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Rhizosphere and Rhizoplane bacteria of Okra (*Abelmuscus esculentus Linn.Moench*) and Cauliflower (*Brassica oleracea* Linn. Var. Botrytis) and their characterization

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Abstract: *The present study is aimed to study the diversity of bacterial flora in the rhizosphere and rhizoplane of Abelmuscus esculentus (Okra) and Brassica oleracea var. Botrytis (Cauliflower) and their morphological and biochemical characterization.*

Rhizosphere is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. The microbe- plant interaction in the rhizosphere can be beneficial, neutral, variable, or deleterious for plant growth. Rhizobacteria that exert beneficial effects on plant development are termed plant growth promoting rhizobacteria (PGPR).

The rhizobacteria from rhizosphere and rhizoplane were isolated by serial dilution techniques and plating onto Nutrient agar medium. The isolates were subjected to various biochemical test.

The results revealed that the population density of Rhizosphere (RS) and Rhizoplane (RP) bacteria in A. esculentus was maximum during August being 3.85 X 10^8CFU/g and 3.975X10^8 CFU/g respectively which declined to 1.95 X 10^8CFU/g and 2.35 X1 0^8CFU/g respectively in December. Similarly, the population density of rhizosphere and rhizoplane bacteria in B. oleracea var. Botrytis was maximum during August being 2.95 X 10^8 and 3.65 X 10^8 CFU/g which declined to 1.95 X 10^8 and 2.15 X 10^8 respectively in December. Twenty bacterial bacterial flora viz. Bacillus. Subtilis, B. megaterium, B. pumilus, B. polymyxa, B. mycoides, Lactobacillus acidophilus, L. plantarum, L. brevis, Micrococcus luteus, Azotobacter beijerinckii, A. chroococcum, Pseudomonas putida, P fluorescence,P. aeruginosa, Streptococcus salivaris, Staphylococcus saprophyticus, Klebsiella sp, Corynebacterium sp, Arthrobacter sp, Citrobacter sp, Rhodococcus erythropolis and Erwinia amylovora were isolated in the rhizosphere and rhizoplane of these vegetable crops.

The isolates viz. Bacillus subtilis, B. megaterium, B. pumilus, B. polymyxa, Lactobacillus acidophilus, L. plantarum, Pseudomonas putida and P. aeruginosa showed catalase negative reaction whereas Bacillus mycoides, Azotobacter chroococcum, A. beijerinckii, Micrococcus luteus, Streptococcus salivaris, Staphylococcus saprophyticus, Klebsiella sp. Corynebacterium sp. Arthrobacter sp. Citrobacter sp. Rhodococcus erythropolis and Erwinia amylovora showed catalase positive reaction. Bacillus mycoides, Micrococcus luteus Pseudomonas fluorescence, P. putida Corynebacterium sp. and Citrobacter sp. showed anaerobic (Hugh- Leifson's O- F) negative result. Other species were non-reactive with Hugh- Leifson's O- F reaction. Micrococcus luteus, Klebsiella sp. and Rhodococcus erythropolis were hemolytic whereas Lactobacillus acidophillus, Azotobacter chroococcum, Pseudomonas fluorescence, P. putida, P. aeruginosa, Corynebacteriumsp. and Citrobacter sp. were non hemolytic. Only Micrococcus luteus and Klebsiella sp. showed Voges Preskaur's negative reaction and others were non-reactive. All rhizobacteria showed non-reactive to methyl red test. All isolates exhibited siderophore production and maximum Phosphate-solubilizing activity and, therefore, can be exploited as bioinoculants/ biofertilizers for improvement of crops.

Key Words: *Rhizosphere, Rhizoplane, Siderophore, Population density, Phosphate solubilization, Abelmuscus esculentus, Brassica oleracea var. Botrytis*

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I. Introduction

Rhizosphere is the narrow region of soil that is directly influenced by root secretions and is considered as a "hot spot" for microbial colonization and activity (Bolton *et al*., 1993) [1]. Soil which is not part of the rhizosphere is known as bulk soil. The rhizosphere contains many bacteria that feed on sloughed-off plant cells,

termed *rhizodeposition*, and the proteins and sugars released by roots. Except for some pathogensic microbes, many rhizosphere microorganisms can improve the growth and health of plants by various mechanisms, such as nitrogen (N2) fixation or production of plant hormones (Patten and Glick 1996; Andrews and Harris 2000) [2, 3]. The different species of plants are also regulators for the diversity (Gelsomino *et al*., 1999; Girvan *et al*., 2003) [4, 5] and biogeography (Normand *et al*., 2007) [6] of the rhizosphere bacteria.

Plants secrete many compounds into the rhizosphere which serve different functions. Strigolactones, secreted and detected by mycorhizal fungi, stimulate the germination of spores and initiate changes in the mycorhiza that allow it to colonize the root. The parasitic plant, *Striga* also detects the presence of strigolactones and will germinate when it detects them; they will then move into the root, feeding off the nutrients present. Symbiotic Nitrogen- fixing bacteria, such as the *Rhizobium* species, detect an unknown compound secreted by the roots of leguminous plants and then produce nod factors which signal to the plant that they are present and will lead to the formation of root nodules, in which the bacterium, sustained by nutrients from the plant, converts nitrogen gas to a form that can be used by the plant. Non-symbiotic (or "free-living") nitrogen-fixing bacteria may reside in the rhizosphere just outside the roots of certain plants (including many grasses), and similarly "fix" nitrogen gas in the nutrient-rich plant rhizosphere. Even though these organisms are thought to be only loosely associated with plants they inhabit, they may respond very strongly to the status of the plants. For example, nitrogen-fixing bacteria in the rhizosphere of the rice plants exhibit diurnal cycles that mimic plant behavior, and tend to supply more fixed nitrogen during growth stages when the plant exhibits a high demand for nitrogen (Sims and Dunigan, 1984) [7].

Some plants secrete allelochemicals from their roots which inhibit the growth of other organisms. For example garlic, mustard produces a chemical which is believed to prevent mutualisms forming between the trees and mycorrhiza (Stinson *et al*., 2006) [8].

The plant root-microbe interaction occurs in soil which leads to destructive, associative or symbiotic associations. The microbe has to pass the rhizosphere region before the start of interaction with plant roots. Beneficial association of microbes with roots may be bacterial, *Actinomycete, Cyanobacterial* or fungal symbiosis. *Azospirillum, Azotobacter* and *Beijerinckia* are known to cause associative symbiosis with the roots of several non-leguminous plants viz., Corn, Wheat, Sorgham etc. *Pseudomonas, Bacillus* and *Streptomyces* are non-symbiotic beneficial rhizobacteria which affect the plant growth favourably (Kloepper, 1980) [9]. These bacteria increase the growth of host plants due to change in balance of *Rhizospheremicro flora* producing an indirect effect on the crops control of pathogens and other harmful microorganisms in the rhizosphere, production of growth hormones like gibberellins and Indole Acetic Acid, release of nutrients nitrogen fixation by *rhizobacteria.*

The rhizosphere is frequently divided into the endorhizosphere, the rhizoplane and the ectorhizosphere (Lynch, 1990) [10]. These respective compartments encompass the root tissues, the root surface and associated soil. Soil further away from the rhizosphere is often termed as bulk soil. Root symbiotic mycorrhizal fungi are also important since they provide a link between bulk soil and plant roots in the mycorrhizosphere (Linderman, 1988) [11]. Observations have shown that the concentration of bacteria found around the roots of plants is generally much greater than in the surrounding soil and that the rhizosphere supports higher microbial growth rates and activities as compared to the bulk soil (Soderberg and Baath, 1998) [12]. One of the main reasons for these higher growth rates is the increased availability of soluble organic compounds that results from plant root exudation. These are typically carbohydrate monomers, amino acids and sugars, but the composition and quantity of root exudates varies depending on plant species and abiotic conditions such as water content and temperature (Martin and Kemp, 1980) [13]. In turn, rhizosphere microorganisms increase root exudation through production of plant hormones or more directly by physically damaging the roots (Grayston *et al*., 1996) [14]. In general, the nutrient-rich rhizosphere is naturally colonized by many beneficial or pathogenic bacteria and fungi which may have a considerable impact on plant growth, development and productivity. The numerous interactions between bacteria, fungi and roots may have beneficial, harmful or neutral effects on the plant, the outcome being dependent on the type of symbiont interaction and the soil conditions (Lynch 1990; Smith and Read, 1997) [10, 15].

The population of microbial species in the rhizosphere may fluctuate from thousands to millions (Nihorimbere *et al*., 2011) [16], and the interactions between roots and soil microbes are often specialized and based on coevolutionary pressures (Dobbelaere *et al*., 2003; Duffy *et al*., 2004; Morgan *et al*., 2005; Morrissey *et al*., 2004) [17, 18, 19, 20]. In the rhizosphere, plant-microbe interactions play important roles in a number of vital ecosystem processes such as carbon sequestration and nutrient cycling (Singh *et al*., 2004) [21]. Positive plant–microbe interactions include symbioses (associations with plant-growth promoting rhizobacteria (PGPR), epiphytes, and mycorrhizal fungi. These interactions have many beneficial impacts on physiological processes of plants, including disease suppression (Haas and Defago, 2005; Mendes *et al*., 2011; Weller *et al*., 2002) [22, 23, 24], increased nutrient availability and uptake (Lugtenberg *et al*., 2002; Morrissey *et al*., 2004) [25, 20], and increased immunity to abiotic (Selvakumar *et al*., 2012; Zolla *et al*., 2013) [26, 27] and biotic stresses (Badri *et*

al., 2013*b*; Zamioudis and Pieterse, 2012) [28, 29], each of which leads to increases in plant productivity (Berg 2009). The root exudates of plants, in turn, are used as substrates and signaling molecules by microorganisms (Bais *et al*., 2006) [30].

The greater Rhizosphere effect is observed with bacteria (R: S values ranging from 10-20 or more) than with Actinomycetes and fungi. Gram-negative, rod shaped, non-sporulating bacteria which respond to root exudates are predominant in the rhizosphere *(Pseudomonas, Agrobacterium).* While Gram-positive, rods, Cocci and aerobic spore forming *(Bacillus, Clostridium)* are comparatively rare in the rhizosphere. The most common genera of bacteria are: *Pseudomonas, Arthrobacter, Agrobacterium, Alcaligenes, Azotobacter, Mycobacterium, Flavobacter, Cellulomonas, Micrococcus* and others have been reported to be either abundant or sparse in the rhizosphere. From the agronomic point of view, the abundance of nitrogen fixing and phosphate solubilizing bacteria in the rhizosphere assumes a great importance. The aerobic bacteria are relatively less in the rhizosphere because of the reduced oxygen levels due to root respiration. The bacterial population in the rhizosphere is enormous in the range from 10^8 to 10^9 per gram of rhizosphere soil. They cover about 4-10% of the total root area occurring profusely on the root hair region and rarely in the root tips. There is predominance of amino acids and growth factors required by bacteria, are readily provided by the root exudates in the region of rhizosphere.

The microbe- plant interaction in the rhizosphere can be beneficial, neutral, variable, or deleterious for plant growth (Baker and Cook, 1974). Rhizobacteria that exert beneficial effects on plant development are termed plant growth promoting rhizobacteria (PGPR) (Kloepper and Scfoth, 1978) [31]. The term rhizobacteria is used for bacteria that aggressively colonize the rhizosphere (SubbaRao, 1999) [32]. Although the mechanisms by which PGPR promote plant growth are not yet fully understood, many different traits of these bacteria are responsible for growth promotion activities (Cattelan *et al*., 1999) [33]. It includes the ability to produce or change the concentration of the plant hormones indoleacetic acid (IAA), gibberellic acid, cytokinins, and ethylene; fix dinitrogen; suppress the growth of deleterious microorganisms by production of siderophore, β-1, 3- glucanase, chitinases, antibiotics, and cyanide; and dissolve phosphates and other nutrients.

Initially, *Azotobacter* and *Azospirillum* were believed to promote plant growth due to their ability to fix dinitrogen. Later, it was known that other plant growth stimulating hormones such as IAA was also involved (Kennedy, 1998) [34]. The use of P- solubilizing bacteria was reported to increase plant growth in some cases, but in other cases it was not. It indicated that other mechanisms may involve in growth response (De Freitas *et al*., 1997 [35].

The plant growth promoting activities of soil bacteria have been studied by Edi Husen (2003) [36]. The Rhizosphere microorganisms have been largely reviewed by Mc Near Jr. David H. (2013); Bais *et al*., (2004); Benfey *et al*., (2010); Genere and Bonfante (2007); Giles *et al*., (2008); Hartmann *et al*., (2008); Zhang *et al*., (2010) [37, 38, 39, 40, 4, 42, 43] etc. Plant growth promoting Rhizobacteria have been studied by Saran and Nehra (2011) [44]; Joseph *et al* ., (2007) [45]; Karakurt *et al*., (2009) [46]; Hameeda *et al*., (2006) [47]; Narula *et al*., (2006) [48]; Mandal *et al*., (2007) [49]; Sridevi and Mallaiah (2007) [50]; Egamberdieva (2008) [51]; Kidoglu *et al*., (2007) [52] etc.

Rhizobacterial association in the improvement of crops has been studied by several authors viz; Assmus *et al*., (1997) [53], Dazzo *et al*., (1996) [54], Downie (1994) [55], Hirsh(1992) [56], Kannenberg and Brewin (1994) [57], Perotto and Boftante (1997) [58], Sabry *et al*., (1997) [59], Schwintzar and Tjepkema (1990) [60], Strobel and Long(1998) [61], Van Rhijn and Vanderleyden (1995) [62], Richa *et al*., (2018) [63] etc.

Biodiversity of bacteria in the Rhizosphere has largely been studied by several workers viz. Seema Rawat *et al*., (2011) [64], Luan *et al*., (2015) [65], Jason A. Peiffer *et al*., (2013) [66], KMari *et al*., (2001) [67], NidhiSakha *et al*., (2016) [68], Javier Pascual *et al*., (2016) [69], Pratibha Prashar *et al*., (2013) 70], Hao Wang *et al*., (2014) [71] etc. The biodiversity of Bacteria in the Rhizosphere of *Abelmuschus esculentus* and *Brassica oleracea* L. var. Botrytis and their biochemical characterization has not been studied so far and hence the present investigation was undertaken.

II. Materials And Methods

The study was conducted at local Okra (*Abelmuschus esculentus*) and Cauliflower (*Brassica oleracea* var. Botrytis) growing garden soil of Patna for five months, from August 2018 to December 2019 at the intervals of 30 days. Soil and root samples of Okra and Cauliflower were collected aseptically in sterile plastic bags from a field at the intervals of 30 days in every month. The various physical characteristics of soil viz., temperature, pH and moisture content were recorded in every month at an interval of 30 days.

Isolation of Rhizosphere and Rhizoplane bacteria

In order to isolate rhizosphere bacteria about 1g of rhizosphere soil of *Abelmuschus esculentus and Brassica oleracea* var Botrytis was transferred separately to 50 mL test tube containing 10 mL sterile distilled water and vortexed vigorously for 10 min. The resulting solution containing the rhizosphere bacteria was serially diluted up to 10^{-4} using sterile distilled water. 100 µl aliquot was taken from each dilution and plated in triplicate onto Nutrient agar medium and incubated at 28°C for 24 - 96 h. After incubation, colony counts were recorded and colonies with distinctive morphologies were selected for further studies. The isolated bacteria were purified by streak plate technique (Beisher, 1991) [72]. The bacterial isolates obtained through this process of isolation were subjected to various biochemical tests according to "Bergey's Manual of Determinative Bacteriology 9th ed. (Holt *et al*., 1994; Robert Breed *et al*.,1962) [73,86].

In order to isolate rhizoplane bacteria roots of *Abelmuschus esculentus* and *Brassica oleracea* were placed in sterile beaker separately containing autoclaved distilled water. It was shaken well and then 10-20 serial washings were given until clear root surface was exposed. Roots were placed with sterile forceps on nutrient agar and were incubated at 28±1^{0}C for 24h- 96h.

Morphological Characterization

The morphological characterization of the bacterial colonies were carried out on the basis of their shape, size, colour, margin, elevation on the media and Gram staining were performed to decide the further determinative protocol.

Chemical Characterization

The pure culture of each isolate was subjected to identification by Bergey's Manual of Determinative Bacteriology, 9th ed.(Holt et al., 1994) using catalase test with 3% hydrogen peroxide, Anaerobic Test, Oxidative Fermentative (O/F test), hemolytic test, methyl red test, Voges Proskaeur test, glucose test, sorbitol test and mannitol test, Oxidase test, and Catalase test. The functional diversity amongst recovered isolates was studied by qualitative screening of their ability to solubilize phosphorus and siderophore production. Isolates exhibiting clearing zone on Pikovskya's agar (Pikovskaya, 1948) [74] after 96-120h of incubation was considered as positive Phosphate solubilization bacteria. Pikovskaya's medium consisted of following composition:

Ingredients	Quantity in (g/L)
Glucose:	10.0
$Ca_3(PO_4)_{2:}$	5.0
$(NH_4)_2SO_{4:}$	0.5
NaCl:	0.2
$MgSO_4.7H_2O$:	0.1
KCl:	0.2
Yeast extract:	0.5
$MnSO_{4:}$	Trace
$FeSO_4.7H_2O$:	Trace
Agar:	15.0

Similarly, siderophore production was assayed according to Schwyne and Neilands (1987) [75]. Isolates exhibiting an orange halo zone on Chromeazurol S agar after 48-72h of incubation were considered as positive. Data were statically analyzed by mean ± S.E and by one-way analysis of variance, ANOVA.The results obtained have been presented in Table1-6 and Figure 1 and 2.

Table- 1: Physical characteristics of Okra and Cauliflower growing garden soil of Patna

Characteristics	Physical characteristics of soil				
	August	September	October	November	December
Temperature in ^{0}C	28±1	25±0.50	23±0.45	17±1.2	13±0.45
pH	8.1±0.04	8.2±0.03	7.9±0.12	7.3±0.13	6.65±0.14
Moisture (%)	78.00±0.21	75.00±0.25	65.00±0.13	64.00±0.16	61.15±0.12

Table- 2: Population density of bacteria in the Rhizosphere and Rhizoplane zone of *Abelmuschus esculentus* in terms of Colony Forming Unit (CFU/g of soil)

Zone	August	September	October	November	December
Rhizosphere	3.84 X 10^8 ±0.034	3.65X 10^8±0.025	2.75 X 10^8 ±0.12	2.25 X 10^8±0.041	1.95X10^8 ±0.065
Rhizoplane	3.97 X10^8 ±0.027	3.90 X10^8 ±0.032	3.85 X10^8 ±0.042	3.55 X10^8 ±0.061	2.35 X10^8 ±0.13

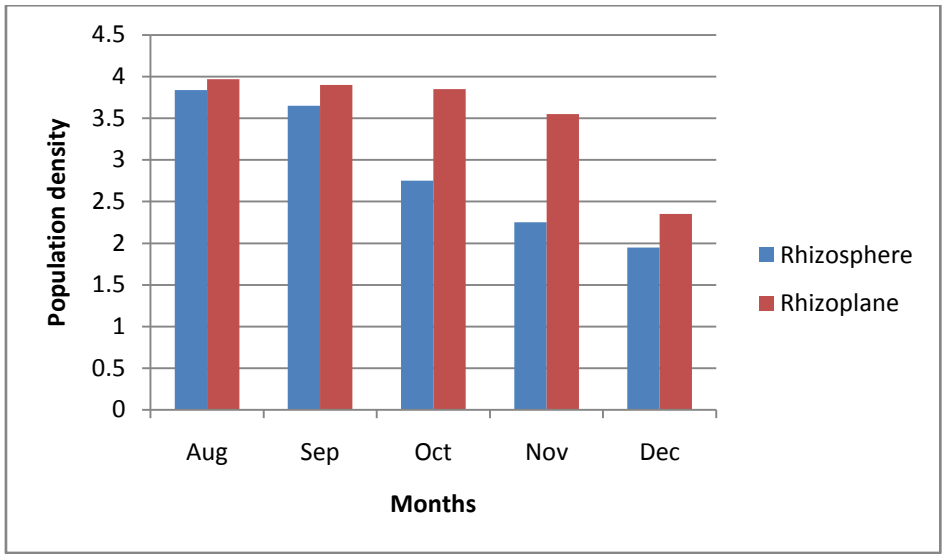


Figure-1: Population density of bacteria in the rhizosphere and rhizoplane of *Abelmuschus esculentus* in terms of Colony Forming Unit (N X 10^8/g of soil)

Table- 3: Population density of bacteria in the Rhizosphere and Rhizoplane zone of *Brassica oleracea* var Botrytis in terms of Colony Forming Unit (CFU/g of soil)

Zone	August	September	October	November	December
Rhizosphere	2.95 X 10^8 ±0.31	2.87X 10^8 ±0.024	2.75 X 10^8 ±0.014	2.35 X 10^8 ±0.12	1.95 X 10^8 ±0.15
Rhizoplane	3.65 X10^8 ±0.33	2.90 X10^8 ±0.54	2.85 X10^8 ±0.37	2.55 X10^8 ±0.021	2.15 X10^8 ±0.12

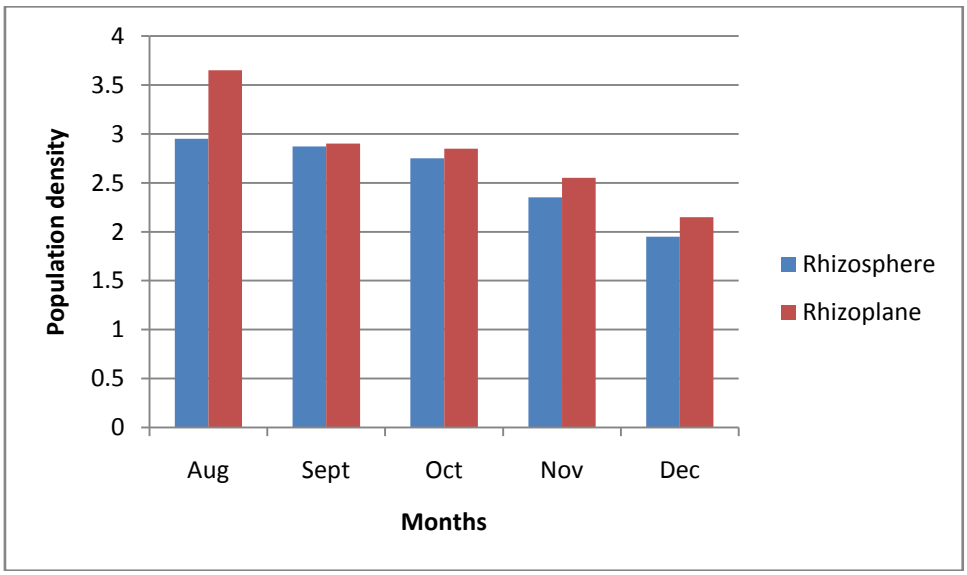


Figure-2: Population density of bacteria in the rhizosphere and rhizoplane of *Brassica oleracea* var Botrytis in terms of Colony Forming Unit (N X 10^8/g of soil)

Table- 4: Bacterial flora isolated from Rhizosphere and Rhizoplane zone of *Abelmuschus esculentus and Brassica oleracea* var Botrytis

S. No.	Bacterial flora	*Abelmuschus esculentus*		*Brassica oleracea* var Botrytis	
		Rhizosphere	Rhizoplane	Rhizosphere	Rhizoplane
1	*Bacillus subtilis*	+	+	+	+
2	*Bacillus megaterium*	+	+	+	+
3	*Bacillus pumilus*	+	-	+	-
4	*Bacillus polymixa*	+	+	+	+
5	*Bacillus mycoides*	+	+	+	+
6	*Lactobacillus acidophilus*	+	-	+	-
7	*Lactobacillus plantarum*	+	-	+	-
8	*Lactobacillus brevis*	+	+	+	+
9	*Micrococcus luteus*	+	-	+	-
10	*Azotobacter beijerinckii*	+	-	+	-
11	*Azotobacter chroococcum*	+	+	+	+
12	*Pseudomonas putida*	+	-	+	-
13	*Pseudomonas fluorescens*	+	+	+	+
14	*Pseudomonas aeruginosa*	-	-	+	+
15	*Streptococcus salivaris*	-	+	-	+
16	*Staphy*lococcus saprophyticus	-	+	-	+
17	*Klebsiella* sp.	+	+	+	-
18	*Corynebacterium* sp.	-	-	+	+
19	*Arthrobacter* sp.	+	+	+	+
20	*Citrobacter* sp.	+	+	+	+
21	*Rhodococcus erythropolis*	+	-	+	+
22	*Erwinia amylovora*	-	-	+	+

Table- 5: Colony characteristics of Rhizosphere and Rhizoplane bacteria

Bacterial flora	Colony characteristics								
	Form	Elevation	Margin	Pigment	Texture	Transparency	Gram staining	Spore staining	Motility
Bacillus polymyxa	Circular	Raised	Entire	Dew drop	Mucoid	Opaque	Positive, Rod	Yes	Yes
Bacillus subtilis	Circular	Raised	Entire	Dew drop	Mucoid	Opaque	Positive, Rod	Yes	Yes
Bacillus megaterium	Circular	Raised	Entire	Dew drop	Mucoid	Opaque	Positive, Rod	Yes	Yes
Bacillus pumilus	Circular	Raised	Entire	Dew drop	Mucoid	Opaque	Positive, Rod	Yes	Yes
Bacillus mycoides	Circular	Raised	Entire	Dew drop	Mucoid	Opaque	Positive, Rod	Yes	Yes
Lactobacillus acidophillus	Circular	Raised	Entire	Buff	Mucoid	Transparent	Positive, Rod	No	Yes
Lactobacillus plantarum	Circular	Raised	Entire	Buff	Mucoid	Transparent	Positive, Rod	No	Yes
Lactobacillus brevis	Circular	Raised	Entire	Buff	Mucoid	Transparent	Positive, Rod	No	Yes
Micrococcus luteus	Circular	Raised	Entire	Colourless	White	Transparent	Positive, Cocci	No	Non motile
Azotobacter chroococcum	Circular	Flat	Entire	Colourless	Waxy	Transparent	Positive, Rod	No	Yes
Azotobacter beijerinckii	Circular	Flat	Entire	Colourless	Waxy	Transparent	Positive, Rod	No	Yes
Pseudomonus fluorescence	Circular	Convex	Entire	Creamy	Mucoid	Transparent	Positive, Rod	No	Yes
Pseudomonas putida	Circular	Convex	Entire	Creamy	Mucoid	Transparent	Positive, Rod	No	Yes
Pseudomonas aeruginosa	Circular	Convex	Entire	Creamy	Mucoid	Transparent	Positive, Rod	No	Yes
Streptococcus salivaris	Circular	Convex	Undulate	White	Waxy	Opaque	Positive, Cocci	No	Non motile
Staphylococcus saprophyticus	Circular	Raised	Entire	Colourless	Mucoid	Transparent	Positive, Cocci	No	Non motile
Klebsiella sp.	Circular	Flat	Entire	Creamy	Mucoid	Opaque	Negative, Rod	No	Non motile
Corynebacterium sp.	Irregular	Flat	Undulate	Creamy	Mucoid	Opaque	Positive, Rod	No	Non motile
Arthrobacter sp.	Circular	Raised	Entire	Yellow	Waxy	Opaque	Positive, Rod	No	Non motile
Citrobacter sp.	Circular	Raised	Entire	Colourless	Mucoid	Transparent	Negative,	No	Motile

							Bacilli		
Rhodococcus erythropolis	Circular	Raised	Entire	Creamy	Waxy	Opaquue	Positive, Cocci	No	Non motile
Erwinia amylovora	Circular	Flat	Entire	Colourless	Mucoid	Opaque	Positive, Rod	No	Motile

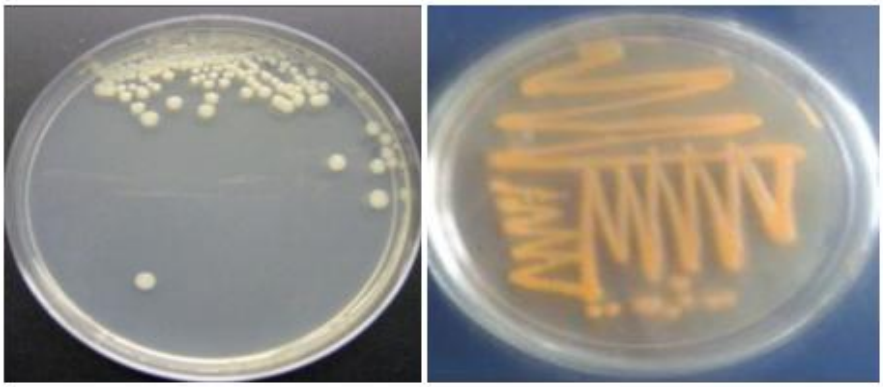
Table- 6: Chemical characteristics of Rhizosphere and Rhizoplane bacteria

Bacterial flora	Chemical characteristics								
	Catalase	Mannitol	Glucose	Anaerobic (Hugh-Leifson's O- F)	Hemolysis	Starch hydrolysis	Sorbitol fermentation	Voges Preskaur's test	Methyl red test
Bacillus subtilis	-	NR	NR	NR	NR	+	NR	NR	NR
Bacillus megaterium	-	NR	+	NR	NR	+	NR	NR	NR
Bacillus pumilus	-	NR	+	NR	NR	+	NR	NR	NR
Bacillus polymyxa	-	NR	NR	NR	NR	+	NR	NR	NR
Bacillus mycoides	+	NR	NR	-	NR	-	NR	NR	NR
Lactobacillus acidophilus	-	-	-	NR	-	-	NR	NR	NR
Lactobacillus plantarum	-	-	-	NR	NR	-	NR	NR	NR
Lactobacillus brevis	-	-	+	NR	NR	-	NR	NR	NR
Azotobacter chroococcum	+	NR	NR	NR	-	NR	NR	NR	NR
Micrococcus luteus	+	+	-	-	+	-	NR	-	NR
Azotobacter beijerinckii	+	-	-	NR	NR	-	NR	NR	NR
Pseudomonus fluorescence	-	NR	NR	-	-	NR	NR	NR	NR
Pseudomonas putida	-	NR	NR	-	-	NR	NR	NR	NR
Pseudomonas aeruginosa	-	NR	NR	-	-	NR	NR	NR	NR
Streptococcus salivaris	+	-	NR	NR	NR	NR	NR	NR	NR
Staphylococcus saprophyticus	+	NR	NR	NR	+	NR	NR	NR	NR
Klebsiella sp.	+	NR	NR	NR	+	NR	NR	-	NR
Corynebacterium sp.	+	NR	NR	-	-	-	NR	NR	NR
Arthrobacter sp.	+	NR	NR	NR	NR	NR	NR	NR	NR
Citrobacter sp.	+	NR	NR	-	-	NR	NR	NR	NR
Rhodococcus erythropolis	+	NR	NR	NR	+	NR	NR	NR	NR
Erwinia amylovora	+	NR	NR	NR	-	NR	NR	NR	NR

Photoplates (Petri plates)

1 2

3 4

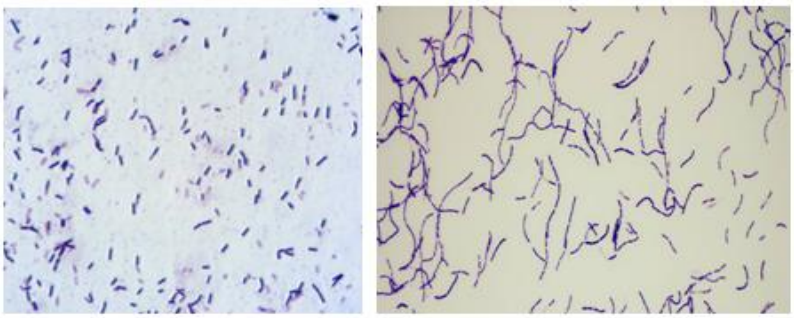


5 6

7 8

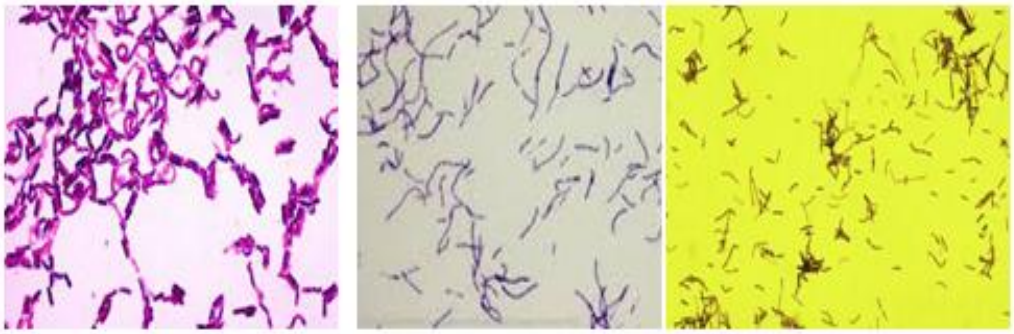
Petri plate 1- 8: Rhizobacteria growing on Nutrient Agar medium

Microphotographs

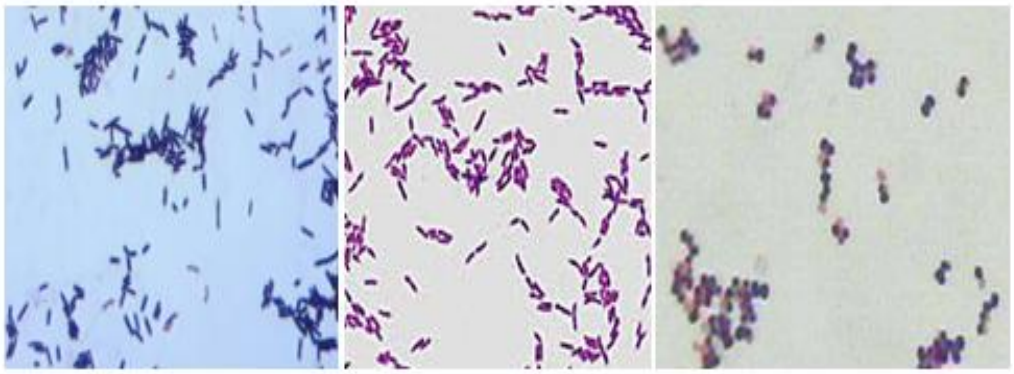


1. *Bascillus subtilis* 2. *Bacillus megaterium*

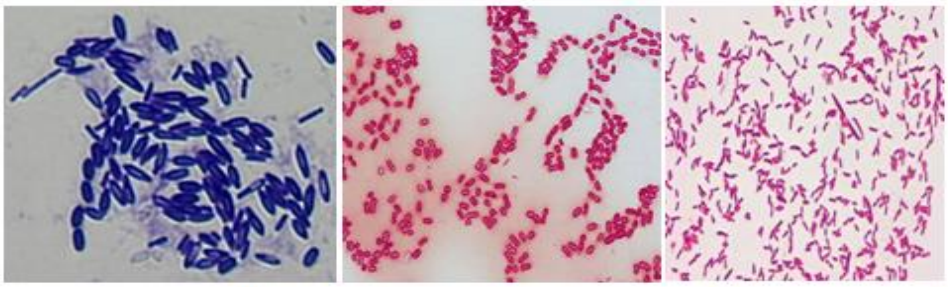
3. *Bacillus pumilus* 4a. *Bacillus polymyxa* 4b. B. polymyxa



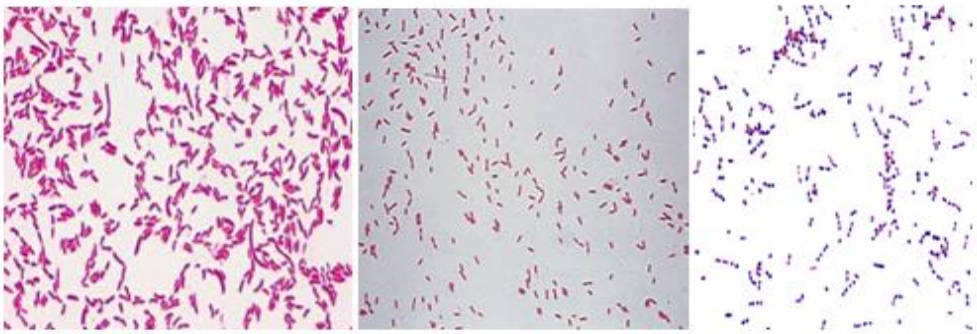
5a. *Bacillus mycoides* 5b. *B. mucoides* 6. *Lactobacillus acidophilus*



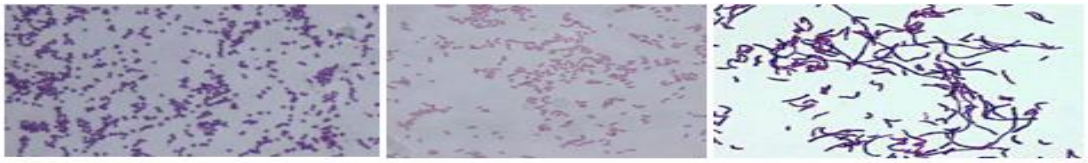
7. *Lactobacillus plantarum* 8. *Lactobacillus brevis* 9. *Micrococcus luteus*



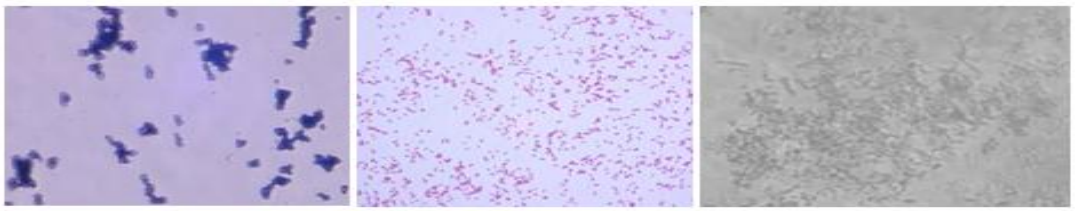
10. *Azotobacter beijerinckii* 11. *Azotobacter chroococcum* 12. *Pseudomonas putida*



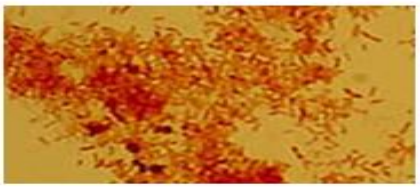
13. *Pseudomonas fluorescence* 14. *Pseudomonas aeruginosa* 15. *Streptoccocus salivaris*



16. *Staphylococcus saprophyticus* 17. *Klebsiella* sp. 18. *Corynebacterium* sp.



19. *Arthrobacter* sp. 20. *Citrobacter* sp. 21. *Rhodococcus erythropolis*



22. *Erwinia amylovora*

III. Results

The physical characteristics for temperature, pH and moisture of garden soil of Patna were studied at monthly intervals from August to December (Table- 1). The temperature varied from 13^0C to 28^0C. At the start of experiment i.e. in the month of August the temperature of garden soil was maximum (28^0C) which gradually declined to 13^0C in January. The pH of the garden soil did not vary significantly and ranged between 6.65 in January and 8.1 in August. Similarly the moisture content of garden soil was maximum in August (78%) which declined to 61% in December (Table- 1).

The population density of Rhizosphere (RS) and Rhizoplane (RP) bacteria of Okra (*Abelmuschus esculentus*) was maximum during August being $3.84X10^8$CFU/g and $3.97X10^8$ CFU/g respectively which declined to $1.95X10^8$CFU/g and $3.15X10^8$CFU/g respectively in December (Table- 2; Figure-1). Similarly, the population density of Rhizosphere (RS) and Rhizoplane (RP) bacteria of Cauliflower (*Brassica oleracea* var. Botrytis) was maximum during August being $2.95X10^8$CFU/g and $3.65X10^8$ CFU/g respectively which declined to $1.95X10^8$CFU/g and $2.15X10^8$CFU/g respectively in December (Table- 3; Figure-2).

In general climate of the Patna is characterized by three distinct seasons, i.e. cool- day winter, hot-day summer and warm wet rainy season. Cool- day season extends from October to February with fairly low temperature varying between 7^0C and 16 ^{0} C, very little rain, clear sky and relatively low humidity. Hot dry Season spreads over March to Mid June with temperatures rising up to $44/45^0$C with low humidity. Warm-wet season is the period of monsoon from mid-June to September. During this period temperatures range from 24^0C to 35^0C with cloudy sky and high humidity. The average annual rainfall varies from 1100 to 1250 mm. The daily temperature comes down to $7\text{-}8^0$C in December – January in north Bihar plains. Since rainfall distribution is dictated by climate and vegetation, the rain fed areas are constrained in their choice of crops, technology and resultant levels of productivity. On an average, the plain region of Bihar records a mean annual total rainfall of 1297 mm which is distributed in the monsoon, autumn, winter and summer seasons as 1039, 32, 110 and 58 mm, respectively.

The bacterial flora isolated and identified in the rhizosphere and rhizoplane zones of *Abelmuschus esculentus* and *Brassica oleracea* var. Botrytis has been presented in Table-4. Twenty two bacterial flora viz. *Bacillus subtilis, Bacillus megaterium, Bacillus polymyxa, B. mycoides, Bacillus pumilus, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus brevis, Azotobacter beijerinckii, Azotobacter chroococcum, Micrococcus luteus, Pseudomonas fluorescence, Pseudomonas putida, Pseudomonas aeruginosa, Streptococcus salivaris, Staphylococcus saprophyticus, Klebsiella sp. Corynebacterium sp. Arthrobacter sp. Citrobacter sp.Rhodococcus erythropolis and Erwinia amylovora* were recorded in the rhizosphere and rhizoplane in August (Petri plates 1- 8; Microphotograph 1-22). Out of these microbes *Bacillus subtilis, B. megaterium, B. polymyxa, B. mycoides, Lactobacillus brevis, Azotobacter chroococcum, Pseudomonas flurorescence, Arthrobacter sp. and Citrobacter sp.* were isolated from rhizosphere and rhizoplane of both

Abelmuschus esculentus and *Brassica oleracea* var. *Botrytis. Bacillus pumilus, Lactobacillus brevis, L. acidiphilus, L. plantarum, Azotobacter chroococcum, A. beijerinckii Pseudomonas fluorescence, P. putida Arthrobacter sp. Citrobacter sp. Micrococcus luteus* were not detected in the rhizoplane of *Abelmuschus esculentus* and *Brassica oleracea* var. Botrytis. Similarly, *Pseudomonas aeruginosa* and *Erwinia amylovora* were not isolated from both rhizosphere and rhizoplane of *Abelmuschus esculentus* and *Brassica oleracea* var. Botrytis. *Staphylococcus saprophyticus* and *Streptococcus salivaris* were not detected in rhizosphere but detected in rhizoplane of both the vegetable crops (Table-4).

The colony characteristics of all the twenty two bacterial isolates have been presented in Table- 5. From the result it is evident that *B. polymyxa, B. subtilis, B. megaterium, B. pumilus B. mycoides, Bacillus polymyxa* and *B. mycoides* exhibited similar colony morphology in terms of form, elevation, margin, pigment, texture and transparency. They were Gram positive and motile, and exhibited endospore production. *Lactobacillus acidophilus, L. plantarus* and *L. brevis* exhibited more or less similar colony characteristics. Their colonies were circular, raised with entire margin, mucoid, transparent, non-spore producing and motile and showed buff pigmentation. *Micrococcus luteus, Azotobacter beijerinckii, A. chroococcus, species of Pseudomonas, Streptococcus, Spaphylococcus, Corynebacterium, Arthrobacter, Rhodococcus* and *Erwinia,* with slight variations in their colony morphology were Gram positive non-spore producing rhizobacteria. Species of *Azotobacter, Pseudomonas, Citrobacter* and *Erwinia* were motile, but *Micrococcus, Streptococcus, Staphylococcus, Klebsiella, Corynebacterium, Arthrobacter* and *Rhodococcus* were non-motile. All isolates except *Corynebacterium sp.*, exhibited circular colonies on nutrient agar medium (Culture plate 1- 8). The isolated *Corynebacterium* sp. showed irregular colonies.

The biochemical tests of these bacterial isolates have been performed and presented in Table- 6. From the result it is evident that the isolates viz. *Bacillus subtilis, B. megaterium, B. pumilus, B. polymyxa, Lactobacillus acidophilus, L. plantarum, Pseudomonas putida and P. aeruginosa* showed catalase negative reaction whereas *Bacillus mycoides, Azotobacter chroococcum, A. beijerinckii, Micrococcus luteus, Streptococcus salivaris, Staphylococcus saprophyticus, Klebsiella* sp. *Corynebacterium* sp. *Arthrobacter* sp. *Citrobacter* sp. *Rhodococcus erythropolis* and *Erwinia amylovora* showed catalase positive reaction.

Only *Micrococcus luteus* showed positive reaction with mannitol. *Lactobacillus acidophilus, L. plantarum, L. brevis, Azotobacter beijerinckii* and *Streptococcus salivaris* showed negative reaction with mannitol, but rest of the bacteria are non-reactive with this reagent. Out of twenty two rhizobacteria only *Bacillus megaterium, B. pumilus* and *Lactobacillus brevis* showed positive reaction with glucose whereas *L. acidophilus, L. plantarum, Micrococcus luteus* and *Azotobacter beijerinckii* showed negative reaction. The rest of the rhizobacteria are non-reactive with glucose. *Bacillus mycoides, Micrococcus luteus Pseudomonas fluorescence, P. putida Corynebacterium sp.* and *Citrobacter sp.* showed anaerobic (Hugh- Leifson's O- F) negative result. Other species were non-reactive with Hugh- Leifson's O- F reaction. Micrococcus luteus, Klebsiella sp. and Rhodococcus erythropolis were hemolytic whereas Lactobacillus acidophillus, *Azotobacter chroococcum, Pseudomonas fluorescence, P. putida, P. aeruginosa, Corynebacteriumsp.* and *Citrobacter sp.* were non hemolytic; others were non-reactive. *Bacillus subtilis, B. megaterium, B. pumilus* and *B. polymyxa* showed positive starch hydrolysis reaction whereas *Bacillus mycoides, Lactobacillus acidophilus, L. plantarum, L. brevis, Micrococcus luteus, Azotobacter beijerinckii* and *Corynebacterium* sp. exhibited negative test. All the rhizobacteria were found to be non-reactive to sorbitol fermentation reaction. Only *Micrococcus luteus and Klebsiella sp.* showed Voges Preskaur's negative reaction and others were non-reactive. All rhizobacteria showed non-reactive to methyl red test (Table-6). All twenty two isolates recovered from rhizosphere and rhizoplane of Abelmuschus esculentus and Brassica oleracea var. Botrytis exhibited siderophore production. All the rhizospheric bacteria showed maximum Phosphate-solubilizing ability and, therefore, can be exploited as bioinoculants/ biofertilizers for improvement of crops.

IV. Discussion

The agro climatic condition of Patna is humid and subtropical being most favourable for the growth of bacteria in the rhizosphere zone. The present findings are in agreement with the work of Seema Rawat *et al*., (2011) [76], Nidhi Sarkhia *et al*., (2016) [77] and Richa Pandey *et al*., (2018) [63] who recorded a more or less similar bacterial diversity and physical characteristics in the Rhizosphere of wheat. Oyeyiola *et al*., (2013) [78] have also recorded similar microbial flora in the rhizosphere and rhizoplane of Okura (Hibischus esculentus). Fazal Mahmood *et al*., (2018) [79] have identified more or less similar rhizobacteria from rhizosphere and rhizoplane of Brassica oleracea var. Botrytis. Kushwaha *et al.,* (2013) [80] have also recorded more or less similar rhizobacteria from the Cauliflower. Alia *et al*., (2013) [81] have isolated phosphate solubilizing bacteria from rhizosphere of several crops. Edi Husen (2003) [36] studied the plant growth promoting activities of rhizobacteria. He investigated the production of IAA (Indole Acetic Acid), phosphate solubilization, dinitrogen fixation, Siderophore production (Fe III-chelating agent) by fourteen rhizobacterial isolates. The present findings gain support from the work of Nidhi Sakhia et al., (2016) [77] who studied the bacterial diversity of

mangroves rhizosphere and found a more or less similar colony characteristics of these bacterial isolates. Richa Pandey et al., (2018) [63] also studied more or less similar colony characteristics of the bacterial diversity of rhizosphere and rhizoplane of *Solanum melongena*.

The rhizosphere is considered as a hot spot of bacterial diversity. This zone harbours bacterial flora whose diversity is mainly expressed in terms of functions adapted to the root presence, and in particular to favour plant growth. This is in turn beneficial to the whole rhizosphere microbiota through the highly nutritive and energetically rhizodepositions. This microbiota consists, besides bacteria, of mycorrhizal fungi and bacteria grazers working in stable synergy (Aragno, 2005) [82]. A continued exploration of the natural biodiversity of soil microorganisms and the optimization and manipulation of microbial interactions in the rhizosphere of crops represents a prerequisite step to develop more efficient bioinoculants.

Siderophore was found to be most prominent characteristic in rhizosphere and rhizoplane which is involved directly or indirectly in influencing plant growth by chelation of iron which is present in very low amount in soil. Siderophore is known to trap insoluble iron (III) and form stable complexes.

Phosphate solubilization was documented to be a major characteristic possessed by isolates. Phosphorus is an essential element for plant growth and development and makes up about 0.2% of dry weight.

Plants absorb Phosphorus from soil solution as phosphate anions. However, phosphate anions are extremely reactive and may be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, Fe^{3+} and $Al3^{+}$ depending on the particular properties of a soil. In these forms, Phosphorus is highly insoluble and unavailable to plants (Tilak et al., 2005) [83]. As a result, the amount available to plants is usually a small proportion of this total. A considerably higher concentration of phosphate solubilizing bacteria is commonly found in the rhizospheric soil. The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatases play a major role in the mineralization of organic phosphorus in soil. The production of organic acids by phosphate solubilizing bacteria had been well documented (Gaur *et al*., 2004; Aragno *et al*., 2005; Berg, 2009) [84, 82, 85]. Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most phosphate solubilizers. *Bacillus polymyxa* and *B. mycoides* were found to be the dominant microbes and thus contribute highest phosphate solubilizing activity. The abundance of particular bacteria in samples was thus indicative of the predominance of the role played by the microflora in their niche. However, no clear relationship could be established between the structural and functional diversity as rhizosphere and rhizoplane bacterial fractions were found to be structurally more diverse.

V. Conclusion

Rhizosphere is regarded as a hot spot of microbial community. It is a unique ecological zone of soil that contains highest concentration of nutrients obtained from plant roots via rhizodeposition. It has a rich pool of potential microbial which favorably influence the growth of plant. Bacteria are the most abundant organisms that reside in rhizosphere of almost all plants and produce plant growth promoting substances (IAA, Siderophores, P-solubilising substance etc.). They are, therefore, called plant growth promoting rhizobacteria (PGPR), and can be exploited as biofertilizers to develop eco-friendly and safe replacement for chemical based fertilizers and pesticides.The PGPR have environmental advantages and are favorably supported by legislative guidelines as well. However, their commercial success is highly dependent on economic and physic-chemical factors of soil. The rhizobacteria isolated in the present investigation have been the most vastly studied PGPR so far, due to their functional properties and predominance in the rhizosphere. These microbes might be exploited to enhance the plant growth and yield so that the use of chemical based agronomic products can be checked to a significant level.

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Conflict of Interest: Authors declare no conflict of interest directly or indirectly.

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