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A Preliminary Study of Soil Bacteria of Kashmir University Campus

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ABSTRACT

A preliminary study on soil bacteria of Kashmir University Campus was carried out to assess their number and diversity. The campus comprises of two distinct areas which differ from each other markedly in terms of biotic and abiotic factors. The total viable count of Naseem Bagh Campus soil (8×10^4 cfu/g) was found to be more than Main University Campus soil (4.2×10^4 cfu/g). A total of 20 isolates of bacteria were recovered out of which 80% were Gram positive cocci. Almost all isolates showed fair utilization of carbohydrates like glucose, fructose. The isolates recovered from Main Campus exhibited higher tolerance to salt (0.5 – 3.5 % NaCl) concentration than those from Naseem Bagh Campus. Antibiotic sensitivity test done against four antibiotics Streptomycin (S), Amoxicillin (Am), Erythromycin (E), and Cloxacillin (Cx) revealed high degree of resistance, with only 8 isolates showing susceptibility.

Key words: Soil bacteria, Kashmir University Campus, diversity

INTRODUCTION

Fertile soil is inhabited by the root system of higher plants, many animal forms (e.g., rodents, insects & worms), and by tremendous numbers of micro-organisms. Soil microbial population is the key element in the biogeochemical cycling of nutrients in nature (Pelczar, *et. al.* 1993). They make possible the cycles of carbon, oxygen, sulphur and nitrogen that take place in terrestrial and aquatic ecosystems. 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). Most of the soil bacteria are decomposers that consume simple organic compounds, such as root exudates and fresh plant litter. The growth of bacteria in soil, like other microbes, is influenced by factors like the amount and type of nutrients available, moisture, degree of aeration, temperature, pH, etc. The existence of roots and extensiveness of the root system in soil also influence the numbers and kinds of bacteria in it.

The Campus of University of Kashmir, situated on the banks of famous Dal lake, comprises of two distinct areas namely Naseem Bagh Campus and Main Campus. The two campuses differ from each other in terms of vegetation cover, temperature, shade, amount of sunlight, etc., and hence differentiated into two

microclimatic habitats. A comparative study carried out on the physico-chemical characteristics of the soils of two campuses (Reyaz and Bhat, 2004; Kangroo and Bhat, 2005) have shown appreciable differences in the same. Since the physico-chemical and structural characteristics of soil provide many microenvironments in which bacterial populations can evolve (Ranjard and Richaume, 2001), a preliminary study on soil bacteria of the campus was considered valuable.

MATERIAL AND METHODS

The present study was conducted between May 2007 to September 2007 in the two comparable microhabitats of Kashmir University i.e., Naseem Bagh (Site A) which is dominated by Chinar trees and can be regarded as a woodland habitat and the Main University Campus (Site B) which is almost a type of open grassland.

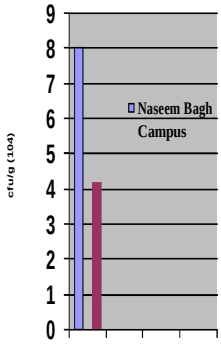
Composite soil samples were collected from the two sites by digging up to a depth of 5 inches with the help of spade. Samples were collected in sterile polythene bags and carried to the laboratory for bacteriological analysis. Analysis was done within 2 to 4 hours until which the samples were stored at room temperature.

Quantitative determination of bacteria was done by dilution plate method using spread plate technique (Cappucinno and sherman., 1992; Taylor *et al.*, 1983). Appropriate dilutions were spread on petri plates, two replicates for each dilution, containing nutrient agar as growth medium. The plates were incubated at 28$\pm$2^{o}C temperature in an incubator. A complete record of all the bacterial colonies appearing in each plate was maintained, and the bacterial count per gram of soil was calculated (Waksman, 1952).

Bacterial cultures isolated were pure cultured by four-way streaking and maintained on nutrient agar slants. Each isolate was given an isolate number on the basis of colony morphology, pigmentation, growth characteristics, etc. Growth behaviour of isolates was evaluated by carbon utilization test and salt tolerance test. Antibiotic sensitivity test of the isolates was carried out using Kirby-Bauer Method using antibiotic discs from Hi Media.

RESULTS AND DISCUSSION

In the present investigation, heterotrophic bacteria were isolated from the soil of Kashmir University Campus. Total bacterial counts of Naseem Bagh Campus (8×10^4 cfu/g) were found to be twice as much as found in Main Campus (4.2×10^4 cfu/g) (Fig 1). This may be attributed to the differences in various biotic and abiotic factors (Reyaz and Bhat, 2004; Kangroo and Bhat, 2005) that have been found to influence the composition and diversity of soil bacterial communities (Piao *et al.*, 2000; Fierer and Jackson, 2006).



Values are the mean of 5 samples
(cfu = colony forming units)
Fig. 1. Bacterial Population of Two Campuses (cfu/gm of soil)

A total of 20 strains were isolated, purified and designated (as A_1, A_2,……., A_{10} for Site A and B_1, B_2,……, B_{10} for Site B) on the basis of colony morphology, pigmentation and microscopic examination as shown in Table 1(a,b). Most of the colonies were small in size and either circular or irregular in shape. About 70% of strains isolated were observed as Gram positive cocci. Some spiral forms were also isolated from Site B. The isolates were further evaluated and characterized for their carbohydrate utilization pattern as shown in Table 2(a, b). All the isolates utilized glucose and fructose fairly well whereas lactose and mannitol were utilized only by 45% and 35% of the strains respectively. Similar results have been reported by Sheikh *et al*. (2006) on *Azotobacter spp*.

The growth behavior of the isolates against different concentrations of salt (NaCl -0.5-3.5%) was also evaluated (Table 3 a, b). It was observed that with the increase in salt concentration from 0.5% to 3.5%, the number of isolates showing growth decreased. For the ten isolates taken from Naseem Bagh campus, no inhibition in growth was observed at 0.5% salt concentration. At 1% concentration, 90% of strains showed maximum growth and at 2% and 3.5% salt concentration only 40% and 30% of the strains were tolerant respectively. Similar trend was observed in isolates from Main University Campus. Such decreasing trends to tolerance towards increasing salt concentrations have also been reported by Raj Kumar (1993) and Sheikh *et, al.,* (2006). However, in case of isolates from Main University campus higher tolerance to the increasing salt concentration was observed than those from Naseem Bagh Campus. This finding can be explained by the fact that this campus is manipulated and maintained for aesthetic purposes for which various types of manures and fertilizers were being continually added. The use of fertilizers leads to the formation of high salt

concentrations which can alter the structure and normal functioning of soil microbial complexes (Kravchenko, 1999; Lapygina *et al*, 2002).

Table 1(a). Colony morphology and microscopic examination of the isolates from Site A

Isolate No	Size	Margin	Elevation	Color	Shape	Gram's Reaction	Cell Shape
A_1	Moderate	Filamentous	Flat	White	Circular	+ve	B
A_2	Very small	Entire	Raised	Yellow	Circular	+ve	C
A_3	Very small	Undulate	Convex	White	Circular	+ve	C
A_4	Small	Lobate	Flat	Red	Biconvex	+ve	C
A_5	Small	Undulate	Raised	Yellow	Biconvex	+ve	C
A_6	Small	Entire	Convex	White	Circular	-ve	C
A_7	Large	Filamentous	Flat	White	Irregular	-ve	C
A_8	Small	Undulate	Flat	Creamish	Irregular	+ve	C
A_9	Small	Entire	Raised	White	Circular	+ve	B
A_{10}	Small	Undulate	Umbonate	Red	Irregular	+ve	C

B Bacilli C Cocci %age of cocci forms = 80%; %age of Bacilli forms = 20% %age of Gram +ve forms = 80%; %age of Gram -ve forms = 20%

Table 1(b). Colony morphology and microscopic examination of isolates from Site B

Isolate No	Size	Margin	Elevation	Color	Shape	Gram's Reaction	Cell Shape
B_1	Small	Entire	Convex	Red	Circular	-ve	C
B_2	Small	Undulate	Flat	White	Irregular	-ve	C
B_3	large	Filamentous	Flat	White	Irregular	+ve	C
B_4	Small	Entire	Convex	Creamish	Circular	-ve	S
B_5	Small	Entire	Convex	White	Circular	+ve	C
B_6	Moderate	Undulate	Flat	Creamish	Irregular	+ve	C
B_7	Small	Lobate	Umbonate	White	Irregular	+ve	B
B_8	Small	Entire	Raised	Creamish	Circular	+ve	B
B_9	Small	Entire	Raised	White	Circular	–ve	S
B_{10}	large	Undulate	Raised	White	Irregular	+ve	C

B Bacilli C Cocci S Spiral
%age of Cocci forms = 60%; %age of Bacilli forms = 20%; %age of Spiral = 20%
%age of Gram +ve forms = 60%; %age of Gram -ve forms = 40%

Table 2(a). Utilization of different carbon sources by isolated strains from Site A

Isolate No.	Carbon source			
	Glucose	Fructose	Lactose	Mannitol
A_1	++	++	+	-
A_2	++	+	+	-
A_3	+	+	-	-
A_4	++	++	+	±
A_5	++	+	±	-
A_6	++	+	-	-
A_7	++	++	+	±
A_8	+	+	+	±
A_9	++	±	-	-
A_{10}	++	++	+	-

++ full growth; + moderate growth; ± suppressed growth; - no growth

Table 2(b). Utilization of different carbon sources by isolated strains from Site B

Isolate No.	Carbon source			
	Glucose	Fructose	Lactose	Mannitol
B_1	++	++	+	±
B_2	++	+	±	-
B_3	++	++	+	±
B_4	+	+	±	-
B_5	++	++	-	-
B_6	++	+	±	±
B_7	++	++	++	-
B_8	++	+	-	-
B_9	++	+	-	-
B_{10}	++	++	++	±

++ full growth; + moderate growth; ± suppressed growth; - no growth

Table 3(a). Growth behavior of isolates at different salt concentrations (Site A)

Strain Codes	Salt concentration (%)			
	0.5	*1*	*2*	*3.5*
A_1	++	++	+	+
A_2	++	++	+	±
A_3	++	+	±	-
A_4	+	±	-	-
A_5	++	+	±	-
A_6	++	++	+	+
A_7	+	-	-	-
A_8	++	+	±	-
A_9	++	++	+	+
A_{10}	++	+	-	-

++ full growth; + moderate growth; ± suppressed growth; - no growth

Table 3 (b). Growth behaviors of isolates at different salt concentrations (Site B)

Strain codes	Salt concentration (%)			
	0.5	*1*	*2*	*3.5*
B_1	++	++	+	+
B_2	++	++	±	-
B_3	++	+	±	-
B_4	++	+	±	-
B_5	++	++	+	+
B_6	++	+	+	-
B_7	++	+	±	-
B_8	++	+	+	-
B_9	++	+	+	±
B_{10}	++	++	+	±

++ full growth; + moderate growth; ± suppressed growth; - no growth

The isolated strains were tested for sensitivity against four antibiotics namely, Streptomycin (S), Amoxicillin (Am), Erythromycin (E) and Cloxacillin (Cx) (Table 4 a, b). The results revealed high degree of resistance against all four antibiotics tested for isolates from both the sites. Amongst the antibiotics tested susceptibility was exhibited only against Streptomycin, 60% in case of Site A and only 30% in case of Site B. In case of other antibiotics almost all the isolates showed resistance which is of the order Am>E>Cx for Site A and Cx> E>Am for Site B. In general, 70% of strains from Site A and 77.5% of strains from Site B were resistant against all four antibiotics tested. About 15% of strains from each site also exhibited intermediate response which implies that the application of a stronger dose would make them susceptible to the drug tested for. Riesenfeld *et al.* (2004) while working on uncultured soil bacteria concluded that soil bacteria are a reservoir of antibiotic resistance genes. Resistance of a single bacterial isolates to more than one antimicrobial drug has also been variously reported (Norelli *et al.*, 1991; Sayah et *al.*, 2005).

Table 4(a). Antibiotic sensitivity behavior of isolates (Site A)

Bacterial strains	Antibiotic Agent			
	S	A_m	E	C_x
A_1	S	R	R	R
A_2	S	R	R	I
A_3	R	R	I	I
A_4	I	R	R	R
A_5	R	R	R	I
A_6	S	R	R	R
A_7	R	R	R	R
A_8	S	I	I	R
A_9	S	R	R	R
A_{10}	R	R	R	R

S - Streptomycin A_m - Amoxicillin E – Erythromycin C_x – Cloxacillin
R – Resistant I – Intermediate S – Susceptible

Table 4(b). Antibiotic sensitivity behavior of isolates (Site B)

Bacterial strains	Antibiotic Agent			
	S	A_m	E	C_x
B_1	S	R	R	R
B_2	R	R	I	R
B_3	I	R	R	R
B_4	R	R	R	R
B_5	S	R	R	R
B_6	R	R	R	R
$0B_7$	I	I	R	R
B_8	R	R	R	R
B_9	R	I	R	R
B_{10}	S	I	R	R

S-Streptomycin A_m-Amoxicillin E–Erythromycin C_x–Cloxacillin
R – Resistant I – Intermediate S – Susceptible

From the present study it may thus be concluded that the bacteria isolated from the soils of the University Campus are mostly gram positive cocci and the counts obtained from Naseem Bagh Campus are twice as much as those obtained from Main University Campus. From carbohydrate utilization test it may be concluded that glucose and fructose are better utilized as sources of carbon than lactose and mannitol. The strains have revealed high resistance patterns against all the drugs tested with the exception of Streptomycin. Almost all isolates from Main Campus showed resistance to Cloxacillin. Since the problem of drug resistance is gaining importance, the present investigation may be of value in this direction. The salt tolerance test done shows some isolates to be more tolerant towards increased concentration of salt (NaCl).This test needs a detailed investigation to reveal the changing salt (NaCl) pattern in the soils and its effect on soil micro-organisms.

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