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Biodiversity of Bacteria in the Rhizosphere of *Solanum melongena* L. (Brinjal) and their Characterization

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A B S T R A C T

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). In the present investigation the biodiversity of bacteria in rhizosphere and rhizoplane of *Solanum melongena* and their physiochemical characteristics was studied. The results revealed that the population density of Rhizosphere (RS) and Rhizoplane (RP) bacteria was maximum during September being $2.85X10^8$CFU/g and $2.95X10^8$ CFU/g respectively which declined to $1.95X10^8$CFU/g and $2.15X10^8$CFU/g respectively in January. Sixteen bacterial flora viz. *Bacillus polymyxa, B. mycoides, Azotobacter chroococcum, Pseudomonas fluorescence, Trichoderma harzianum, Staphylococcus sp, Streptococcus sp, Klebsiella sp, Micrococcus sp, Corynebacterium sp, Arthrobacter sp, Lactobacillus sp, Clostridium sp, Enterococcus sp, Escherichia coli* and *Citrobacter sp* were recorded in the rhizosphere and rhizoplane of *Solanum melongena*. Among these only three isolates viz. *Bacillus polymyxa, Pseudomonas fluorescence,* and *Lactobacillus* showed catalase negative reaction. *Bacillus mycoides, Pseudomonas fluorescence, Corynebacterium,* and *Citrobacter* showed anaerobic (Hugh- Leifson's O- F) negative result. *Azotobacter chroococcum, Pseudomonas fluorescence, Corynebacterium* and *Lactobacillus* were non hemolytic whereas *Trichoderma, Staphylococcus, Klebsiella* and *Micrococcus* were recorded as hemolytic microbes. *Trichoderma, Klebsiella* and *Micrococcus* showed Voges Proskaur negative reaction. All isolates recovered from rhizoplane exhibited siderophore production. All the rhizospheric bacteria showed maximum Phosphate-solubilizing ability and, therefore, can be exploited as bioinoculants/ biofertilizers for improvement of crops.

Introduction

Rhizosphere is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. 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.

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).

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).

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). 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). 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). 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). 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 (Smith 1976) and abiotic conditions such as water content and temperature (Martin and Kemp 1980). In turn, rhizosphere microorganisms increase root exudation through production of plant hormones or more directly by physically damaging the roots (Grayston *et al.*, 1996). 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).

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). The term rhizobacteria is used for bacteria that aggressively colonize the rhizosphere (SubbaRao, 1999).

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). 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). 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).

The plant growth promoting activities of soil bacteria have been studied by Edi Husen (2003). The Rhizosphere microorganisms have been largely reviewed by David H. Mc Near Jr. (2013); Bais *et al.,* (2004); Benfey *et al.,* (2010); Genere and Bonfante (2007); Giles *et al.,* (2008); Hartmann *et al.,* (2008); Kosuta *et al.,* (2003); Zhang *et al.,* (2010) etc. Plant growth promoting Rhizobacteria have been studied by Saran and Nehra (2011); Joseph *et al.,* (2007); Karakurt *et al.,* (2009); Hameeda *et al.,* (2006); Narula *et al.,* (2006); Mandal *et al.,* (2007); Sridevi and Mallaiah,(2007); Egamberdieva (2008); Kidoglu *et al.,* (2007) etc.

Rhizobacterial association in the improvement of crops has been studied by several authors viz; Assmus, *et al.,* (1997); Dazzo *et al.,* (1996); Downie (1994); Hirsh (1992); Kannenberg and Brewin (1994); Perotto and Boftante (1997); Sabry *et al.,* (1997); Schwintzar and Tjepkema (1990); Strobel and Long (1998); Van Rhijn and Vanderleyden (1995) etc.

Biodiversity of bacteria in the Rhizosphere has largely been studied by several workers viz. Seema Rawat *et al.,* (2011); Luan *et al.,* (2015); Jason A. Peiffer *et al.,* (2013); Mari *et al.,* (2001); NidhiSakha *et al.,* (2016); Javier Pascual *et al.,* (2016); Pratibha Prashar *et al.,* (2013); Hao Wang *et al.,* (2014) etc. The biodiversity of Bacteria in the Rhizosphere of *Solanum melongena* L. and their biochemical characterization has not been studied so far and hence the present investigation was undertaken.

Materials and Methods

The study was conducted at local brinjal growing garden soil of Patna for five months, from September 2017 to January 2018 at the intervals of 30 days. Soil and root samples of brinjal 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 bacteria

In order to isolate rhizosphere bacteria about 1g of rhizosphere soil of *Solanum melongena L.* was transferred 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). The bacterial isolates obtained through this process of isolation were subjected to various biochemical tests according to "Bergey's Manual of Determinative Bacteriology"7th ed.

Isolation of rhizoplane bacteria

In order to isolate rhizoplane bacteria roots of *Solanum melongena* were placed in sterile beaker 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.

Morphologica 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.

Biochemical characterization

The pure culture of each isolate was subjected to identification by Bergey's Manual of Determinative Bacteriology(1962) using catalase test with 3% hydrogen peroxide, Anaerobic Test, Oxidative Fermentative (O/F test),hemolytic test, methyl red test, Voges Proskaur test, glucose test, sorbitol test and mannitol test, Oxidase test, and Catalase test.

Functional characterization

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 Pikovaskya's agar after 96-120h of incubation were considered as positive solubilization bacteria. Similarly, siderophore production was assayed according to Schwyne and Neilands (1987). Isolates exhibiting an orange halo zone on Chromeazurol S agar after 48-72h of incubation were considered as positive.

The results obtained have been presented in Tables 1–5; Figure 1; Petri plate 1–5 and Microphotographs 1–16.

Results and Discussion

The physical characteristics for temperature, pH and moisture of garden soil of Patna were studied at monthly intervals from September to January (Table- 1). The temperature varied from 12^0C to 28^0C. At the start of experiment i.e. in the month of September the temperature of garden soil was maximum (28^0C) which gradually declined to 12^0C in January. The pH of the garden soil did not vary significantly and ranged between 6.70 in January and 8.3 in September. Similarly the moisture content of garden soil was maximum in September (75%) which declined to 62.15% in January (Table- 1).

The population density of Rhizosphere (RS) and Rhizoplane (RP) bacteria was maximum during September being $2.85X10^8$CFU/g and $2.95X10^8$ CFU/g respectively which declined to $1.95X10^8$CFU/g and $2.15X10^8$CFU/g respectively in January (Table- 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 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) who

recorded a more or less similar bacterial diversity and physical characteristics in the Rhizosphere of wheat.

The bacterial flora isolated and identified in the rhizosphere and rhizoplane zones of *Solanum melongena* has been presented in Table- 3; Fig- 1. Sixteen bacterial flora viz. *Bacillus polymyxa, B. mycoides, Azotobacter chroococcum, Pseudomonas fluorescence, Trichoderma harzianum, Staphylococcus sp, Streptococcus sp, Klebsiella sp, Micrococcus sp, Corynebacterium sp, Arthrobacter sp, Lactobacillus sp, Clostridium sp, Enterococcus sp, Escherichia coli* and *Citrobacter sp* were recorded in the rhizosphere and rhizoplane of *Solanum melongena* in September (Petri plates 1- 16). Out of these microbes only *Staphylococcus, Enterobacter* and *Escherichia coli* were not recorded in the rhizoplane zone of *Solanum melongena*. The incidence of *Bacillus polymyxa* and *B. mycoides* was high in both rhizosphere and rhizoplane. In rhizosphere zone the incidence of B. polymyxa and B. mycoides were 25.5% and 20.7% respectively. Similarly in rhizoplane zone these two Gram positive bacteria were in 27.6% and 22.5% respectively (Table 3; Fig. 1). The incidence of other bacteria in these two zones was relatively low in the range of 2.0 to 8.0%. Seema Rawat *et al.,* (2011) have also found a high incidence of Bacillus in the rhizosphere zone of wheat. *Bacillus polymyxa* and *B. mycoides* thus documented as dominant microflora of both rhizosphere and rhizoplane of *Solanum melongena* in the month of September.

The colony characteristics of all the sixteen bacterial isolates have been presented in Table- 4. From the result it is evident that *Bacillus polymyxa* and *B. mycoides* exhibited similar colony morphology in terms of form, elevation, margin, pigment, texture and transparency. Both species were Gram positive and motile, and exhibited endospore production. Out of sixteen isolates only three species viz. *B. polymyxa, B. mycoides* and *Clostridium* exhibited endospore production. Other isolated showed negative spore staining reaction. *Azotobacter chroococcum, Pseudomonas fluorescence, Trichoderma harzianum, Lactobacillus* sp and *Clostridium* sp were Gram positive rods and motile. *Streptococcus, Staphylococcus, Micrococcus* and *Enterococcus* were reported as Gram positive, cocci with positive motility test. *Citrobacter* was Gram negative, rod shaped and motile (Table- 4). All isolates except *Trichiderma harzianum, Corynebacterium* and *Clostridium* exhibited circular colonies on nutrient agar medium (Culture plate 1- 5).

These three isolated showed irregular colonies. The present findings gain support from the work of Nidhi Sakhia *et al.,* (2016) who studied the bacterial diversity of mangroves rhizosphere and found a more or less similar colony characteristics of these bacterial isolates.

The biochemical tests of these bacterial isolates have also been performed and presented in Table- 5. From the result it is evident that only three isolates viz. *Bacillus polymyxa, Pseudomonas fluorescence,* and *Lactobacillus* showed catalase negative reaction. *Bacillus mycoides, Pseudomonas fluorescence, Corynebacterium,* and *Citrobacter* showed anaerobic (Hugh-Leifson's O- F) negative result. *Azotobacter chroococcum, Pseudomonas fluorescence, Corynebacterium* and *Lactobacillus* were non hemolytic whereas *Trichoderma, Staphylococcus, Klebsiella* and *Micrococcus* were recorded as hemolytic microbes. *Trichoderma, Klebsiella* and *Micrococcus* showed Voges Proskaur negative reaction. More or less similar observations have been noticed by Nidhi Sarkhia *et al.,* (2016) for these microbes.

Table.1 Physical characteristics of garden soil of Patna

Characteristics	Physical characteristics of soil				
	September	October	November	December	January
Temperature in ^{0}C	28±1	26±0.50	22±0.45	16±1.2	12±0.45
pH	8.3±0.03	8.1±0.02	7.8±0.12	7.2±0.15	6.70±0.10
Moisture (%)	75.00±0.25	71.00±0.35	65.00±0.15	62.00±0.14	62.15±0.11

Table.2 Population of bacteria in the Rhizosphere and Rhizoplane zone of *Solanum melongena* in terms of Colony Forming Unit (CFU/g of soil)

Zone	September	October	November	December	January
Rhizosphere	2.85 X 10^8	2.81X 10^8	2.75 X 10^8	2.15 X 10^8	1.95 X 10^8
Rhizoplane	2.95 X10^8	2.90 X10^8	2.82 X10^8	2.35 X10^8	2.15 X10^8

Table.3 Bacterial flora isolated from Rhizosphere and Rhizoplane zone of *Solanum melongana* in the month of September 2017

Bacterial flora	Rhizosphere	% incidence	Rhizoplane	% incidence
Bacillus polymixa	+	25.5	+	27.6
Bacillus mycoides	+	20.7	+	22.5
Azotobacter chroococcum	+	5.0	+	4.5
Pseudomonas fluorescence	+	8.0	+	7.5
Trichoderma harzianum	+	4.5	+	4.0
Streptococcus sp.	+	7.0	+	6.5
Staphylococcus sp.	+	3.5	-	0.0
Klibsiella sp	+	4.5	+	3.7
Micrococcus sp	+	2.5	+	2.0
Corynebacterium	+	6.5	+	5.0
Arthrobacter sp.	+	3.7	+	3.5
Lactobacillus sp	+	3.9	+	3.7
Clostridium sp	+	2.5	+	2.1
Enterococcus sp	+	2.5	-	0.0
Escherichia coli	+	2.5	-	0.0
Citrobacter sp.	+	5.5	+	6.5

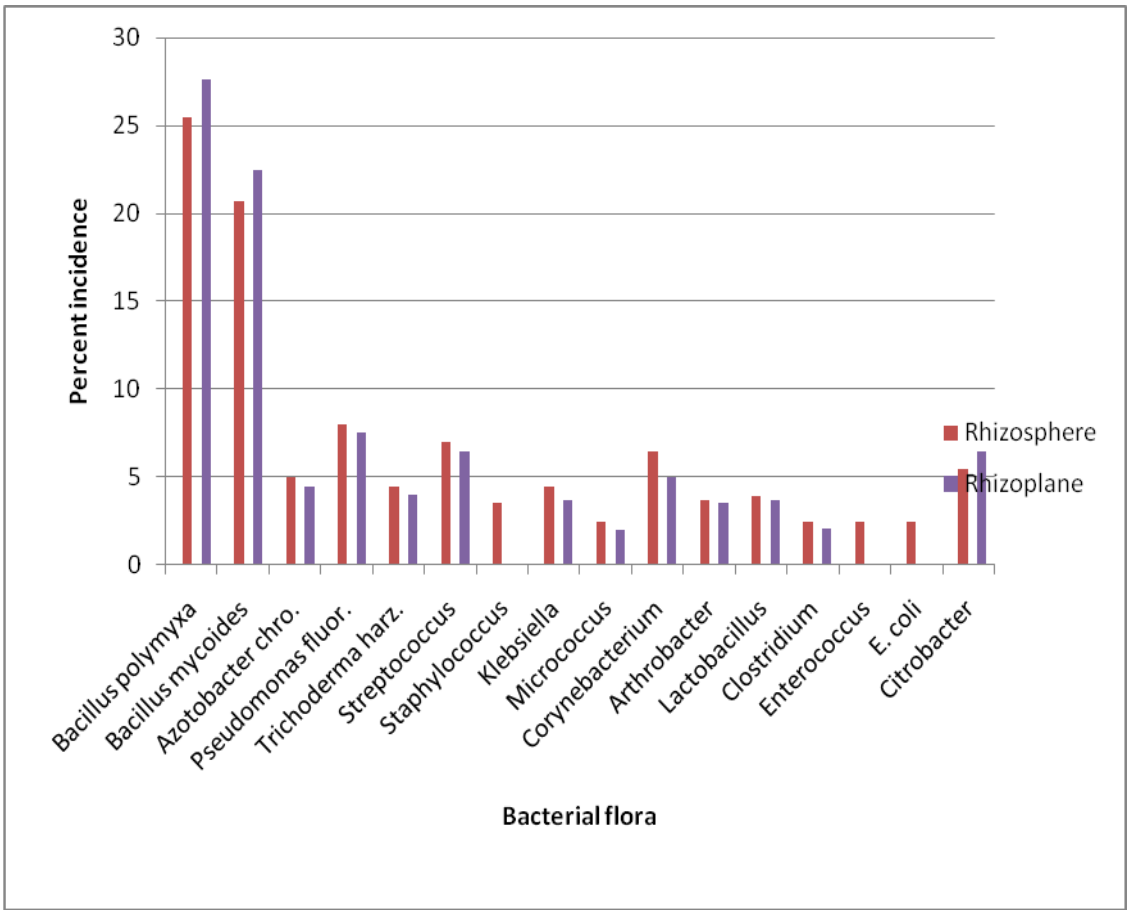
Table.4 Colony characteristics of rhizosphere and rhizoplane bacteria of *Solanum melongena*

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 mycoides	Circular	Raised	Entire	Dew drop	Mucoid	Opaque	Positive, Rod	Yes	Yes
Azotobacter chroococcum	Circular	Flat	Entire	Colourless	Waxy	Transparent	Positive, Rod	No	Yes
Pseudomonus fluorescence	Circular	Convex	Entire	Creamy	Mucoid	Transparent	Positive, Rod	No	Yes
Trichoderma harzianum	Irregular	Convex	Undulate	White	Waxy	Opaque	Positive, Rod	No	Yes
Streptococcus sp.	Circular	Convex	Undulate	White	Waxy	Opaque	Positive, Cocci	No	Non motile
Staphylococcus sp.	Circular	Raised	Entire	Colourless	Mucoid	Transparent	Positive, Cocci	No	Non motile
Klebsiella sp.	Circular	Flat	Entire	Creamy	Mucoid	Opaque	Negative, Rod	No	Non motile
Micrococcus sp.	Circular	Raised	Entire	Colourless	White	Transparent	Positive, Cocci	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
Lactobacillus sp.	Circular	Raised	Entire	Buff	Mucoid	Transparent	Positive, Rod	No	Motile
Clostridium sp.	Irregular	Convex	Entire	Creamy	Mucoid	Translucent	Positive, Rod	Yes	Motile
Enterococcus sp.	Circular	Raised	Entire	Colourless	Mucoid	Translucent	Positive, Cocci	No	Motile
Escherichia coli	Circular	Raised	Entire	Colourless	Mucoid	Opaque	Negative, Rod	No	Motile
Citrobacter sp.	Circular	Raised	Entire	Colourless	Mucoid	Transparent	Negative, Bacilli	No	Motile

Table.5 Chemical characteristics of rhizosphere and rhizoplane bacteria of *Solanum melongena*

Bacterial flora	Chemical characteristics								
	Catalase	Mannitol	Glucose	Anaerobic (Hugh-Leifson's O- F)	Hemolysis	Starch hydrolysis	Sorbitol fermentation	Voges Proskaur's test	Methyl red test
Bacillus polymyxa	-	NR	NR	NR	NR		NR	NR	NR
Bacillus mycoides	+	NR	NR	-	NR	-	NR	NR	NR
Azotobacter chroococcum	+	NR	NR	NR	-	NR	NR	NR	NR
Pseudomonus fluorescence	-	NR	NR	-	-	NR	NR	NR	NR
Trichoderma harzianum	+	-	-	NR	+	NR	NR	-	NR
Streptococcus sp.	+	-	NR	NR	NR	NR	NR	NR	NR
Staphylococcus sp.	+	NR	NR	NR	+	NR	NR	NR	NR
Klebsiella sp.	+	NR	NR	NR	+	NR	NR	-	NR
Micrococcus sp.	+	+	-	-	+	-	NR	-	NR
Corynebacterium sp.	+	NR	NR	-	-	-	NR	NR	NR
Arthrobacter sp.	+	NR	NR	NR	NR	NR	NR	NR	NR
Lactobacillus sp.	-	-	-	NR	-	-	NR	NR	NR
Clostridium sp.	+	NR	NR	+	+	NR	NR	NR	NR
Enterococcus sp.	+	NR	NR	NR	+	NR	NR	NR	NR
Escherichia coli	+	NR	NR	NR	-	NR	NR	NR	NR
Citrobacter sp.	+	NR	NR	-	-	NR	NR	NR	NR

Fig.1 Percent incidence of Bacterial flora of the Rhizosphere and Rhizoplane of *Solanum melongena*



Petri plate 1- 5 Rhizobacteria growing on Nutrient Agar medium

1

2

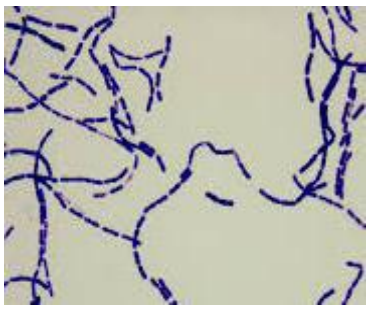
3

4

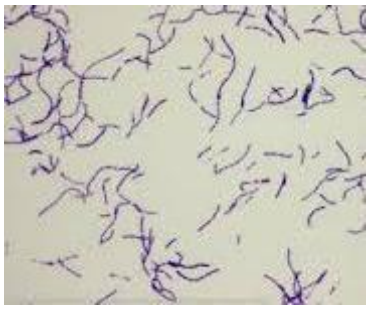
5

Photoplates (Petri plates)

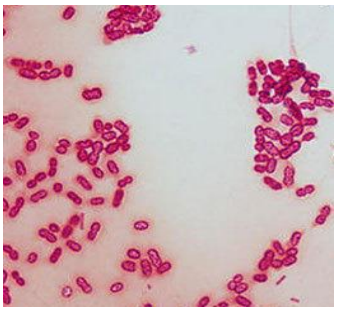
Microphotographs



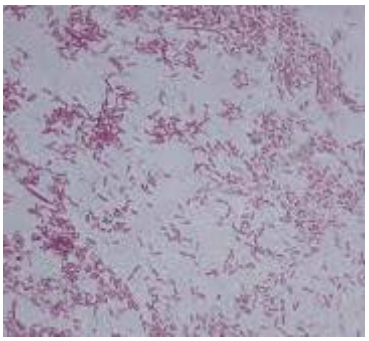
1. Bacillus polymyxa



2. B. mucoides

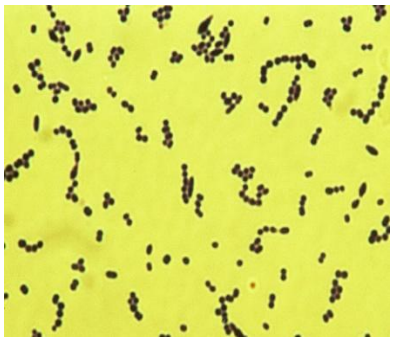


3. Azotobacter chroococum

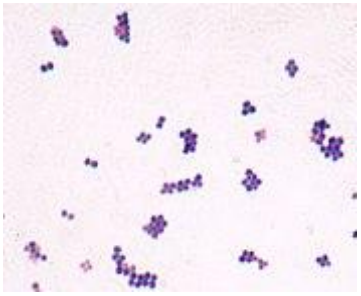


4. *Pseudomonas fluorescence*

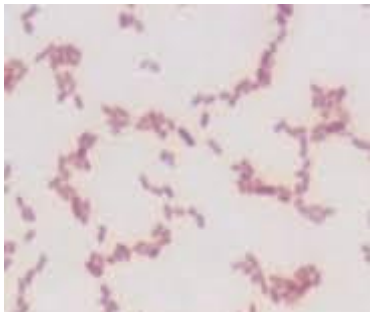
5. *Trichoderma harzianum*



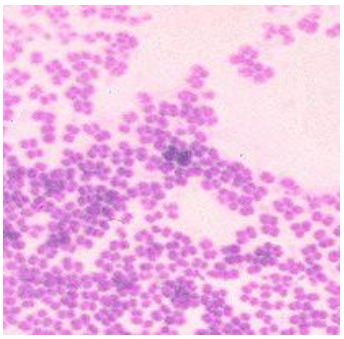
6. Streptococcus sp



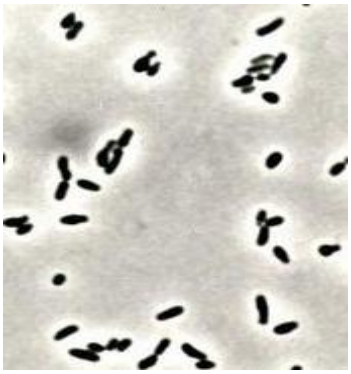
7. *Staphylococcus sp.*



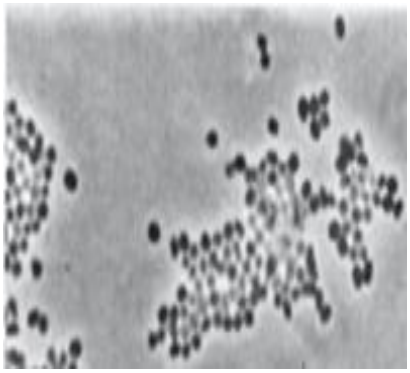
8. Klebsiella sp



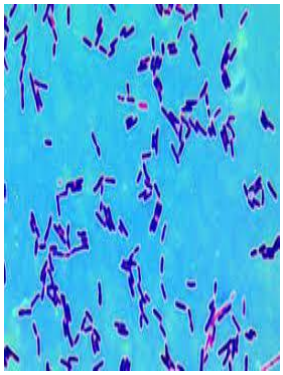
9. Micrococcus



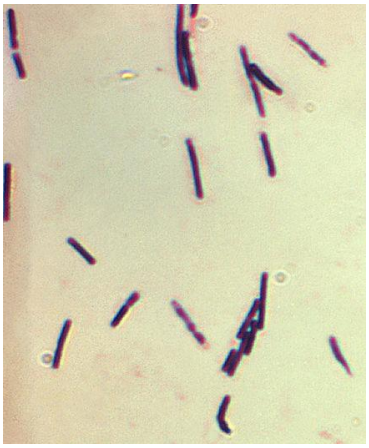
10. *Corynebacterium sp.*



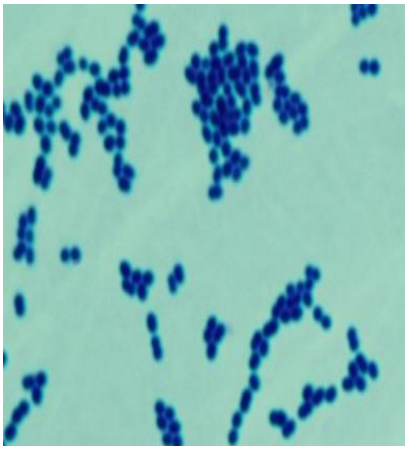
11. Arthrobacter sp.



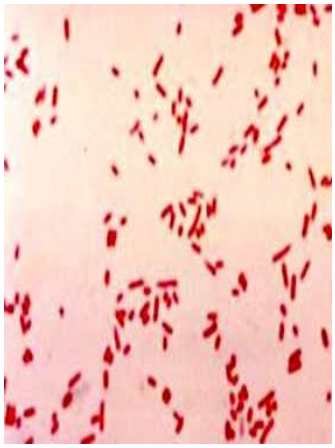
12.Lactobacillus sp.



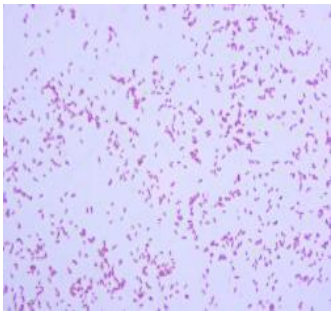
13. Clostridium sp



14. Enterococcus sp.



15. Escherichia coli



16. Citrobacter sp.

All isolates recovered from rhizoplane exhibited siderophore production. All the rhizospheric bacteria showed maximum Phosphate-solubilizing ability and, therefore, can be exploited as bioinoculants/ biofertilizers for improvement of crops.

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). A continued exploration of the natural biodiversity of soil microrganisms 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). 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). 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.

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