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Isolation and Enumeration on Beneficiary Microbes from the Soils of Punjab

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Abstract: ***Certain beneficiary microbes viz. free living Nitrogen fixer (Azotobacter), associative Nitrogen fixer (Azospirillum) and Pseudomonas fluorescens were isolated from the soil samples of Ashoka in Ludhiana and Kharar, Punjab. During microbial population enumeration certain antagonistic interactions by bacteria (Bacillus) against other microbes have been reported. The population of all the microbes was more in rhizosphere than that of non-rhizosphere. The highest population of bacteria was reported in Model Town, Ludhiana (280 x 10^4/g soil) followed in Pakhowal Road, Ludhiana (65 x 10^4/g soil) and least was reported in Kharar (60 x 10^4/g soil). Azotobacter showed maximum frequency than that of Azospirillum and Pseudomonas. The highest percentage of Azotobacter was reported in Pakhowal Road, Ludhiana (29.23%) followed by Model Town, Ludhiana (17.14%) and least in kharar (15.00%). The population of Azospirillum was also highest in Pakhowal Road, Ludhiana (53.84%) and least in Kharar (3.33%). Similarly, Pseudomonas also showed higher frequency in Pakhowal road, Ludhiana (24.1%).***

Keywords: Beneficiary microbes, Ashoka, Soil samples

1. Introduction

Soil is an ecosystem that contains a diverse community of microorganisms in rhizosphere. These microbes provide precious life to soil system catering to some growth requirements to plants. The microbes work in cognito to maintain ecological balance by active participation in carbon, nitrogen, sulphur and phosphorus cycles in nature.Interacting with the underground compounds of plants, soil microbes form a particularly important part of rhizosphere[1]. Some of these interactions such as attack by disease causing microbes are undesirable, as they usually result in decrease in crop productivity. There are many interactions which are beneficial for plant productivity. From the abundance of beneficial soil microbes, the so called free-living nitrogen fixer Azotobacter, associative nitrogen fixer Azospirilliumand plant growth promoter Pseudomonas are probably of highest scientific interest.

These days, there is an upsurge in isolation and selection of beneficial microbes for development of plant growth promoting formulations for restoration forestry at distributed sites. Different media encourage the growth of different types of microorganisms [2]. These beneficiary microbial inoculants have been also used for ecological amelioration of degraded forest areas, waste lands, bioremediation of mining sites and areas degraded by industrial effluents.

Thus, present investigation was envisaged with the Objectives

- To collect and analyze soil samples from specific sites of Punjab.
- To isolate and enumerate the general bacterial microflora from these samples.
- To isolate and identify the diazotrophs viz. Azotobacter, Azospirillium and plant growth promoter (Pseudomonas) on specific media.

2. Materials and Methods

2.1 Soil Sampling

Surface soil samples (upto 30 cm depth) were collected from various soil classes viz. rhizosphere (with roots) and non-rhizosphere (roots free soil) of feeder roots ofSaracaasoca (Ashoka) tree at Ludhiana (Pakhowal Road and Model Town) and Kharar (Punjab). Composite soil sample of each class was mixed thoroughly and air dried. These samples were passed through 100-mesh sieve for soil Microbiological study. Sieved soil samples were used for determination of soil pH in 1:2 soil water suspensions, Electrical Conductivity (E.C), Organic Matter (O.M.) and total Nitrogen (N) using standard AOAC (Association of Official Agricultural Chemists) methods.

2.2 Microbial Enumeration:

Total bacterial and fungal population from these soil samples was determined by serial dilution on Nutrient agar media (NAM) by following pour plating method.

The media were sterilized by autoclaving at 15 lbs pressure for 20 minutes.

2.3 Isolation of Nitrogen Fixers microorganisms

Jensen's media [3] was used for isolation of Azotobacter, Nitrogen Free Bromothymol Blue (NFB) agarmedia (Dobereiner's agar) [4] for Azospirilliumand King's B media for isolation of Pseudomonas [5].

The frequency ofAzotobacter, Azospillium, Pseudomonasrespectivelywere calculated as the ratio of number ofspecificcolonies to total number of bacterial colonies on NAM at same dilution and expressed as percentage.

The flat, soft, milky and mucoid colonies of Azotobacter were picked and subcultured on NAM.

The morphological divergent Azospirillium colonies (white, yellow and pink) were isolated and preserved on NAM media.

The green-yellow colonies, exhibiting fluorescence under Ultraviolet light were transferred on NAM.

3. Results and Discussion

3.1 Analysis of Soil Samples

All the soil samples collected from the different areas viz. Model Town (Ludhiana), Pakhowal Road (Ludhiana) and Kharar were tested for their physiochemical properties. Most of soils under study were normal to alkaline with pH varying from 7.1 to 8.0. The electrical conductivity (E.C.) values from different locations indicated that all the soil samples were normal in nature with E.C. value ranging between 0.23 to 0.30 mmhos/cm. The soils were rich in organic carbon (0.38% to 0.50% O.C.) content. The O.C. was higher in rhizosphere than that of non-rhizosphere. The O.C. was maximum in Model Town, Ludhiana while the least in Kharar. The total nitrogen percentage (N %) was highest in Model Town, Ludhiana (0.038%) while minimum in Pakhowal Road, Ludhiana and Kharar (0.028%) (Table1).

3.2 Enumeration and Identification of Microorganisms (Bacteria)

All the collected soil samples were used to enumerate bacteria including beneficiary microbes viz. Azotobacter, Azospirillum and Pseudomonas to determine their inter-relationship in various zones of Saracaasoca (Ashoka tree) in Punjab. Dilution pour plate techniques were used to study the relative abundance of soil bacteria. Clear differences were reported between microbial population of corresponding rhizospheric and non-rhizospheric zones. The rhizosphere microbes behaved differently from non-rhizosphere both in terms on number.

Table 1: Characterisation of soil fertility in Punjab

Area		Soil sample	pH	E.C.[Electrical conductivity (mmhos/cm)]	Organic Carbon(O.C.) C%	Organic Matter percentage (O.M.%)	Total Nitrogen percentage (N%)
Punjab	Model Town, Ludhiana	R	8.0	0.30	0.50	0.86	0.038
		NR	7.6	0.24	0.47	0.81	0.036
	Pakhowal Road, Ludhiana	R	7.8	0.26	0.47	0.81	0.028
		NR	7.7	0.24	0.43	0.74	0.024
	Kharar	R	7.2	0.24	0.44	0.75	0.028
		NR	7.1	0.23	0.38	0.65	0.026

R- Rhizosphere
NR- Non-Rhizosphere

more in rhizosphere than that of non-rhizosphere. So rhizosphere showed a positive influence on microbial population. This may be due to the production of growth promoting substances such vitamins, amino acids etc. by the root of Saracaasocatree resulting in enhancing competitive ability of the beneficiary microbes. This zone also results in more survival rate of microbes. The results were supported by the finding of certain workers[6] who also reported the higher population of microbes in rhizosphere.

Growth of Microbes on General Media

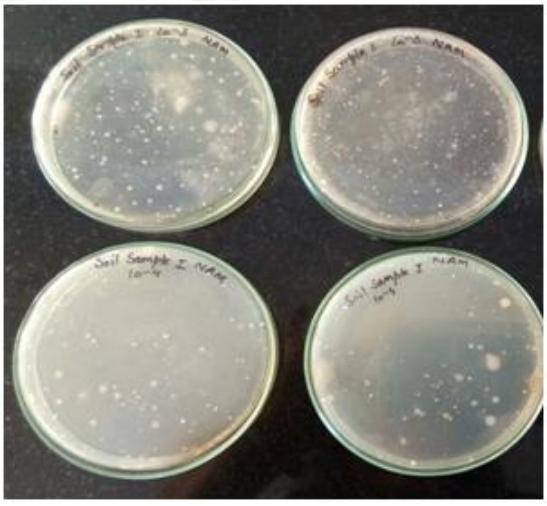


Figure 1: Bacterial colonies

The highest bacterial community in rhizosphere was recorded in Model Town, Ludhiana (280 × 10^4/g soil) followed by Pakhowal Road, Ludhiana (65 × 10^4/g soil) and the least in Kharar (60 ×10^4/g soil). Similar trend was also reported in non-rhizosphere zone. In non-rhizosphere, highest population also observed in Model Town, Ludhiana (120 × 10^4/g soil) followed by Pakhowal Road, Ludhiana (35 × 10^4/g soil) while the least in Kharar (30 ×

10^4/g soil) (Table 2). Thus the population of total bacteria in rhizosphere was reported to be highest as compared as to non-rhizosphere. Majority of bacterial isolates were Gram negative and non-motile and frequently belonged to Bacillus species. Besides this other microbes viz. Serratia and Micrococcus were also observed.

Antagonism was also reported in case of certain bacteria. The antagonistic action can be due to production of antibiotics, hormone like substances, competition for nutrients and colonization sites etc. as observed also by some scientists [7]. These studies show that microbial antagonist can be used in agricultural practice for improvement of soil and ultimately can prove beneficial for harnessing maximum benefits from crop.

Figure 2: Microorganisms showing Antagonism

3.3 Isolation and Frequency Determination of Nitrogen Fixing Bacteria (NFB)

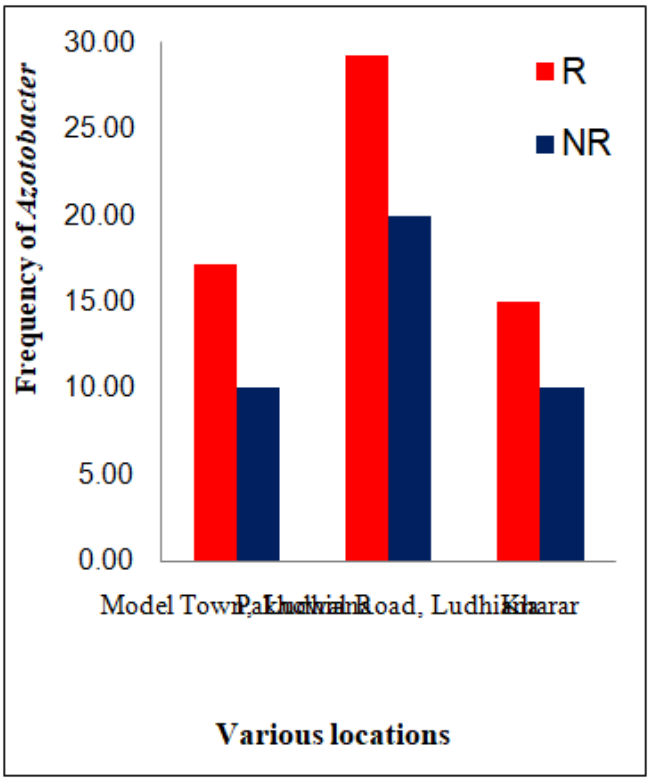
Nitrogen fixing bacteria (NFB) associated with Saracaasocain soils from rhizosphere and non-rhizosphere of different locations was also isolated and their frequency was also determined.

3.3.1 Frequency of Free Living Nitrogen Fixer (Azotobacter)

The population count of Azotobacter was also more in rhizosphere than non-rhizosphere sites. The positive rhizospheric effect of perennial plants on microbial activity has been widely reported by some scientists [8].

On the Jensen agar media the community of free living Nitrogen fixer (Azotobacter) in rhizosphere was higher in Pakhowal Road, Ludhiana (29.23%) followed by Model Town, Ludhiana (17.14%) and the least in the Kharar (15.00%). In the non-rhizosphere, community was also reported to be highest in Pakhowal Road, Ludhiana (20.00%) followed by Model Town, Ludhiana and Kharar (10.00%) (Table 2) (Figure 3) (Graph 1). The population ofAzotobacterwas higher in Pakhowal Road, Ludhiana in rhizosphere than that of non-rhizosphere zone. Our results are in corrobation with the result of some workers [9]. The results indicate that there will be more fixation of Nitrogen in the rhizosphere sites.

Figure 3: Azotobacter forming cyst during unfavourable conditions



Graph1: Frequency of Azotobacter in different locations of Punjab

3.3.2 Frequency of Associative Nitrogen Fixers (Azospirillum)

On the Dobereiner agar media, the community of associative Nitrogen fixer (Azospirillum) in rhizosphere and non-rhizosphere was reported. The population density of Azospirillum was higher in Pakhowal Road, Ludhiana (53.84%) followed by Model Town, Ludhiana (10.71%) and the least in the Kharar (8.33%). In the non-rhizosphere community was also reported to be highest Pakhowal Road, Ludhiana (31.42%) followed by Model Town, Ludhiana (6.66%) and the least in Kharar (3.33%) (Table 2) (Figure 4) (Graph 2). The population ofAzospirillumwas higher in Pakhowal Road, Ludhiana in rhizosphere than that of non-rhizosphere and our results are in corrobation with the results of various scientists [10].

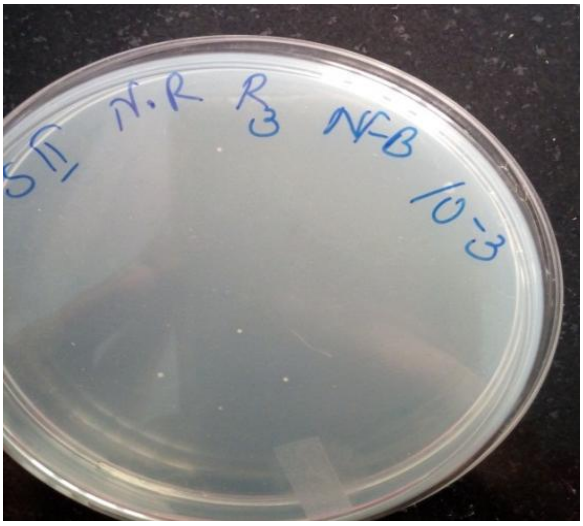
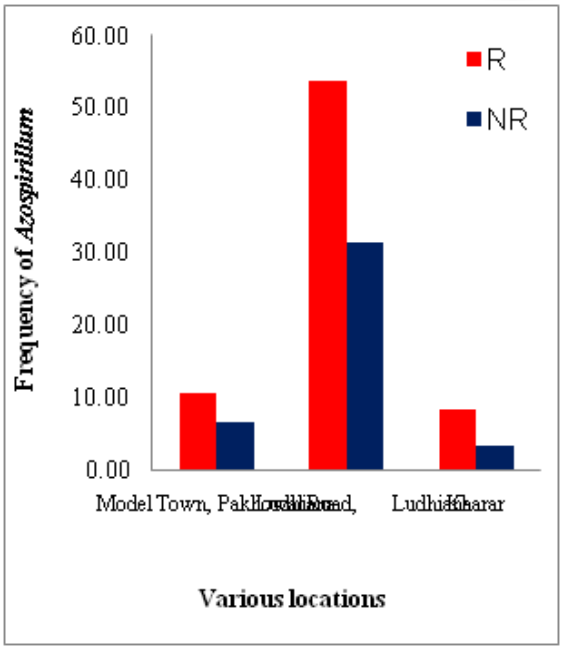
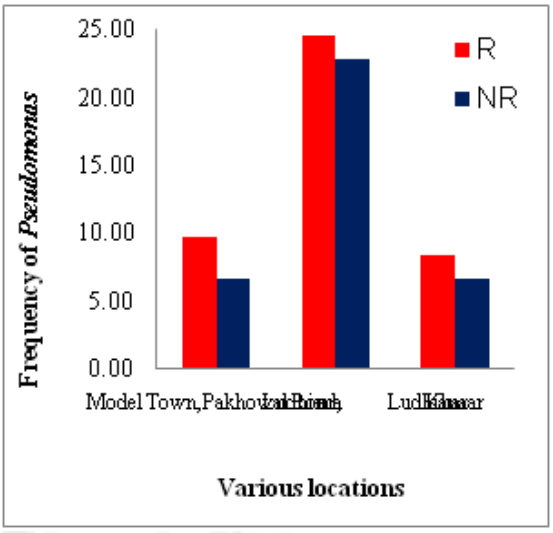


Figure 4: Azospirillum colonies on Dobereiner Agar Media

Figure 5: Pseudomonas colonies on King's B Media



Graph 2: Frequency of Azospirillum in different locations of Punjab



Graph 3: Frequency of Pseudomonas in different locations of Punjab

3.3.3 Frequency of Plant Growth Promoting Bacteria (Pseudomonas)

On the King's B agar media the community of plant growth promoting bacteria (Pseudomonas) in rhizosphere was highest in Pakhowal Road, Ludhiana (24.61%) followed by Model Town, Ludhiana (9.64%) and the least in the Kharar (8.33%). In the non-rhizosphere zone, the population of Pseudomonas was also reported to be highest in Pakhowal Road, Ludhiana (22.85%) followed by Model Town, Ludhiana and Kharar (6.66%) (Table 2) (Figure 5) (Graph 3). The population of Pseudomonas was higher in Pakhowal Road, Ludhiana on rhizosphere than that of non-rhizosphere. Our results are similar to the results of [11].

The highest population of these beneficiary microorganisms in rhizosphere zone than that of non-rhizosphere may be due to their highest competitive ability in the rhizosphere part. It may be due to their more antagonistic properties which allowed their much more survival is this region only, inhibiting the growth of other microorganisms. The low total viable count of bacteria and fungi as well as the low viable count of N_2 fixers and PGPR in the soil sample of Kharar were possible due to temperature and moisture factors coupled with fertility level [12].

The microorganisms proliferated in rhizosphere better than anywhere else as they obtain their nutrition from root exudates, plants mucigel and root lysates as reported by various worker [13].

Table 2: Frequency of Free Living Nitrogen Fixing Bacteria (Azotobacter) from the natural habitat of Saracaasoca (Ashoka tree) from Punjab

Area		Soil Sample	Bacterial Mean Plate count (x 10^4/g soil) on NAM	Azotobactercount (x10^4/g soil) onJensen's Agar	Azospirillum count (x 10^4/g soil) on Dobereiner's Agar	Pseudomonas count(x 10^4/g soil) on King's B media	Percentage of Azotobacter	Percentage of Azospirillum	Percentage of Pseudomonas
Punjab	Model Town, Ludhiana	R	280	48	30	27	17.14	10.71	9.64
		NR	120	12	8	8	10.00	6.66	6.66
	Pakhowal Road, Ludhiana	R	65	19	35	16	29.23	53.84	24.61
		NR	35	7	11	8	20.00	31.42	22.85
	Kharar	R	60	9	5	5	15.00	8.33	8.33
		NR	30	3	1	2	10.00	3.33	6.66

4. Conclusions

The work done would result in development of technology package for large scale multiplication and field delivery/application of efficient beneficiary strains for restoring vegetation in Punjab area. Mass multiplication of the native growth promoting microbes with wide ecological adaptability would be of wide practical utility in Agroecologicalprogrammes on recolonization and conservation of soils of this area. The microbial formulations and selected protocols would find practical utility in various governmental and non-governmental organisation/agencies involved in agricultural programme.

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Author Profile

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