	0	0	0	0	17
B_2	8	0	0	0	20	0	0	0	28
B_3	0	0	0	0	0	0	10	0	10
B_4	7	0	0	0	0	0	0	0	7
B_5	0	0	0	0	8	0	9	0	17
B_6	9	0	0	0	0	0	0	0	9
B_7	10	14	14	0	0	8	11	4	61
B_8	13	12	12	10	5	10	8	3	73
B_9	14	0	0	0	0	0	0	0	14
B_{10}	0	0	15	0	0	0	0	0	15
B_{11}	0	0	0	15	0	8	0	0	23
B_{12}	0	0	0	16	0	0	12	0	28
B_{13}	0	0	0	5	0	0	0	0	5
B_{14}	0	0	0	5	15	0	0	5	25
B_{15}	0	10	6	6	0	10	0	0	32
B_{16}	0	0	15	15	0	0	20	0	50
B_{17}	0	0	4	4	0	0	0	0	8
B_{18}	0	0	3	3	20	0	0	0	26
B_{19}	0	0	12	12	10	0	0	0	34
B_{20}	0	0	0	0	5	0	0	0	5
B_{21}	0	15	0	0	20	6	0	3	44
B_{22}	0	0	0	0	20	0	0	0	20
B_{23}	0	0	0	0	20	0	0	0	20
B_{24}	0	0	0	0	15	0	0	0	15
B_{25}	0	15	0	15	10	15	10	0	65
B_{26}	0	0	2	0	0	0	20	0	22
B_{27}	0	11	0	5	0	5	0	10	31
B_{28}	0	9	0	10	0	0	0	0	19
B_{29}	0	8	0	10	0	0	0	5	23
B_{30}	0	10	0	10	0	4	0	4	28
B_{31}	0	15	0	4	0	6	0	3	28
B_{32}	0	0	0	0	0	5	0	0	5
B_{33}	0	0	0	0	0	4	0	0	4
B_{34}	0	0	0	10	0	0	0	0	10
B_{35}	0	0	0	0	0	0	0	6	6
B_{36}	0	0	0	0	0	0	0	4	4

Table 2. Colony count, number of isolates and cfu/g at the four sites in the months of November and December 2010.

Site	November			December			Grand total
	Number of isolates	Colony count	Cfu/g	Number of isolates	Colony count	Cfu/g	
Site I (Fenced Area)	7	70	0.7×10^4	10	104	1.0×10^4	174
Site II (Grazing Area)	12	101	1.0×10^4	11	97	0.9×10^4	198
Site III (Deforested Area)	12	178	1.8×10^4	11	78	0.8×10^4	256
Site IV (Dense forest)	8	100	1.0×10^4	10	44	0.4×10^4	144

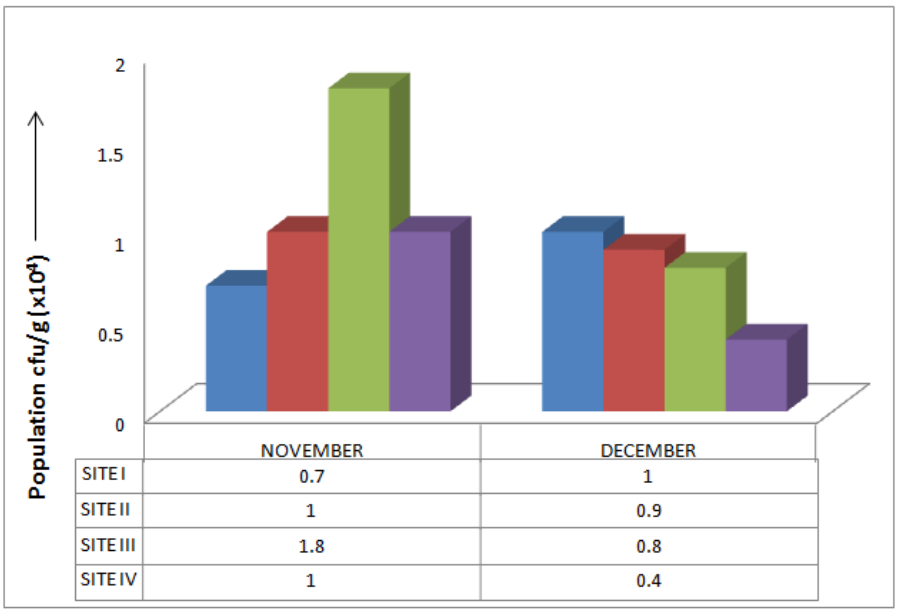

Fig 2. Comparison of cfu/g at the four sites in the months of November and December 2010.

Table 3. Colony Forming Units (cfu/g) of isolates at site 1 during November and December 2010

S. No.	ISOLATE NUMBER	NOVEMBER (cfu/g)	DECEMBER (cfu/g)
1.	B_1	0.9×10^3	0
2.	B_2	0.8×10^3	0
3.	B_4	0.7×10^3	0
4.	B_6	0.9×10^3	0
5.	B_7	1.0×10^3	1.4×10^3
6.	B_8	1.3×10^3	1.2×10^3
7.	B_9	1.4×10^3	0
8.	B_{15}	0	1.0×10^3
9.	B_{21}	0	1.5×10^3
10.	B_{25}	0	1.5×10^3
11.	B_{27}	0	1.1×10^3
12.	B_{28}	0	0.9×10^3
13.	B_{29}	0	0.8×10^3
14.	B_{30}	0	1.0×10^3
15.	B_{31}	0	1.5×10^3

Table 4. Colony Forming Units (cfu/g) of isolates at site II during November and December 2010

S. No.	ISOLATE NUMBER	NOVEMBER (cfu/g)	DECEMBER (cfu/g)
1.	B_1	0.8×10^3	0
2.	B_7	1.0×10^3	0.7×10^3
3.	B_8	0	0.5×10^3
4.	B_{10}	1.5×10^3	0
5.	B_{11}	1.6×10^3	0
6.	B_{12}	0.5×10^3	0
7.	B_{13}	0.5×10^3	0
8.	B_{14}	0.6×10^3	0
9.	B_{15}	1.5×10^3	1.0×10^3
10.	B_{16}	0.4×10^3	0
11.	B_{17}	0.3×10^3	0
12.	B_{18}	1.2×10^3	0
13.	B_{21}	0	0.4×10^3
14.	B_{25}	0	1.5×10^3
15.	B_{26}	0.2×10^3	0
16.	B_{27}	0	0.5×10^3
17.	B_{28}	0	1.0×10^3
18.	B_{29}	0	1.0×10^3
19.	B_{30}	0	1.0×10^3
20.	B_{31}	0	0.4×10^3
21.	B_{34}	0	1.0×10^3

Table 5. Colony Forming Units (cfu/g) of isolates at site III during November and December 2010

S. No.	ISOLATE NUMBER	NOVEMBER (cfu/g)	DECEMBER (cfu/g)
1.	B_2	2×10^3	0
2.	B_5	1.8×10^3	0
3.	B_7	0	0.8×10^3
4.	B_8	0.5×10^3	1.0×10^3
5.	B_{11}	0	0.8×10^3
6.	B_{14}	1.5×10^3	0
7.	B_{15}	0	1.0×10^3
8.	B_{18}	2.0×10^3	0
9.	B_{19}	1.0×10^3	0
10.	B_{20}	0.5×10^3	0
11.	B_{21}	2.0×10^3	0.6×10^3
12.	B_{22}	2.0×10^3	0
13.	B_{23}	2.0×10^3	0
14.	B_{24}	1.5×10^3	0
15.	B_{25}	1.0×10^3	1.5×10^3
16.	B_{27}	0	0.5×10^3
17.	B_{30}	0	0.4×10^3
18.	B_{31}	0	0.6×10^3
29.	B_{32}	0	0.5×10^3
20.	B_{33}	0	0.4×10^3

During the study the temperature and *p*H was recorded at the four sites under consideration, the maximum soil temperature (12.5^0**C)** was at site I in November while as it was minimum (0.3^0**C)** at site IV in December. The average soil temperature decreased from 10.75^0**C** in November to 1.6^0**C** in December. Average *p*H also decreased from 5.7 to 5.3. The total monthly bacterial population decreased from November to December at the four sites under consideration except site I where the population increased from November to December (Table 2 and Fig 2). This decrease in the count may be attributed to the difference in various biotic and abiotic factors that have been found to influence the density and diversity of soil bacterial communities. The results obtained in the present study are confirmed by the findings of Piao *et al.*, 2000; Fierer and

Table 6. Colony Forming Units (cfu/g) of isolates at site 1V during November and December 2010

S. No.	ISOLATE NUMBER	NOVEMBER (cfu/g)	DECEMBER (cfu/g)
1.	B_3	1.0×10^3	0
2.	B_5	0.9×10^3	0
3.	B_7	1.1×10^3	0.4×10^3
4.	B_8	0.8×10^3	0.3×10^3
5.	B_{12}	1.2×10^3	0
6.	B_{14}	0	0.5×10^3
7.	B_{16}	2.0×10^3	0
8.	B_{21}	0	0.3×10^3
9.	B_{25}	1.0×10^3	0
10.	B_{26}	2.0×10^3	0
11.	B_{27}	0	1.0×10^3
12.	B_{29}	0	0.5×10^3
13.	B_{30}	0	0.4×10^3
14.	B_{31}	0	0.3×10^3
15.	B_{35}	0	0.6×10^3
16.	B_{36}	0	0.4×10^3

Table 7. Temperature and *p*H recorded at four sites during November and December 2010.

Site	Temperature (^{0}C)		*P*h	
	Nov.	Dec.	Nov.	Dec.
I	12.5	2.5	6.26	6.48
II	11.5	2.3	6.6	5.9
III	10.0	1.2	5.25	4.5
IV	9.0	0.3	4.86	4.7
Average	10.75	1.6	5.7	5.3

Jackson, 2006. However the average variation in temperature and *p*H at the four sites in the months of November and December may also be attributed for the decrease in the bacterial population. The physical properties of soils like temperature and *p*H recorded at the four sites under consideration during the study period showed a variation of about 10^{o}C in soil temperature from November to December and also a *p*H change of 0.3(Table 7). So from the values of temperature and *p*H (Table 7) it is quite clear that the average temperature varied from 10.75^{o}C in November to 1.6^{o}C in December, a variation of about 10°C. Similar results were shown by Murphy, 2000 who showed that the bacteria grow faster at higher temperatures; the growth rate slows dramatically at low temperatures. The present findings are also confirmed by Pettersson 2004 who reported that the soil bacterial community had an optimum temperature for growth and diversity. Another reason for the decrease of bacterial population from November to December may be attributed to the decrease in *p*H because the average *p*H varied from 5.7-5.3.The present study is confirmed by the findings of Lauber *et al.,* 2009 who reported that the effect of soil *p*H on bacterial community composition is evident at even relatively coarse levels of taxonomic resolution. Similar results were also shown by Rousk *et al.,* 2010 who reported that the composition of the bacterial communities is closely defined by the soil *p*H, the apparent direct influence of *p*H on bacterial community composition is probably due to the narrow *p*H ranges for optimal growth of bacteria. The increase in the bacterial population at site 1 from November to December may be attributed to the increase in *p*H from 6.26 to 6.48, the grazing rate also decreased from November to December at this site. A study was carried out by Kohler *et al.,* 2005 to study the effect of cattle grazing on bacterial communities in pastures and he showed that bacterial community changes due to simulated effects of cattle grazing. The cattle activities may induce changes in the bacterial community structure.

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