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# Isolation and Characterization of Plant Growth Promoting Rhizobacteria from Banana Rhizosphere

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**A B S T R A C T**

Plant Growth Promoting Rhizobacteria (PGPR) are free living soil microorganisms that exert beneficial effects on plants. In the present study 8 strains of fluorescent pseudomonas and 4 strains of *Bacillus* were isolated from Banana rhizosphere of Nanded, Maharashtra. These strains were characterized morphologically and biochemically and studied for their plant growth promoting activities such as IAA production, GA production, Phosphate solubilization. Biocontrol traits of the isolates were also studied such as siderophore production and HCN production. These strains were tested for antifungal activity against plant pathogens *Alternaria solani* and *Fusarium oxysporum*.Yps 8 exhibited the most effective antifungal activity against *Alternaria* and Yps1 exhibited the most effective antifungal activity against *Fusarium oxysporum.*

## Introduction

Rhizosphere, the layer of soil influenced by plant root (Saharan and Nehra 2011), is known to play pivotal role in plant growth and development (Hrynkiewicz and Baum 2012). Rhizobacteria aggressively colonize roots of plants, able to multiply and colonize on the roots at all stages of plant growth, survive in the presence of a competing microflora (Antoun and Kloepper, 2001). The rhizobacteria in the rhizosphere can be neutral, detrimental or beneficial for plant growth.

About 2 to 5% of rhizobacteria, when reintroduced in rhizosphere, have beneficial effect on plant growth and are termed as plant growth promoting rhizobacteria. (PGPR) (Kloepper and Schroth, 1978). Plant growth promoting rhizobacteria (PGPR) have been studied for long. The well known genera of PGPR are *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, and *Pseudomonas.*

The means by which PGPR enhance the nutrient status of host plants can be categorized into five areas: (1) biological nitrogen fixation, (2) increasing the availability of nutrients in the rhizosphere, (3) inducing root surface area, (4) enhancing other beneficial symbiosis of the host, and (5) combination of modes of action. Fluorescent *Pseudomonas* are among the most influencing plant growth promoting rhizobacteria in plants rhizosphere and it is an excellent root colonizers (Raaijmakers and Weller, 2001) have been frequently reported as successful biological control agents (Leeman *et al*., 1995).

There are several PGPR inoculants currently commercialized that seem to promote growth. The use of PGPR inoculants as biofertilizers and/or antagonists of phytopathogens provide a promising alternative to chemical fertilizers and pesticides.

## Materials and Methods

### Rhizospheric Soil Collection

Rhizospheric soil samples were collected from banana plants of Nanded.Plants were selected from agriculture fields showing good, healthy plant growth. Plants were carefully uprooted from the soil so that the roots and the attached soil were removed intact. Thereafter, roots with the adherant soil were transferred to sterile sample collection bags and packed for transport to the lab.

### Isolation of Fluorescent *Pseudomonas*

Fluorescent pseudomonads were isolated from soil using King's B (KB) agar medium following serial dilution and plating technique. The plates were incubated at $30^0$ C for 24 h. Colonies were observed under UV light on a ransilluminator. The fluorescent colonies under UV light were picked up, purified by repeated streaking on same medium and checked for their fluorescence. Well isolated single colonies were transferred to KB slants for preservation.

### Isolation of *Bacillus* sp

The method followed was essentially that of Travers *et al*. (1987) with minor modifications. One g of soil was taken in 10 ml of sterile water and subjected to heat treatment at $65^0$C for 30 min. One ml of heat treated suspension was then serially diluted, 0.1 ml aliquot from each dilution was plated on nutrient agar and plates were incubated at $30^0$ C for 48 h. Isolates showing different colony characters were selected, purified and stored on NA slants for identification.

### Growth Promoting Activities by the Isolates of Fluorescent *Pseudomonas* and *Bacillus*

### Determination of Indole Acetic Acid

Isolates were inoculated in 100 ml King's B broth supplemented 0.1mg/ml tryptophan and incubated at 27 ± 2 °C for 4 days. Supernatant was centrifuged, acidified to pH 2.5 and extracted with 10 ml of ethyl acetate. Ethyl acetate fraction was evaporated at 40 °C under vacuum and residue was suspended in 2 ml ethanol and mixed with Fe-HClO4 reagent. The absorbance was measured at 530nm after 25 min (Gordon and Weber, 1951).

### Estimation of GA

Twenty-five ml of the culture filtrate was taken in a test tube to which two ml of zinc acetate was added. After two minutes, 2 ml of potassium ferrocyanide was added and centrifuged at 1000 rpm for 15 minutes. To five ml of this supernatant, five ml of 30 per

cent HCl was added and incubated at 200 C for 75 minutes. The blank sample was treated with five per cent HCl and the absorbance of the samples as well as blank was measured at 254 nm in a UV-vis spectrophotometer. The amount of GA present in the extract was calculated from the standard curve and expressed as µg/ml of the medium. The standard curves of IAA and GA were prepared by using graded concentrations of IAA and GA. (Paleg, 1965).

## Phosphate Solubilization

The active bacterial cultures were spot inoculated on pikovaskay's media plate and incubated at $30^0$c for 5 days The isolates showing clear zone of solubilization around the colony were taken as P solubilizers. The diameter of the zone of solubilization was measured and expressed in centimeters.

## Biocontrol Activities of Fluorescent *Pseudomonas* and *Bacillus*

## HCN production

Whatman No. 1 filter paper pads were placed on the lid of petriplate and the plates were sterilized. TSA medium amended with glycine (4.4 g/l) was sterilized and poured into the sterile plates. The isolates were streaked on the medium. The filter paper padding in each plate was soaked with 2 ml sterile picric acid solution. The plates were sealed with parafilm in order to contain gaseous metabolites produced by the antagonists and to allow for chemical reaction with picric acid present in the filter padding. After incubation for a week time at 300 C, the colour change of the filter paper was noted and the HCN production potential of the antagonists was assessed (Wei *et al.*, 1991).

## Siderophore Production

Production of siderophore by bacterial antagonists was assessed by plate assay. Chrome Azurol S blue agar medium (CAS) was used to detect siderophore production by the isolates of fluorescent pseudomonads and *Bacillus* sp. (Schwyn and Neilands, 1987).

## Antifungal Activity

Fungal pathogen was grown on a Potato Dextrose Agar plate. A disc of fungal growth from this plate was taken with the help of a sterile cork borer and placed at the center of a fresh PDA plate. Twenty four hour old bacterial culture was then streaked parallelly on both side of the fungal disc 2-3 cm away from the disc. The plates were incubated at $30^oC$ for 96 hours. Inhibition of fungal growth was recorded after 96 hours of incubation in comparison control. (Ganesan and Gnanamanickam, 1987).

## Results and Discussion

A total of 12 strains of which, 8 were fluorescent *pseudomonas* and 4 *bacillus* were isolated and identified by morphological and biochemical characters.

## Plant Growth Promoting Traits of Isolates

## IAA Production

All the isolates of fluorescent pseudomonas were positive for IAA production. IAA production by fluorescent pseudomonas ranged from 89 µg/ml to 108 µg/ml. The highest production of IAA was observed in Yps6 (108 µg/ml). The isolates of bacillus Yb1 and Yb2 were positive for IAA production and Yb3 and Yb4 were negative for IAA production.Yb1 produced 51 µg/ml IAA(Table 2).

### *GA Production*

All the isolates of fluorescent pseudomonas were positive for GA production.GA

production by fluorescent pseudomonas ranged from 44 µg/ml to 72 µg/ml.Yps2 was the highest producer of GA(72 µg/ml).All the isolates of Bacillus were positive for GA production.Yb1 was the highest producer of GA(52 µg/ml) (Table 2).

## Phosphate Solubilization

All the isolates of fluorescent *pseudomonas* and *Bacillus* were positive for phosphate solubilization (Table 2). Many genera of bacteria such as *Achromobacter, Agrobacterium, Bacillus, Pseudomonas, Serratia* and several others have been reported to solubilize varying quantities of phosphorus depending on the efficiency of the strains. The most efficient and dominant solubilizers belong to genera *Bacillus* and *Pseudomonas* (Gaur, 2002).

**Table.1** Morphological Characters of the Isolates

| Sr.No | Isolate code | Grams Nature | Morphology | Motility | Endospore | Fluorescent Pigment |
|---|---|---|---|---|---|---|
| 1 | Yps1 | Gram Negative | Rod shaped | Motile | _ | + |
| 2 | Yps2 | Gram Negative | Rod shaped | Motile | _ | + |
| 3 | Yps3 | Gram Negative | Rod shaped | Motile | _ | + |
| 4 | Yps4 | Gram Negative | Rod shaped | Motile | _ | + |
| 5 | Yps5 | Gram Negative | Rod shaped | Motile | _ | + |
| 6 | Yps6 | Gram Negative | Rod shaped | Motile | _ | + |
| 7 | Yps7 | Gram Negative | Rod shaped | Motile | _ | + |
| 8 | Yps8 | Gram Negative | Rod shaped | Motile | _ | + |
| 9 | Yb1 | Gram positive | Rod shaped | Motile | + | _ |
| 10 | Yb2 | Gram positive | Rod shaped | Motile | + | _ |
| 11 | Yb3 | Gram positive | Rod shaped | Motile | + | _ |
| 12 | Yb4 | Gram positive | Rod shaped | Motile | + | _ |

**Table.2** Plant Growth Promoting Traits of Isolates

| Sr.No | Isolate code | IAA Production | GA Production | Quantitative IAA Production (µg/ml) | Quantitative GA Production (µg/ml) | Phosphate solubilization |
|---|---|---|---|---|---|---|
| 1 | Yps1 | + | + | 105 | 56 | + |
| 2 | Yps2 | + | + | 98 | 72 | + |
| 3 | Yps3 | + | + | 99 | 44 | + |
| 4 | Yps4 | + | + | 100 | 45 | + |
| 5 | Yps5 | + | + | 102 | 46 | + |
| 6 | Yps6 | + | + | 108 | 58 | + |
| 7 | Yps7 | + | + | 95 | 62 | + |
| 8 | Yps8 | + | + | 89 | 61 | + |
| 9 | Yb1 | + | + | 51 | 52 | + |
| 10 | Yb2 | + | + | 50 | 51 | + |
| 11 | Yb3 | _ | + | _ | 42 | + |
| 12 | Yb4 | _ | + | _ | 46 | + |

**Table.3** Biocontrol Traits of Isolates

| Sr.No | Isolate code | Siderophore Production | HCN Production |
|---|---|---|---|
| 1 | Yps1 | + | + |
| 2 | Yps2 | + | ++ |
| 3 | Yps3 | _ | + |
| 4 | Yps4 | + | _ |
| 5 | Yps5 | + | ++ |
| 6 | Yps6 | _ | + |
| 7 | Yps7 | + | ++ |
| 8 | Yps8 | + | + |
| 9 | Yb1 | _ | + |
| 10 | Yb2 | _ | + |
| 11 | Yb3 | _ | + |
| 12 | Yb4 | _ | + |

No colour change – No HCN production(-), Brownish colouration – Weak HCN production(+), Brownish to orange - Moderate HCN production(++), Complete orange - Strong HCN production(+++)

**Table.4** Antifungal Activity of Isolates

| Sr. No. | Isolate wheat | Mean zone of inhibition In (cm) | |
|---|---|---|---|
| | | Fungal plant pathogen | |
| | | ***Alternaria solani*** | ***Fusarium oxysporum*** |
| 1 | Yps1 | 6.45 | 5.95 |
| 2 | Yps2 | 6.95 | 5.25 |
| 3 | Yps3 | - | - |
| 4 | Yps4 | - | - |
| 5 | Yps5 | - | 5.05 |
| 6 | Yps6 | 6.85 | - |
| 7 | Yps7 | 6.9 | 5.45 |
| 8 | Yps8 | 7.1 | - |
| 9 | Yb1 | 6.75 | 4.85 |
| 10 | Yb2 | - | - |
| 11 | Yb3 | - | - |
| 12 | Yb4 | - | - |

## Biocontrol Traits of Isolates

### Siderophore Production

The isolates of fluorescent *pseudomonas* Yps1, Yps2, Yps4, Yps5, Yps7, Yps8 were positive for Siderophore production. All the isolates of *bacillus* were negative for Siderophore production(Table3). Suryakala *et al.* (2004) observed that siderophores produced by *Pseudomonas fluorescens* were antagonistic to fungal pathogens like

*Fusarium oxysporum, Alternaria* sp. and *Colletotrichum capsicii*

## HCN Production

Weak HCN production was observed by Yps1, Yps3., Yps6, Yps8.Moderate HCN production was observed by Yps2, Yps5, Yps7. All the isolates of *Bacillus* were weak producer of HCN(Table 3). HCN is produced by many rhizobacteria and is postulated to play a role in biological control of pathogens (Defago *et al.*, 1990).

## Antifungal Activity

Antifungal activity of isolates was tested against *Alternaria* and *Fusarium oxysporum*. The highest zone of inhibition was given by Yps8 against *alternaria solani* and highest zone of inhibition was given by Yps1 against *Fusarium oxysporum*. No zone of inhibition was observed by Yb2, Yb3, Yb4. Spore forming *Bacillus* sp. isolated from naturally occurring compost was found to be antagonistic to *Fusarium solani* causal agent of black root rot of chickpea (Sriveni *et al.*, 2004).

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