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Bacterial Diversity in Sugarcane *(Saccharum Officinarum)* Rhizosphere of Saline Soil

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Abstract

A bacterium including PGPR plays a very important role in plant growth promotion and increase yield of crops. Most of the bacteria produce phytohormones, fixes atmospheric nitrogen, solublizes the phosphates and resist phytopathogens by production of siderophores. An understanding of microbial diversity perspectives in agricultural contest, is important and useful to know soil quality and also helpful for taking measures for soil management and increased plant productivity. It is also important to understand the relationship of soil and plants with the diversity of associated bacteria for their better exploitation. Therefore, it is important to know the microflora and their diversity. Most of the rhizospheric bacterial diversity from normal soil have been studied and organisms explored for their use as bioinoculents. However, saline soil rhizospheric microfloras remain unexplored. By considering this, in the present study fourty three bacterial isolates including PGPR have been isolated from saline soil of Kolhapur district of southern Maharashtra, India. Isolates were identified up to genus and species level. Few isolates were studied their nitrogen fixing and phosphate solublizing activity. Present study showed that amongst nitrogen fixing bacteria Azotobacter chroococcum found to be most dominant and Bacillus subtilis was found to be most dominant phosphate solublizer. Study indicated the importance of these organism as bioinoculents for saline soil and can be explored for biofertilizer production.

Keywords: Diversity, PGPR, Saline soils, Rhizosphere, sugar cane.

Introduction

A number of bacterial species associated with plant rhizosphere belonging to genera *Azospirillum, Bacillus, Pseudomonas, Rhizobium, Serratia, Arthrobacter, Aceinetobacter, Alcaligenes, Erwinia, Flavobacterium, Burkholderia*, Which exerts a beneficial effect on plant growth[1]. They enhance growth of the plant by phosphate solublization, Nitrogen fixation, Phytohormone and exopolymer production[2-4].

Plant play a important role in selecting and enriching the types of bacteria by the constituents of their root exudates, thus depending on the nature and concentration of organic constituents of exudates and corresponding ability of bacteria to utilize these as sources of energy, the bacterial community develops in the rhizosphere[5-8].

Soil is highly heterogenous and complex microhabitat, which is reflected in the spatial distribution and enormous diversity of microorganisms and their metabolic versatility. The importance of soil microorganisms for sustenance of all other forms of life needs no emphasis, in fact it is the presence of microorganisms that modifies the habitat and makes it possible for other life forms to survive and function. Soil organisms contribute to the critical soil function by acting as primary driving agents of nutrients of nutrient cycling, regulating the dynamics of soil organic matter.

The indigenous species and strains of bacteria are very useful in production of bioinoculents for local crops because these organisms have already been adapted to local environmental conditions, hence they can be explored as bioinoculents for local crops. It is also important to study the organisms from saline rhizosphere habitats because these organisms have adapted to osmoregularity mechanisms which are still not well known. Studying diversity of such soil will contribute towards long term goal of improving plant-microbe interactions for salinity affected fields and crop productivity.

Soil microorganisms also play an important role in soil processes that determine plant productivity. Therefore it is necessary to determine the microbial diversity of indigenous community, their distribution and behavior in soil habitats. By considering this in the present study sugarcane rhizosphere is explored for bacterial biodiversity from saline soils of Kolhapur district of Western Maharashtra, India.

Material and Methods

Collection of soil samples: Soil samples from rhizosphere regions of Sugarcane crop were collected from sixteen different sites asceptically in sterile plastic bags from saline soils of Kolhapur district of western Maharashtra, India. Samples were collected at 90d of crop.

Physiochemical analysis: Physicochemical analysis were carried out as per the methods described by[9].

Isolation of Microorganisms: One gram of rhizospheric soil was separated from roots with help of brush in petridish. It was dissolved in 100ml buffered saline and placed on shaker for 30 min. From this different dilutions Viz 10^{-2},10^{-4},10^{-6},10^{-8},10^{-10} were prepared. From each dilutions 0.1 ml was spread on Nutrient agar for isolation as well as enumeration of different bacteria, 0.1ml was spread on Ashbys Mannitol agar for *Azotobacter* spp.,Congored yeast extract agar for *Rhizobium*spp., Nitrogen free agar for *Azospirillum* spp respectively. Individual colonies showing different morphology from respective medium were transferred on slants of respective media and further used for identification and other studies. Unless otherwise stated experiment was conducted in triplicates.

Identification of Microorganisms: All the isolates were identified as per the Bergeys Mannual of Systematic bacteriology[10] and Micro IS software[11].

Functional Characterization: Functional characterization of isolates was studied by qualitative screening for their Phosphate solublization on KB medium, Nitrogen fixation by Acetylene reduction assay[12-14].

Results and Discussion

Table 1and 2 indicates the physicochemical characters of saline soils.

Table-1
Physicochemical analysis of Saline Soils

Villege	Sr. No.	ECe	pH	Moisture %	Organic matter %	Organic carbon %	Nitrogen content %	Available 'P' Kgha^{-1}
Arjunwad	1	06	8.7	21.8	1.26	0.73	0.02	5.4
	2	04	8.61	18.2	1.4	0.81	0.05	5.2
	3	07	8.72	22.4	1.34	0.78	0.04	5.5
	4	09	8.76	22	1.12	0.65	0.06	5.4
		6.50	**8.70**	**21.10**	**1.28**	**0.74**	**0.04**	**5.38**
Kurundwad	5	04	8.9	18.6	1.45	0.84	0.07	5.8
	6	05	8.95	19.2	1.98	1.15	0.06	5.4
	7	08	8.97	21.4	1.76	1.02	0.05	5.5
	8	07	8.98	17.2	1.69	0.98	0.02	5.4
		6.00	**8.95**	**19.10**	**1.72**	**1.00**	**0.05**	**5.53**
Udgaon	9	05	8.66	23.7	1.34	0.78	0.06	5.7
	10	07	8.71	22.8	1.09	0.63	0.04	6.9
	11	06	8.73	22.2	1.24	0.72	0.05	7.2
	12	04	8.79	21.4	1.19	0.69	0.03	6.4
		5.50	**8.72**	**22.53**	**1.22**	**0.71**	**0.05**	**6.55**
Chinchwad	13	08	8.68	27.4	1.38	0.8	0.04	4.69
	14	04	8.72	30.8	1.28	0.74	0.06	4.63
	15	05	8.76	28.2	1.17	0.68	0.03	4.54
	16	06	8.78	26.4	1.07	0.62	0.06	4.58
		5.75	**8.74**	**28.20**	**1.23**	**0.71**	**0.05**	**4.61**

Table-2
Physicochemical analysis of normal soil

Villege	Sr. No.	ECe	pH	Moisture %	Organic matter %	Organic carbon %	Nitrogen content %	Available 'P' Kgha^{-1}
Arjunwad	1	0.90	7.80	15.20	1.58	1.84	0.12	24.00
Kurundwad	2	1.50	7.70	10.40	1.48	2.55	0.10	22.00
Udgaon	3	1.20	7.75	14.60	1.48	2.55	0.11	23.00
Chinchwad	4	1.40	8.92	12.30	1.39	2.39	0.10	24.00
Average of Total Village		1.25	8.04	13.13	1.48	2.33	0.11	23.25

Results indicated that saline soils contain less quantity available nutrients as compared to normal soil because of which after some years these soils become non productive.

Microbiological analysis: Enumeration of Bacteria: Results of bacterial count in the saline and normal soils are presented in table 3 and 4. The data showed that the average bacterial population was 95.37 x 10^4 g^{-1} in saline soil and in normal soil sample was 663.75 x 10^4 g^{-1}. This indicated that the population of bacteria was far below than the bacterial population of normal soil which is eight times less than normal soil. It was highest 119.75 x 10^4 g^{-1} in soils from Kurundwad and lowest 55.50 x 10^4 g^{-1}, in soil from Chinchwad.

Enumeration of Actinomycetes, *Azotobacter* Sp, Rhizobium and phosphate solublizing bacteria: The average population of *Azotobacter* was 1.43 x 10^4 g^{-1}, which was significantly less than the normal soils. *Azotobacter* population was highest 3.75 x 10^4 g^{-1} in soil samples collected from Kurundwad and the lowest number 0.25 x 10^4 g^{-1} was recorded in soil samples collected from Chinchwad. The average *Rhizobium* population was 2.37 x 10^4 g^{-1}. It was highest i.e. 4 x 10^4 g^{-1} in the soil samples collected from Kurundwad and was lowest i.e. 0.25 x 10^4 g^{-1} in the soil samples collected from Chinchwad areas.The average population of Phosphate solublizers was 3.18 x 10^4 g^{-1}. It was highest 4.25 x 10^4 g^{-1}in the soil samples collected from Kurundwad and was lowest 2.0 x 10^4 g^{-1} in the soil samples collected from Chinchwad areas. The average population of Phosphate solublizers in the normal soil was 135.5 x 10^4 g^{-1}.

Overall results of enumeration of different microorganisms from sugarcane saline soil rhizosphere indicated that bacterial population was eight times less than normal soil.

Table-3
Microbial Population of Saline Soils (Microbial Population x 10^4/gm)

Village	Sr. No.	Bacteria	Actinomycets	*Azotobacter*	*Rhizobium*	Phosphate Solublizers
1.	2.	3.	4.	5.	6.	7.
Arjunwad	1	120.00	24.00	1.00	4.00	4.00
	2	128.00	27.00	3.00	6.00	5.00
	3	113.00	22.00	1.00	3.00	4.00
	4	109.00	21.00	1.00	2.00	3.00
Avg.		**117.50**	**23.50**	**1.25**	**3.75**	**4.00**
Kurundwad	5	138.00	28.00	10.00	8.00	7.00
	6	125.00	24.00	3.00	5.00	4.00
	7	111.00	21.00	1.00	2.00	3.00
	8	105.00	19.00	1.00	1.00	3.00
Avg.		**119.75**	**23.00**	**3.75**	**4.00**	**4.25**
Udgaon	9	103.00	18.00	1.00	1.00	4.00
	10	98.00	18.00	1.00	2.00	2.00
	11	85.00	16.00	0.00	2.00	3.00
	12	69.00	16.00	0.00	1.00	2.00
Avg.		**88.75**	**17.00**	**0.50**	**1.50**	**2.50**
Chinchwad	13	65.00	15.00	1.00	1.00	3.00
	14	55.00	12.00	0.00	0.00	2.00
	15	54.00	12.00	0.00	0.00	2.00
	16	48.00	11.00	0.00	0.00	1.00
Avg.		**55.50**	**12.50**	**0.25**	**0.25**	**2.00**
Avg. population		**95.37**	**19.00**	**1.43**	**2.37**	**3.18**

Table-4
Microbial Population in normal soils (Microbial Population x 10^4/gm)

Village	Sr. No.	Bacteria	Actinomycets	*Azotobacter*	*Rhizobium*	Phosphate Solublizers
1.	2.	3.	4.	5.	6.	7.
Arjunwad	1	660.00	120.00	22.00	16.00	140.00
Kurundwad	2	627.00	110.00	15.00	12.00	133.00
Udgaon	3	710.00	148.00	26.00	21.00	138.00
Chinchwad	4	658.00	178.00	18.00	17.00	131.00
Avg.		**663.75**	**139.00**	**20.25**	**16.50**	**135.50**

Table-5
List of Identified Bacterial isolates

Isolate No.	Name of the bacterial Isolate	Isolate No.	Name of the bacterial Isolate	Isolate No.	Name of the bacterial Isolate
1	*Bacillus firmus*	16	*Bacillus brevis*	30	*Planococcus citreus*
2	*Bacillus sphaericus*	17	*Bacillus subtilis*	31	*Arthrobacter species*
3	*Peptostreptococcus productus*	18	*Streptomyces species*	32	*Azotobactor chroococcum*
4	*Pseudomonas pseudomallei*	19	*Bacillus stearothermophilus*	33	*Serratia marcescens*
5	*Alcaligens species*	20	*Azospirillum brasilens*	34	*Pseudomonas fluorescens*
6	*Bacillus subtilis*	21	*Azotobacter chroococcum*	35	*Bacillus firmus*
7	*Bacillus megaterium*	22	*Rhizobium species*	36	*Bacillus firmus*
8	*Flavobacterium multivorum stearothermophilus*	23	*Rhizobium species*	37	*Planococcus citreus*
9	*Chromobacterium violaceum*	24	*Azotobactor chroococcum*	38	*Micrococcus luteus*
10	*Pseudomonas mallei*	25	*Bacillus brevis*	39	*Azotobactor chroococcum*
11	*Peptostreptococcus productus*	26	*Pseudomonas fluorescens*	40	*Bacillus subtilis*
12	*Bacillus subtilis*	27	*Arthrobacter species*	41	*Azospirillum lipoferum*
13	*Bacillus brevis*	28	*Arthrobacter species*	42	*Bacillus megatarium*
14	*Bacillus subtilis*	28	*Azotobactor venelandii*	43	*Pseudomonas fluorescens malleiffpfluore scenspseudomonallei*
15	*Bacillus firmus*	29	*Azospirillum lipoferum*		

Table 5 indicates the list of identified bacteria from sugarcane rhizosphere of saline soils. Amongst all the bacterial isolates genera *Bacillus* was found to be the most dominant followed by Pseudomonas and amongst species *Bacillus subtilis* was found to be most dominant, which correlates with[15].

The strains from the genera *Bacillus ,Pseudomonas, Rhizobium* are amongst the most phosphate solublizers. *Pseudomonas* was dominant at 60d, 90d[16]. Some researcher studied the maize PGPR and their role in plant growth promotion[17,18]. They found that *Azotobacter chroococcum* and phosphate solublizer *Bacillus* megaterium as most dominant Nitrogen fixer and phosphate solublizer. I report *Bacillus subtilis* as most dominant phosphate solublizer and *Azotobacter chroococcum* Nitrogen fixer.

Different PGPR in Sugarcane crops in Brazil was studied[19,20], they showed presenceof *Aceinetobacter* diazotrophicus, *Azospirillum brasilence, Azospirillum lipoferum, Burkholderia, Herbaspirillum, Rhizobium leguminosarum* as dominant nitrogen fixer while my results indicated that *Azotobacter chroococcum, Azospirillum lipoferum Rhizobium leguminosarum* as dominant nitrogen fixers, However, species of *Burkholderia*, and *Herbaspirillum* were found to be absent in saline soils of Kolapur district.

Number of bacterial species associated with sugarcane rhizosphere was isolated[3]. These belonging to *Azospirillum, Alcaligens, Arthrobacter, Acinetobacter, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium,* and *Serratia.* My investigation also detected presence of *Azospirillum, Alcaligens, Arthrobacter, Bacillus, Flavobacterium, Pseudomonas, Rhizobium, and Serratia.*

While *Burkholderia, Enterobacter, Acinetobacter, Erwinia*,were found to be absent in saline soils of Kolhapur district.

It was reported the accumulation of compatible solutes such as Glutamate, Proline, Glycine, Betaine and Trehalose in response to salinity/ osmolarity in *Azospirillum* and *Azotobacter* species which indicated that these strains can be used as bioinoculents for saline soils[21-24].

Conclusion

There is a scope for use of nitrogen fixing *Azotobacter chrococcum* and Azospirillum lipoferum as potential Nitrogen fixing biofertilizer and Bacillus subtilis as potential phosphate solublizer for reclamation of saline soils. On presenting this work, I am impressed with the ability of *Azotobacter chrococcum* and Azospirillum lipoferum to grow in the presence of salts. Further there is lack of comparative results primarily due to difficulty in comparing results obtained, my work will encourage researcher to obtain comparative results. It may hope that my investigations may inspire others to carry out work on salt tolerant nitrogen fixing *Azotobacter chrococcum* and *Azospirillum lipoferum, and Bacillus subtilis* other aspects

which have not yet studied. Detail microbiological analysis of saline soil carried out with respect to PGPR Bacteria, , which could serve as Basic data for further research. A survey of available literature, suggests that microbiology of saline soil and exploitation of microorganisms from these soil has not been dealt extensively. Considering this lacuna, investigations were focused on the microbiology of saline soil and potential of these microorganisms for commercially important bioinoculents for saline soils.

There is scope for use of nitrogen fixer *Azotobacter chroococcum, Azospirillum lipoferum, Azospirillum brasilense* and phosphate solublizing *Bacillus subtilis, Pseudomonas fluoescens* as potential Biofertilizers for reclamation saline soils of local area because isolates belongs to same soil. On completing this investigatation, I am impressed with the wide diversity of microorganisms present in saline soils.

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