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Microbial diversity in rhizospheric soils of Chilli crop

Microbiology

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ABSTRACT

A tremendous interest has emerged with respect to the importance of microbial diversity in rhizospheric soil which depends on soil health and quality. The objective of this study was designed using an annual crop Chilli (Capsicum annuum L) and the diversity was constantly monitored and studied for the species dominance, richness and evenness of plant growth promoting microorganism. Expecting such an empirical relationship from the single Chilli is useful to test diversity predictions in natural sites. Although there appears to be a great deal of work on plant growth promoting microorganism, there are very few reports on such associations with Chilli plants. The findings of the present investigation will highlight the plant growth promoting microorganism from the local soil of chilli plants. Diversity of plant growth promoting microorganism in chilli soils collected from various places includes 7 different criteria's such as Bacteria, Azospirillum, Azotobacter, Pseudomonas, Actinomycetes, Rhizobia, and Fungi. Diversity indices was analysed using Pearson's correlation matrix. Among all the 7 different criteria the most significant was Rhizobia and Actinomycetes respectively in the soil samples collected from different region.

KEYWORDS:

Chilli, diversity, plant growth, CFU

Introduction

Capsicum annuum L. is a dicotyledonous flowering plant, with different general names in English, such as hot pepper, chilli, chilli, sweet pepper, hot pepper chile pepper, and bell pepper. In Spanish the usual name is chile, which results in the plurals of chillies, chilies, and chiles and the pre-Columbian indigenous Nahua (Aztec) Amerindian name for the plant was transcribed as chilli or chilli (Bosland, 2000).

Chilli is commonly grown worldwide either outside in fields or in greenhouses. The ability to produce a quality crop in such a wide range of climates and conditions has helped to make chilli globally a common crop. It is also known around the world for its flavour, degree of pungency, nutritive value, colour, and as a spice - vegetable, due to its taste and unlimited utility, it is of high demand all over India and the world (Tiwari et al, 2005). Because of the great diversity and wide variability in colour, shape, size, and degree of pungency, India is the biggest producer of chilli.

Microbial diversity is a potential goldmine and a vast frontier for the industry because it offers biochemical pathways and countless new genes to probe for enzymes, antibiotics and other useful molecules (Singh & Agrawal 2002). Of late, interest in soil microbes as a diverse group of soil organisms that prevent the attack of soil-borne plant pathogens (Chanway, 1997) and that promote plant growth (Bashan, 1998) has taken prominence. Those beneficial free living soil bacteria are usually referred to as Plant Growth Promoting Rhizobacteria or PGPR (Kloepper et.al, 1989).

Rhizosphere the dynamic zone in soil is under the influence of plant root includes the existence of complex interactions between plants and soil microflora with plant-beneficial, -neutral and -detrimental (pathogenic) interactions. The rhizosphere extends several centimeters from the root region (Darrah 1993) and includes root exudates such as sugars, amino acids, organic acids, and minerals stimulating the soil microflora to colonize the rhizospheric region and exceeds soil microflora 1000 times the population observed in non-rhizosphere soil (Bowen and Rovira 1976, Jones 1998), which also enhances plant growth by producing phytohormones, siderophores that chelate essential metabolites, suppression of pathogenic microorganisms by the production of antifungal metabolites, and induce resistance in plants against pathogens.

Materials and methods

Soil analysis: The rhizosphere soil samples were collected from different chilli plant cultivating areas: Mysore, Hassan regions of Karnataka, India. Soil samples were collected in order to isolate, characterize and investigate the diversity of plant growth promoting microorganisms associated with the roots of chilli plants. Top soil was removed, roots were pulled out soil (15cms depth) surrounding the roots were collected in sterile plastic bags and preserved at lower temperature. Analysis for microflora was done within 2 days of soil sample collection.

Isolation

The soil samples were desiccated, compacted and sieved, from which 1g soil was suspended in suspended in 9ml saline and swen at 150rpm for 20 minutes at 37°C. Supernatant was serially diluted (10-1 to 10-10) in triplicates and inoculated using spread plate technique and pour plate technique on different media such as

a. Yeast Extract Mannitol Agar with Congo Red (CRYEMA) for Rhizobia was incubated at 28°C for 2days.
b. Ashby's Mannitol Agar for *Azotobacter* was incubated at 28°C for 3-4 days.
c. Nitrogen free Bromothymol Blue (NfB) agar for *Azospirillum* was incubated at 30°C for 7-8days.
d. Modified Rose Bengal Agar (MRBA) for fungal counts was incubated at room temperature for 3-5days.
e. Pikovskaya's Agar for isolating of phosphate solubilizers was incubated at 30°C for 3-7days.
f. Soil Extract Agar(SEA) medium for *Actinomycetes* was incubated at 30°C for 8-12days
g. King's BV(KB) medium for *Pseudomonas* was incubated at 37°C for 24-48 hrs.
h. Nutrient Agar(NA) for nonspecific organisms was incubated at 37°C for 24-48 hrs.(Aneja, K.R. 2001)

Enumeration

Plant growth promoting microorganisms populations were evaluated by using colony forming unit (CFU). Number of bacteria per ml of the original sample was calculated using the formula:

CFU/g = number of colonies on a plate ×dilution factor (reciprocal of the dilution)

Screening of plant growth promoting microorganism:

Statistical analysis : The diversity of Plant growth promoting microorganism that occurred in different samples were subjected to Pearson's Correlation matrix.

Result and discussion

Diversity of Plant growth promoting microorganisms in chilli soils collected from various places (Mysore and Hassan) includes 7 different criteria's such as Bacteria, *Azospirillum, Azotobacter, Pseudomonas, Actinomycetes, Rhizobia, and* Fungi (Table 1&2).

Pearson's Correlation matrix (Table 1.2 & 2.2) among microorganisms such as Bacteria, *Azospirillum, Azotobacter, Pseudomonas, Actinomycetes, Rhizobia* and Fungi was compared. Rhizobia and Azotobacter (Table 1.2) were found to be significant in the chilli soil samples collected from Mysore region indicating their dominance and richness against the other microorganisms such as Bacteria, *Azospirillum, Pseudomonas, Actinomycetes,* and Fungi.

Actinomycetes (Table 2.2) was found to be significant in the chilli soil samples collected from Hassan region indicating their dominance and richness against the other microorganisms such as Bacteria, *Azospirillum, Azotobacter, Pseudomonas, Rhizobia* and Fungi.

Table 1: Isolation of microorganism in rhizospheric chilli soils collected from Mysore region

Organisms	Dilutions									
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Bacteria	432	396	349	259	147	135	76	21	9	2
	371	362	312	261	201	112	91	37	12	7
	401	389	309	272	196	142	102	42	32	15
Azospirillum	32	34	29	24	21	14	11	9	7	2
	37	32	30	21	18	15	13	11	8	7
	42	37	32	31	27	23	26	18	13	9
Azotobacter	46	39	31	21	18	15	9	8	7	4
	57	52	49	42	32	27	18	13	7	2
	51	43	39	34	28	27	21	19	5	-
Pseudomonas	211	113	97	85	63	54	41	39	21	18
	182	132	110	91	69	56	49	37	25	15
	189	149	123	95	67	59	46	30	29	16
Actinomycetes	22	19	14	19	13	9	5	4	1	1
	23	21	19	17	14	8	4	2	4	-
	25	24	18	18	16	9	3	1	-	-
Rhizobia	12	7	5	2	4	3	1	-	-	-
	9	7	4	3	2	1	-	-	-	-
	12	5	7	6	4	2	1	-	-	-
Fungi	24	15	9	5	5	4	3	2	1	-
	22	19	7	4	3	3	3	4	2	2
	19	14	11	10	9	7	5	5	3	2

Table 1.2 Pearson's Correlation matrix of organisms isolated from chilli soils of Mysore region

	1	2	3	4	5	6	7
1	**1**						
2	**.983****	**1**					
3	**.987****	**.991****	**1**				
4	**.953****	**.952****	**.962****	**1**			
5	**.981****	**.966****	**.969****	**.926****	**1**		
6	**.930****	**.925****	**.935****	**.992****	**.915****	**1**	
7	**.883****	**.887****	**.894****	**.966****	**.840****	**.959****	**1**

**. Correlation is significant at the 0.01 level (2-tailed),

1. Bacteria 2. Azospirillum 3. Azotobacter 4. Pseudomonas 5. Actinomycetes 6. Rhizobia 7. Fungi

Table 2: Diversity of microorganism in rhizospheric chilli soils collected from Hassan region

Organism	Dilutions									
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
Bacteria	249	219	135	112	91	59	19	7	6	4
	272	216	142	122	97	65	32	21	15	3
	279	206	149	129	89	69	42	15	16	4
Azospirillum	124	119	97	86	61	72	55	31	25	14
	129	121	99	91	69	59	41	29	15	9
	122	117	94	81	71	69	62	39	27	19
Azotobacter	31	27	21	11	14	10	8	5	3	2
	32	29	19	17	16	12	10	5	2	-
	39	25	21	14	14	12	09	4	3	-
Pseudomonas	9	8	8	7	5	2	1	1	-	-
	15	12	11	9	8	5	3	2	-	-
	12	13	10	7	7	3	2	2	1	-
Actinomycetes	42	39	24	19	13	9	5	4	1	1
	45	39	29	21	19	15	12	9	6	4
	49	41	27	25	21	16	10	9	6	2
Rhizobia	8	4	4	1	-	-	-	-	-	-
	7	3	5	4	3	1	-	-	-	-
	7	2	4	3	2	1	-	-	-	-
Fungi	29	15	9	8	6	3	2	1	-	1
	24	11	9	6	5	3	2	2	-	-
	20	10	9	8	8	4	3	2	-	-

Table 2.3 Pearson's Correlation matrix of organisms isolated from chilli soils of Hassan region

	1	2	3	4	5	6	7
1	**1**						
2	**.961****	**1**					
3	**.987****	**.968****	**1**				
4	**.957****	**.960****	**.949****	**1**			
5	**.996****	**.971****	**.990****	**.964****	**1**		
6	**.942****	**.886****	**.926****	**.919****	**.920****	**1**	
7	**.958****	**.879****	**.955****	**.882****	**.943****	**.961****	**1**

**. Correlation is significant at the 0.01 level (2-tailed).

1. Bacteria 2. Azospirillum 3. Azotobacter 4. Pseudomonas 5. Actinomycetes 6. Rhizobia 7. Fungi

Conclusion

Diversity of plant growth promoting microorganismin chilli soils collected from various places includes 7 different criteria's such as *Bacteria, Azospirillum, Azotobacter, Pseudomonas, Actinomycetes, Rhizobia, and Fungi.* Diversity indices was analysed using Pearson's correlation matrix. Among all the 7 different criteria the most significant was Rhizobia and *Actinomycetes* respectively in the soil samples collected from different region.

One contribution of this study is an increase in the knowledge about the microbial diversity in rhizospheric region of different soil samples. Based on these data about the diversity of microbes in soil, it should be able to isolate particular species from soil and reveal their interactions with plant and other microbes in future. Though the variety of abundance of species in a defined unit is known as biodiversity it is also synonymous with species richness and relative species abundance in space and time. To comprehend a perfect diversity species richness and species diversity is to be studied, where in heterogeneity can be understood.

The present work is also aimed to find the alternative approaches like investigating indigenous microbes for the presence of plant growth promoting traits and to select those microbes which can be used to increase the yield of crop plants is in progress.

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