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A Comparative Evaluation of the activities of a leguminous and non-leguminous rhizoflora and non rhizosphere microflora

SubTitle: Comparison of microbial activities

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Abstract

Soil is the best environment for the growth of so many microbes. Of these some are seen colonized in the rhizosperic region of plants. This happens in response to the exudates of plant roots. The organisms are generally called rhizoflora. These rhizoflora are beneficial to the plants by secreting various Plant Growth Promoting substances. Hence they are called Plant Growth Promoting Rhizoflora (PGPR). PGPR also induce resistance in host plants by the production of antibiotics, by the production of compounds called siderophores etc. This compound also helps the host plants to absorb iron. Thus it helps plant to increase their growth and control plant pathogens. This study was conducted for comparing the activities of rhizoflora of a leguminous and a non-leguminous plant with a non-rhizosperic soil sample. From the microbes islated from the said sources, a total of 24 isolates (7 from leguminous rhizoflora, 10 from non-leguminous rhizoflora and 7 from non-rhizosperic soil) were taken for screening of the activities mentioned. Two colonies isolated from leguminous rhizosphere and one colony isolated from non-leguminous rhizosphere showed anti-microbial activity. Only a single culture, that too from leguminous rhizosphere showed siderophore production. And regarding phosphate solubilization, 6 isolates from leguminous rhizosphere, 3 from non-leguminous rhizosphere and 3 from non-rhizosphere showed positive activity.

Key Words: rhizoflora, rhizosphere, leguminous, non-leguminous, antimicrobial, siderophore, phosphate solubilization, PSB

I. Introduction

The soil is a rich source of diverse microbes like protozoa, fungi, viruses and bacteria. These microorganisms are involved in a variety of interactions in soil, (Antoun and Kloepper 2001). Much of these are beneficial to the growth and sustenance of plants in soil. Of the soil microbes some live in near the root of plants and are able to multiply and colonize at all stages of plant growth. There organisms are called rhizospheric organisms. The varied genetic and functional activities of the microbial populations have impacts on soil functions, (Sandra and Ayona, 2015; Nannipieri *et al*., 2003). It has been demonstrated that the soil borne microbes interact with plant roots and soil constituents at the soil interface (Barea *et al*., 2002). The plants secrete some metabolites to the soil which attracts the soil bacteria to it. They use these as food and in turn secrete some growth promoting substances needed by plants and also some anti microbial substances and thus preventing the growth of microbial pathogens.

Previous works have shown that the capacity of rhizoflora of biofertilizer applied rhizosphere is much greater than that of chemical fertilizer applied rhizosphere, (Sandra and Ayona, 2015). The diversity of microbes as well as microbial interactions in rhizosphere is found to be different in both leguminous and non-leguminous plants (Hooper and Gordon, 2001). Recently the importance of rhizoflora has been recognized as their capacity for phosphate solubilization, nitrogen fixation, induced resistance and plant growth enhancements, (Hayat *et al*., 2010; Berg, 2009; Choi *et al*.,

2008; Rodriguez *et al.*, 2004; Bloemberg and Lugtenberg, 2001). It is reported that soil contains phosphorus to about 0.05% w/w but of this only 0.1% can be used by plants, (Scheffer and Schachtschabel, 1992). There are some rhizosphere bacteria which causes soil acidification which will release phosphate in soluble form. These are called Phosphate Solubilizing Bacteria (PSB). The capacities of rhizoflora of a leguminous plant as well as that of a non-leguminous plant were compared in terms of soil respiration, siderophore production as well as production of antimicrobials and phosphate solubilization by doing and initial screening for the activities mentioned and the results are discussed in this paper.

II. Materials and Methods

Study Materials

One leguminous plant and one non-leguminous plant were selected for the study. The leguminous plant selected was *Pisum sativum* and non-leguminous plant, *Capsicum annuum*. The seeds were sown on two separate pots filled with soil of clay loam type, with 23.48% sand, 38.57% clay and 38% silt, and a pH of 7.17. Sixty days old seedlings of *Pisum sativum* (leguminous) and *Capsicum annuum* (non-leguminous) were selected for the study.

Collection of soil

Using sterile spatula, soil samples were collected from the pot (away from the rhizosphere), from the rhizhospere of leguminous plant and the rhizosphere of non-leguminous plant and transferred to sterile fresh air tight bags. Rhizosphere samples were collected from uprooted plants. After vigorous shaking the root portion of the plant was cut and chosen for the study. It was then taken to the laboratory for further analysis and isolation.

Enrichment and Enumeration of rhizoflora

The soil samples were subjected to serial dilution and microbes were isolated by pour plate method. Morphologically distinct bacterial colonies were selected as mentioned below for further studies and subjected to grams staining technique.

Identification of Microbial Activity

Antimicrobial Activity Screening

The isolated bacteria were screened for antimicrobial activity by plate assay method. Sterile filter paper discs were soaked with the broth cultures of isolated bacteria and were placed on a lawn culture of *Psuedomonas fluorescence*. After overnight incubation, petri plates were looked for clearance zone around filter paper discs.

Phosphate solubilization Activity

The samples collected from non-rhizospheric soil, rhizosphere of leguminous and that of non-leguminous soil were screened for phosphate solubilization using media supplemented with $Ca_3(PO_4)_2$. The medium was incubated for I week at 28°C and the zone size were determined for the three sets .

Siderophore Production

Iron is important for plant health and metabolism. It is found in proteins such as nitrogenase, ferredoxins, cytochromes, and leghemoglobin. Plant Growth Promoting Rhizospheric bacteria (PGPR) could perform uptake of iron from soil and provide plant with this element. Siderophore production was analyzed for the two samples sing CAS agar media.

III. Results and Discussion

Bacteria from a leguminous and non-leguminous rhizosphere were enriched and enumerated. For a reference, bacteria were also enriched and enumerated from a similar soil without either kind of plants. The results of this are as follows: *Pisum sativum* rhizosphere contained 66 colony forming units (CFU) at 10^{-5} dilution. Hence the sample was calculated to contain $66x10^{-5}$ CFU/g. *Capsicum annuum* rhizosphere contained 44 colony forming units (CFU) at 10^{-4} dilution. Hence the sample was calculated to contain $44x10^{-4}$ CFU/g and non-cultivated soil contained 28 colony forming units (CFU) at 10^{-5} dilution. Hence the sample was calculated to contain $28x10^{-5}$ CFU/g. Liu and Sinclair, (1993) proposed that plant and soil type, both have influence on the microbial diversity and community structure in the rhizosphere.

From this, microorganisms for study were selected as follows:

I. 7 colonies from leguminous rhizosphere.
II. 10 colonies from non –leguminous rhizosphere.
III. 7 colonies from the soil sample.

A total of 24 colonies were selected for further studies

Colony Morphology and Gram's reaction

The morphology of the isolated Colony Forming Units (CFU) was noted and the results for microorganisms from rhizosphere of *Pisum sativa*, *Capsicum annuum* and non-cultivated soil sample are presented as Table 1, Table 2 and Table 3. The selected bacterial colonies from leguminous and non-leguminous rhizosphere and from uncultivated soil were subjected to gram staining and the results are shown in Table 4, Table 5 and Table 6 respectively.

Soil Respiration Method for measuring microbial activity in soil

Antimicrobial Activity

`The selected microbes were screened for anti-microbial activity by plate assay method. Two colonies isolated from leguminous rhizosphere and one colony isolated from non-leguminous rhizosphere showed clearance zone (Table 6 and 7). None of the isolates from raw soil showed zone of incubation (Table 7 and Table 8). The results show that the microorganisms present in the rhizosphere of leguminous plants are more potent when compared to the microorganisms from non-leguminous as well as from raw samples.

Phosphate Solubilization

All the bacterial isolates (from rhizosphere of leguminous plant, rhizosphere of non-leguminous plant and non-rhizosperic soil were analyzed for phosphate solubilization activity and the results are tabulated (Table 9, 10 and 11). The results shows that there is a difference in the capacity of microorganisms isolated from rhizosphere of leguminous plant, rhizosphere of non-leguminous plant and the rhizosphere of non-rhizospheric soil. The number of bacterial isolates showing the activity was maximum in leguminous rhizoflora, whereas, the number was minimum in non-rhizospheric soil. The non-leguminous rhizosphere comes in between these. Also the zone of solubilization was also larger in the positive cultures of rhizoflora from leguminous plants.

The zone is the clearance of phosphate supplied through the media. There are many organisms which are capable of solubilizing phosphate, but this depends on the ecological conditions as well as vegetation, Zhu, *et al.*, (2011).

Siderophore Production

The isolates were screened for siderophores production potential and it was found that out of 24 isolates only one colony, L10 from leguminous rhizosphere showed siderophores production. Earlier studies have shown that it is difficult to grow fastidious microorganisms on CAS agar plates because of some ingredients in the said medium having antimicrobial activity. This may be the reason why most of the cultures did not give positive results while screening for siderophore production. The Plant Growth Promoting rhizoflora produces particular compounds for plants which will help them to grow, facilitates the uptake of nutrients from soil and prevents disease causing microbial attacks. An indirect facilitation to microbial growth is provided by preventing the effects of deleterious microorganisms. This is achieved by the production substances such as siderophores, which are small metal binding proteins.

IV. Conclusion

According to the results obtained as per this study and the observations made, it is concluded that the number and diversity of microorganisms are greater in rhizosphere of leguminous plant followed by non-leguminous plant when compared to non-rhizospheric soil.

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Tables:

Table: 1 (Isolated colonies- leguminous)

Culture	Form	Size	Surface	Texture	Colour	Elevation	Margin
L1	Irregular	Medium	Dull	Moist	Opaque	Raised	Undulate
L2	Rhizoid	Large	Dull	Dry	Transparent	Flat	Rhizoid
L3	Irregular	Medium	Glistening	Viscous	Opaque	Umbonate	Lobate
L4	Irregular	Large	Dull	Dry	Opaque	Flat	Lobate
L5	Circular	Small	Glistening	Moist	Opaque	Convex	Entire
L6	Circular	Small	Glistening	Moist	Opaque	Convex	Entire
L7	Circular	Small	Dull	Dry	Opaque	Flat	Entire
L8	Irregular	Medium	Glistening	Viscous	Opaque	Umbonate	Lobate
L9	Circular	Punctifom	Dull	Dry	Transparent	Flat	Entire
L10	Circular	Small	Glistening	Moist	Translucent	Convex	Entire

Culture	Form	Size	Surface	Texture	Color	Elevation	Margin
NL1	Filamentous	Large	Glistening	Viscous	Opaque	Umbonate	Lobate
NL2	Rhizoid	Medium	Wrinkled	Dry	Opaque	Flat	Rhizoid
NL3	Irregular	Large	Dull	Moist	Translucent	Flat	Undulate
NL4	Irregular	Small	Dull	Dry	Opaque	Flat	Undulate
NL5	Circular	Small	Dull	Dry	Opaque	Flat	Entire
NL6	Irregular	Medium	Glistening	Moist	Opaque	Pulvinate	Curled
NL7	Circular	Small	Dull	Dry	Opaque	Flat	Entire

Table : 2 (Non-Leguminous)

Culture	Form	Size	Surface	Texture	Color	Elevation	Margin
S1	Irregular	Medium	Dry	Moist	Opaque	Raised	Undulate
S2	Irregular	Medium	Dry	Dry	Transparent	Flat	Undulate
S3	Irregular	Medium	Dry	Moist	Opaque	Flat	Undulate
S4	Irregular	Small	Dry	Moist	Transparent	Pulvinate	Curled
S5	Circular	Small	Glistening	Viscous	Opaque	Convex	Entire

S6	Circular	Large	Glistening	Moist	Translucent	Convex	Undulate

Table: 3. Isolates from soil

Culture	Gram (+/-)	Shape
L1	'+'ve	Cocci
L2	'+'ve	Bacilli
L3	'-'ve	Cocci
L4	'+'ve	Cocci
L5	'-'ve	Bacilli
L6	'+'ve	Bacilli
L7	'+'ve	Cocci
L8	'-'ve	Cocci
L9	'-'ve	Cocci
L10	'-'ve	Bacilli

Table: 4. Isolates from rhizosphere of *Pisum sativum*

Culture	Gram (+/-)	Shape
NL1	'-'ve	Cocci
NL2	'-'ve	Cocci
NL3	'+'ve	Bacilli
NL4	'-'ve	Cocci
NL5	'-'ve	Bacilli
NL6	'+'ve	Cocci
NL7	'+'ve	Cocci

Table: 5. Isolates from rhizosphere of *Capsicum annuum*

Culture	Gram (+/-)	Shape
S1	'+'ve	Cocci
S2	'+'ve	Cocci
S3	'+'ve	Cocci
S4	'+'ve	Rod shaped
S5	'-'ve	Cocci
S6	'-'ve	Cocci

Table: 6. Isolates from rhizosphere of raw soil

Culture (Isolates)	*Pseudomonas fluorescens*
L1	NIL
L2	NIL
L3	NIL
L4	NIL
L5	NIL
L6	NIL
L7	NIL
L8	10 mm
L9	NIL
L10	8 mm

Table: 7 Antibiotic productions in leguminous root

Culture (Isolates)	*Pseudomonas fluorescens*
NL1	NIL
NL2	NIL
NL3	NIL
NL4	NIL
NL5	5 mm
NL6	NIL
NL7	NIL

Table: 8. Antibiotic production in leguminous root

Culture (Isolates)	*Pseudomonas fluorescens*
S1	NIL
S2	NIL
S3	NIL
S4	NIL
S5	NIL
S6	NIL
S7	NIL

Table: 9 Antibiotic production in soil sample

Culture (Isolates)	Zone Size
L1	1.2
L2	0.8
L3	1.5
L4	0
L5	0
L6	1.3
L7	0
L8	1.7
L9	0.7
L10	0

Table: 10: Phosphate Solubilization (Leguminous Plant)

Culture (Isolates)	Zone Size
NL1	0.6
NL2	0
NL3	0
NL4	1.0
NL5	1.2
NL6	0
NL7	0

Table 11: Phosphate Solubilization (Non-Leguminous Plant)

Culture (Isolates)	Zone Size
S1	0
S2	0
S3	1.0
S4	0
S5	0.8
S6	0
S7	0.6

Table 12: Phosphate Solubilization (Non-Rhizospheric soil)

