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Characterization of Multiple Plant Growth Promoting Traits of Serratia Spp. Associated With Vegetables Rhizospheric Soil

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Abstract: ***The rhizosphere zone of 1-2 mm around plant roots is rich in nutrients and provides niches different from those in bulk soil for bacteria to thrive. Plant growth promoting rhizobacteria (PGPR) are known to influence plant growth by various direct or indirect mechanisms. The current research assessed the plant growth promoting (PGP) potential of Serratia spp isolated from the fields growing horticultural crops of the rhizosphere of 6 different crops viz., eggplant, cabbage, ladies finger, tomatoes, green chili and pea plants from both Namakkal and Erode districts of Tamil Nadu state. 45 soil samples were collected from the rhizospheric soil of vegetable plants and Ten isolates of Serratia spp were isolated. IAA production was shown in all the isolates of Serratia spp (100%). Ser 1, 4 and 9 produced the highest level of IAA as compared to other isolates while Ser 2,3,7 and 8 produced moderate amount of IAA. All the isolates produced yellow color indicating their ability to produce ammonia. For all the ten isolates, HCN production was seen in Ser 1,4,5,7 and 10 while the rest produced little or no significant amounts of HCN. The formation of clear zones on the medium was an indication of phosphate solubilization. The isolates exhibited more than one PGP trait which may promote plant growth directly or indirectly or synergistically. The results provide insights into biotechnological approaches to using PGPR as an alternative to chemicals.***

Keywords: Indole-3-acetic acid, Hydrogen Cyanide, Plant growth- promoting rhizobacteria, Serratia spp

1. Introduction

Plants play a key role in the terrestrial ecosystem by being major or what is commonly known as primary producers. They also have the ability to fix atmospheric carbon to be used by animals and human beings. These plants do release photosynthates which can be up to 30%. These photosynthates create better environment for more microorganisms to thrive. This phenomena was first described as rhizosphere (Morgan et al., 2005; Hiltner, 1904).

There are different strategies plants have evolved to meet the challenge of the limited occurrence of macro and micro nutrients in the soil, the challenge of environmental stressors have all made plants to form mutualistic association with microorganisms. The microbes benefit by getting carbon and plants benefit by acquiring nutrients and protection from the environmental stressors as drought, salinity, temperature, pathogens etc. (Parniske, 2008).

Rhizobacteria have a common trait of colonizing the root (Lugtenberg et al., 2002). In the soil environment, two common activities namely; nutrient cycling and soil fertility are carried out by bacteria which form the most common microbes. The bacteria survive better in the root environments because it's rich in nutrients. This contributes to the reason as to why there is a variety of microorganisms in the rhizosphere. Amid this diversity, there is multiple interactions between different microorganisms. Such interactions involve competition for space and nutrients, antibiosis, parasitism and predation. The resulting effect can result in harming the plant, protecting and promoting the plant or neutral effects. The microorganisms that bring beneficial effects are known as plant growth promoting rhizobacteria (PGPR)- the free living rhizosphere bacteria. They have been known to promote growth and seed germination (Kloepper *et al,*. 1978).

These PGPR bacteria influence plant growth either directly or indirectly. In indirect promotion of plant growth, the PGPR will prevent or decrease the deleterious effects of phytopathogenetic microbes, while in direct plant growth promotion, auxins (plant growth regulators) are produced, there is also boosted availability of nutrients to the host plant by siderophores production, phosphate solubilization, fixation of atmospheric nitrogen (Lucy *et al,*., 2004).

There is vast literature showing the ability of rhizobacteria to synthesize auxins in vitro with Indole acetic acid (IAA) being one of the most physiologically active auxins. PGPR isolated from sugarcane rhizosphere have been established to produced IAA of up to 2.42 µg IAAml-1. IAA as a plant growth promoter helps in root elongation, increased number of roots involved in taking up of nutrients from the soil (Arshad and Frankenberger, 1993).

Rhizobacteria also perform a vital process called nitrogen fixation. Nitrogen is a vital nutrient to plants, gaseous nitrogen (N_2) is not available to them due to the high energy required to break the triple bonds between the two atoms. Rhizobacteria, through nitrogen fixation, are able to convert gaseous nitrogen (N_2) to ammonia (NH_3) making it an available nutrient to the host plant which can support and enhance plant growth. The host plant provides the bacteria with amino acids so they do not need to assimilate ammonia

(Benizri et al,.2001). Studies done by Farag (2000) indicate that Phosphate solubilizing bacteria (PSB) have the ability to increase available Phosphates for plant through production of organic acids. So far, rhizobacteria which stimulate plant growth directly have received little attention.

With the ever increasing world population, the agricultural sector a center of focus with the principal goal being the production of better, sufficient, safe and affordable food. Also, famers and producers face the challenge of sustainability and economic profit thus the increased use of synthetic chemicals in agriculture but this approach has led to bigger worries over environmental contamination and has resulted in the continuous dependency on pesticides regularly because they an indispensable part of an agricultural cycle and resulting in development of resistance to specific pesticides over time thus requiring a continual development of new pesticides (Fernando et al, 2004). In most parts of India, farmers depend on soil that lack enough nutrients as nitrogen, phosphorus and iron to support plant growth. Farmers have for many years used chemical products to help obtain high yield from crops. The use of these chemicals come with long term side effects to humans, animals and the environment. Many a times farmers experience low yield has been witnessed over a number of years. There is need to direct our attention to alternative crop enhancing techniques as plant growth promoting microorganisms. Unfortunately, rhizobacteria that stimulate plant growth directly have received much less attention than biocontrol rhizobacteria. Therefore, this study aimed to screen bacterial isolates belonging to the genera *Serratia spp*, for their multiple plant growth promoting activities namely; IAA production, Ammonia production, phosphate Solubilization and Hydrogen cyanide production.

2. Methodology

The study took place in and around Namakkal and Erode districts for a period of three months (May-April 2009).

Soil sampling

For isolation of PGPR, soils were collected from the fields growing horticultural crops. The rhizosphere of 6 different crops viz., eggplant, cabbage, ladies finger, tomatoes, green chili and pea plants in both Namakkal and Erode districts of Tamil Nadu state. The soils strongly adhering to the roots and within the space explored by the roots were considered as the rhizosphere soil (Garcia *et al.,* 2005). Soil samples were collected from randomly selected crops and immediately transported in sterile ice boxes meant for transport. In the laboratory, the plant material with its rhizospheric soil was prepared for isolation of *Serratia spp.*

Isolation of Serratia spp

In the laboratory, under sterile conditions, *Serratia spp.* was isolated and preliminarily identified on the basis of morphological, microscopic and biochemical tests. The bacterium was maintained on Nutrient Agar (Himedia) slants. The samples were streaked on Luria-Bertani (LB) medium plates. The plates were incubated at 30˚C for 2 to 4 days. Typical of *Serratia spp* strains were purified by repeated sub culturing of single colonies. The cell morphology and motility of bacterial strains were observed under a light microscope. Bacterial cultures were maintained on the respective slants.

Biochemical characterization of Serratia spp

Characterization of *Serratia spp* was done by Gram's reaction, carbohydrate fermentation, oxidase test, O-F test, H2S production, IMViC tests, as per the standard methods (Cappuccino and Sherman, 1992).

Production of Indole acetic acid

Indole acetic acid (IAA) produced by the Serratia isolates was determined as described by Brick et al,.(1991) whereby the isolates were grown in their specific media 28 °C for 4 days. After the 4 days the cultures were prepared and centrifuged at 3000 rpm for half an hour. The supernatant was put aside and 1ml mixed with 2 ml of Salkowski's reagent (50 ml, 35% of perchloric acid,1 ml 0.5 M FeCl3 solution) then it was kept in the dark. It was observed for the development of pink color as an indication of IAA production. The optical density (OD) was recorded at 530 nm after 30 min and 120 min.

Production of ammonia

The isolates were tested for the production of ammonia by growing them in peptone water (Dye 1962). Test tubes were prepared with 10ml of peptone water each, and the cultures were inoculated in them and incubated for 2-3 days at 30±2 °C. After the 3 days, Nessler's reagent (0.5 ml) was taken and added in each tube. Development of faint yellow color indicates small amounts of ammonia and deep yellow to brownish color indicates maximum amount of ammonia production (Cappuccino and Sherman, 1992).

Production of HCN

For screening Hydrogen Cyanide production by the isolates, we adapted the method of Lorck (1948). Nutrient broth was amended with 4.4 g of glycine/l and the isolate was streaked on the modified agar plate. The plates were taken and a Whatman`s filter paper no. 1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed at the top of the plate then sealed with parafilm and incubated at 28±2 °C for 96 hours. Color change of the filter paper from deep yellow to orange and orange to brown indicates the production of HCN.

Phosphate solubilization

The cultures were streaked on Pikovaskya's (Pikovaskya, 1948) agar medium and incubated at 28±˚C for 6 days. Zones of clearance which formed around the colonies were used to assess the potential of Phosphate solubilization. The mineral phosphate solubilizing (MPS) ability was arrived at by subtracting the colony diameter from the total diameter of the zone of solubilization. The Mineral Phosphate Solubilization ability by *Serratia spp* is due to lowering of the pH of the medium either by H^+ extrusion (Illmer and Schinner 1995) or by secretion of organic acids and chelating metabolites (Gaur, 1990;Tinker, 1984).

3. Results

Ten isolates of *Serratia spp* were isolated from the 45 soil samples.

Table 1: Showing number of Serratia spp isolates obtained from the collected samples

Type of Specimen	*No of Specimen*	*No Isolated*
Tomato soil	10	2
Eggplant soil	8	3
Cabbage soil	2	1
Ladies finger soil	5	0
Beet root soil		3
Carrot soil	10	0
Chilly soil	8	1
Pea plant soil	2	0
Total	45	10

Isolation and Identification of rhizospheric isolates

10 bacterial isolates were successfully isolated and identified as *Serratia spp.* The isolates were identified based on morphological observation and biochemical characterization (Table 2). Bergey's manual of determinative of bacteriology was used as a reference to identify the isolates (MacFaddin, 2000). The isolates were identified as *Serratia spp.*

Table 2: Morphological and Biochemical characterization of rhizospheric *Serratia isolates*

Characteristics	*SER 1*	*SER 2*	*SER 3*	*SER 4*	*SER 5*	*SER 6*	*SER 7*	*SER 8*	*SER 9*	*SER 10*
Gram staining	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Catalase	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Oxidase	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Indole	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
VP	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
MR	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Sucrose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Mannitol	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Lactose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Glucose	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve

Characterizarization of Serratia spp for PGP traits

The *in vitro* screening for plant growth promoting activities like, production of IAA, ammonia production, hydrogen cyanide production, and phosphate solubilization in the present was done as follows;

Production of Indole Acetic Acid

IAA production was shown in all the isolates of *Serratia spp* (100%). Ser 1, 4, 6 , 9 and 10 produced the highest level of IAA as compared to other isolates while Ser 2, 3,7 and 8 produced moderate amount of IAA as shown in table 3. This was shown by measuring the amount of IAA production through increased OD values by using spectrophotometer. Highest diameter was observed in isolate Ser 9 (3.1) followed by Ser 1 (3.0), Ser (10), Ser 4 (2.9) and Ser 6 (2.5)

Table 3: Showing production of IAA by *Serratia spp* with different tryptophan by filter paper assay

Isolates	*Intensity of color*	*Diameter (in cm)*
Ser 1	+++	3.0±0.10
Ser 2	++	2.4±0.06
Ser 3	+	1.8±0.09
Ser 4	+++	2.9±0.03
Ser 5	++	2.3±0.01
Ser 6	++	2.5±0.11
Ser 7	+	1.7±0.08
Ser 8	+	1.3±0.03
Ser 9	+++	3.1±0.05
Ser 10	++	2.8±0.11

Key: + light; ++ moderate; +++ high intensity

Production of ammonia

All the isolates produced yellow color indicating their ability to produce ammonia. Ser 4 and 5 produced the maximum amounts of ammonia as evidenced by the deep yellow and brownish color while Ser 1,2,3,8 and 9 produced moderate amount of ammonia and Ser 6.7 and 10 produced small amounts because of the faint yellow color.

Table 4: Showing Ammonia production by *Serratia spp*

Test isolate	*Color change*	*Remarks*
Ser 1	Yellow	++
Ser 2	Yellow	++
Ser 3	Yellow	++
Ser 4	Yellow	+++
Ser 5	Yellow	+++
Ser 6	Yellow	+
Ser 7	Yellow	+
Ser 8	Yellow	++
Ser 9	Yellow	++
Ser 10	Yellow	+

Key: +++ - high, ++ - medium, + - low

1) Hydrogen Cyanide production

For all the ten isolates, HCN production was seen in Ser 1,4,5,7 and 10 while the rest produced little or no significant amounts of HCN.

Table 5: Showing HCN production

Test isolate	*Color development*	*Interpretation*
Ser 1	Red to pink	+
Ser 2	No color change	-
Ser 3	No color change	-
Ser 4	Red to pink	+
Ser 5	Red to pink	+
Ser 6	No color change	-
Ser 7	Red to pink	+
Ser 8	No color change	-
Ser 9	No color change	-
Ser 10	Red to pink	+

Key: + positive with HCN production, - Negative without HCN production

Phosphate solubilization

The formation of clear zones on the medium was an indication of phosphate solubilization. Ser 4 and 9 formed wider and clearer zones as compared to all other isolates. Ser 1, 2, 5 and 6 formed no zones at all indicating inability to solubilize phosphates.

4. Discussion

In the present study, microorganisms were isolated from vegetable rhizospheric soil of Namakkal and Erode district. The rhizospheric soil was found to have a total of 10 isolates belonging to *Serratia spp.*

The isolates were first characterized based on their morphological characteristics, cultural and chemical reactions based on Bergy's Manual of Systematic Bacteriology (Tein et al., 1979). All isolates were gram negative, rod shaped, motile, catalase positive, oxidase negative, indole negative, VP negative, MR positive, sucrose positive, mannitol positive, lactose and glucose positive.

IAA production was detected in all the isolates. There was increase in the level of IAA with the increasing concentration of tryptophan (200-650 µg/ml). A similar study by Barazani and Friedman (2000) reported similar findings. Ser 1,2,4,6, 9 and 10 produced relatively high concentration of IAA compared to other isolates. IAA production was found to be dependent upon bacterial isolate and concentration of tryptophan.

Ammonia production was a common trait among all the isolates. *Serratia spp* is one of the chief ammonifying bacteria. They release protein hydrolyzing enzymes-proteases. The nitrogenous compounds in the soil are mineralized into inorganic form of nitrogen, mainly ammonia.

HCN production was detected in all the isolates. Cyanide is a secondary metabolite produced by *Serratia spp* that protects several plants from root diseases caused by soil borne fungi and suppressing root pathogens.

Phosphate solubilization was not a common trait among in all the isolates. Ser 4 and 7 were found to be good phosphate solubilizers among the 10 isolates. 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 phosphorous in soil. Phosphorus is a major essential macronutrients for biological growth and development. P in soils is immobilized or becomes less soluble either by absorption, chemical precipitation, or both. Although P content in an average soil is 0.05%, only 0.1 % of the total P present is available for to the plant because of its chemical fixation and low solubility. Microorganisms offer a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available for the plants.

Most of the isolates showed multiple traits for PGP with IAA being the dominant trait, then ammonia production, phosphate solubilization and HCN production. Bacteria inoculants like *Serratia spp* have the potential to improve plant growth (Lugtenberg et al., 2002).

5. Summary

In this present investigation, 45 samples were collected from the rhizosphere soil of vegetables and isolated ten *Serratia spp.* Further, *Serratia spp.* Were confirmed by appropriate cultural and biochemical characteristics. Characterization of the *Serratia spp* for their multiple plant growth promoting traits as indole acetic acid, ammonia production, hydrogen cyanide production and phosphate solubilization were performed using appropriate steps. IAA production was not shown in all the isolates and at different concentrations of tryptophan, the amount of IAA produced varied depending on the concentration of tryptophan. It was noted that as the concentration of tryptophan increased, the level of IAA production increased also. Ser 4 produce the highest amount of IAA. All the isolates produced ammonia. HCN production was not seen in all the isolates. Ser 4 and 10 were best HCN producers based on the intensity of the pink color produced. Phosphate solubilization was not a common trait to many of the isolates. Based on the diameter of the zones of solubilization, the isolates Ser 4 and 8 were termed to be the best solubilizers. So, some isolates exhibited more than one PGP trait thus they can be used as multiple plant growth promoting rhizobacter.

6. Conclusion

The present study, based on cultivation and biochemical methods, confirmed the occurrence of *Serratia sp.* In the rhizospheric soil associated with vegetables grown in Namakkal and Erode districts. All the *Serratia sp.* Isolates possess the capacity for IAA production, Ammonia production, HCN production and phosphate solubilization. The isolates may be used as plant growth promoters for improving plant yield under poor soil conditions. However, further studies need to be done by adding the rhizobacterium inoculant as biofertilizer on the potential of this isolate to enhance plant growth at field level and also further evaluation of *Serratia spp* isolates exhibiting PGP traits is needed and the different in PGP traits in each *Serratia* strains.

7. Other recommendations

Equalize the length of your columns on the last page. If you are using *Word*, proceed as follows: Insert/Break/Continuous.

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