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Assessment of Bacterial Diversity and PGP Activity of Rhizobacteria in Rhizosphere of *Vigna mungo*

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Little information is known about the composition of bacterial diversity associated with roots of *Vigna mungo* plant. With this background, the present study was carried out to investigate the diversity of bacterial isolates and PGP activity of rhizobacteria from the soils of *Vigna mungo*, grown in different regions of Indo Gangetic plains. Investigations of the rhizobacterial population associated with collected soils done by the means of colony forming units using serial dilution agar plate technique. In this context, total bacterial count, occurrence percentage, rhizospheric to soil (R: S) ratios were examined. Total bacterial count of all soil samples were found to be in the range of $12x10^5$ to $98x10^5$ with occurrence percentage of 1.07% to 92.85%. The values of R: S ratio lies in between 0.85 to 3.25 which is above 1 clearly indicates presence of positive rhizospheric effect in soil samples of Indo Gangetic plains. From the rhizosphere of selected plant, a total of seventeen bacteria were isolated and checked for Indole-3 acetic acid production colorimetrically using Salkowski reagent. Among all the isolates, IAA production was positively exhibited by majority of bacterial isolates and were found to produce IAA in the range of 34.97 and 90.64 μg/ml. In our findings, two isolates IGV6 and IGV7 showed best positive result for indole-3-acetic acid production. Therefore, these promising isolates can be considered for as possible phytostimulator and bio-inoculant in rhizosphere of *Vigna mungo* plant. In addition to this, bacterial communities associated with rhizosphere of *Vigna mungo* plant acting as a reservoir of species which can be further explored for their bioprospecting potential and its application in agriculture and industry.

Key words: IAA, phytostimulator, rhizosphere and rhizobacterial population.

Plant roots play a key role in selecting, enriching and harbouring bacterial diversity in the particular root soil environment[1]. Variety and type of bacterial diversity associated with roots of plant generally depend upon the nature and concentrations of organic substances released by root exudates in the rhizosphere[2, 3]. Rhizosphere is the narrow zone of soil surrounding the roots of plant in which the microbial population stimulated by plant root activities or where growth of plant enhanced by microorganisms present in the soil. In the rhizosphere, the plant-microbe interactions can be either beneficial, neutral or negative[4, 5]. Rhizosphere concept was firstly introduced by Hiltner in 1904[6]. Rhizobacteria (bacteria associated with roots) exert the beneficial effects on the growth of host plant by variety of mechanisms known as

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(PGPR) Plant growth promoting rhizobacteria[7, 8, 9]. Rhizobacteria is a common term used for bacteria that colonize the rhizosphere. PGPR use one or more type of mechanisms i.e. direct & indirect to enhance the growth of plants[4]. PGPR directly enhance the plant growth by different type of mechanisms such as synthesis of phytohormones i.e. IAA (Indole-3-acetic acid), cytokinins, ethylene and gibberellic acid, fixation of atmospheric N_2 that is transferred to the plant, synthesis of enzymes i.e. ACC deaminase which modulate the proper level of phytohormones, production of siderophores that chelate iron, mineral solublization like phosphorus, and make it available to the plant root[8, 10].

In addition to this, IAA is one of the important plant growth promoting trait of rhizobacteria that directly enhance the growth of plant in the presence of physiological precursor tryptophan[11, 4]. Indole-3 Acetic Acid (IAA) is the most important principle auxin found in higher plants which regulate various physiological processes including root initiation, cell enlargement, cell division stimulation and resulting in increase the root surface area enabling the plant to acquire significantly more nutrients from soil[2, 5]. In the last few years, more number of rhizosphere bacteria (PGPR) were studied and identified because the rhizosphere play important role in the functioning of the ecosystem[5]. Various species of bacteria like *Pseudomonas, Bacillus, Azospirillum, Azotobacter, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Serratia* and *Klebsiella* have been reported to enhance the plant growth[6].

Vigna mungo commonly referred to as black gram dal, Urad, black lentil, and white lentil and is one of the important pulse crop grown throughout in several regions of Indian subcontinent. *Vigna mungo* first time originated and cultivated in India from the ancient times and used by majority of Indian peoples for make dal (*dal makhani*) and different culinary preparations such as dosa, idli, vada and paapad. *Vigna mungo* is highly nutritious and proteinaceous in nature and is good for diabetic patients[12].

A wide variety of microorganisms in the vicinity of crop plants are being exploited in the production of microbial inoculant formulations or biofertilizers to reduce chemical fertilizer costs[13]. Biofertilizer or microbial inoculants are biological products which contain living cell of efficient strains of microorganisms (PGPR) that increase the nutrient status of the host plant, enhance crop productivity and help in minimize the negative impact of chemical fertilizer for sustainable agriculture[14]. Significant increases in crop productivity or yield have been reported by applying soil bacteria or PGPR as microbial inoculants[13]. So, keeping all this in view, this study was aimed to assess the bacterial diversity and PGP activity of rhizobacteria from the rhizospheric soils of *Vigna mungo* for plant growth promotion.

MATERIALS AND METHODS

The present investigation was conducted in the department of Biotechnology, MNNIT Allahabad, India.

Collection of soil sample

Vigna mungo was selected as a test crop for the present study from three different sites of different sites i.e. Chandpur, KVK and Sarokhanpur in Indo Gangetic plains. Plant with intact root system was dug out and carefully taken in plastic bags and stored at 4^0C for further microbiological analysis. In the present study, A total of six soil samples rhizospheric and non-rhizospheric were collected to assess the bacterial diversity and PGP potential.

Isolation of bacterial isolates

Total bacterial counts were determined using serial dilution agar plate technique by using 1g soil[14]. The CFU (colony forming units) were recorded after 24-48 h of incubation at 28±1ºC by using the formula given below:

$$CFUg^{-1} \text{ dry soil} = \frac{\text{Average number of colonies}}{\text{Dry weight of soil X Dilution factor}}$$

Occurrence %

Relative abundance of dominant bacteria from collected soil sample was also calculated on nutrient agar medium with the formulae given below:

$$\text{Occurrence (\%)} = \frac{\text{Number of colonies of a species}}{\text{Total number of colonies}} \text{ X } 100$$

R: S ratio

Rhizospheric to soil (R: S) ratio[15] can be calculated by the formula :

$$\text{R: S ratio} = \frac{\text{CFU of rhizosphere bacteria}}{\text{CFU of non rhizosphere bacteria}}$$

PGP activity of rhizobacteria

IAA production

IAA production was assayed colorimetrically using Salkowski reagent (ferric chloride-per chloric acid) according to the methodology[16] described by Gordon and Weber, 1951. Fifty milliliter NB containing 0.1% DL-tryptophan was inoculated with 500 µl of 24 h old bacterial cultures and incubated in refrigerated incubator Shaker at 28±0.1 °C and 180 rpm for 48 h in dark. The bacterial cultures were centrifuged at 10,000 rpm for 10 min at 4 °C. Estimation of indole-3-acetic acid (IAA)-like auxins in the supernatants was done using colorimetric assay.

Colorimetric estimation

One millilitre of supernatant was mixed with 4 ml Salkowski reagent and absorbance of the resultant pink color was read after 30 min at 535 nm in UV/Visible Spectrophotometer. Appearance of pink color in test tubes indicated IAA production described by Gordon and Weber in 1980. A standard curve was prepared by dissolving 1mg of IAA in few drops of methanol and made to 10 ml volume with de-ionized water and considered as stock solution. Added 4 ml of Salkowski reagent to 50, 100, 150, 200, 300, 400 and 500 µl aliquots of the stock solution taken in sterile test tubes and made to 5 ml volume with de-ionized water. The blank consisted of 4 ml Salkowski reagent and 1 ml de-ionized water. The absorbance of the resultant pink color was read at 535 nm after 30 min. Values of concentration versus optical density were plotted and regression equation used to calculate the indole-derivates. The values of IAA-like auxins were expressed as µg ml^{-1} over control.

RESULTS AND DISCUSSION

Plant root exudates secrete variety of organic substances into soil and play very critical role in selecting, enriching and harbouring bacterial diversity in the vicinity of root soil environment[1]. Several reports in the literature indicating that presence of bacterial diversity in the rhizosphere is mainly depend on the concentration of the nutrients available[21]. Hence, the total bacterial count in the rhizospheric soils ranged from 74 to $96x10^5$ whereas bacterial counts in control soils (non-rhizosphere) were 12 to $20x10^5$. In our study, we obtained significantly relatively higher bacterial counts in the rhizospheric soils of *Vigna mungo* than in the non-rhizospheric control soils (Fig. 1). Our result supports the finding of various researchers which stated that plant release a wide variety of organic compounds in the rhizosphere through root exudates and provides a rich environment for microbial activity[2, 3]. Fig. 1 clearly indicates that rhizospheric soil of Sarokhanpur region showed higher CFU in comparison to rhizospheric soil & non rhizospheric soil of KVK & Chandpur. The higher CFU indicates the presence of maximum number of viable cells in that

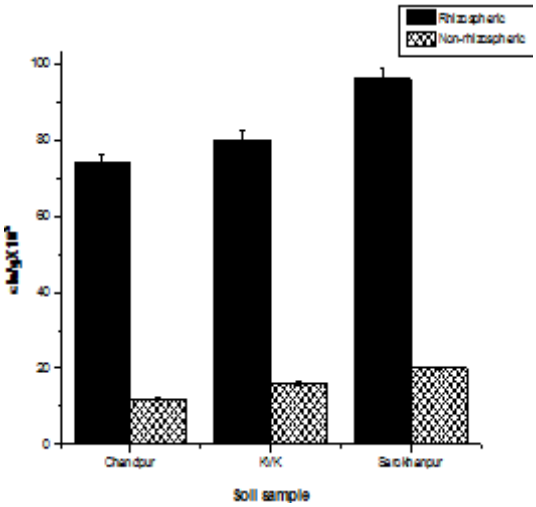

Fig. 1. Total bacterial count of rhizospheric and non rhizospheric soil sample of *Vigna mungo*

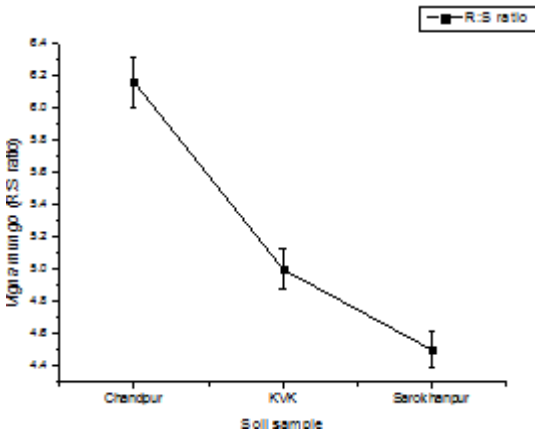

Fig. 2. R: S ratio of soil samples collected from *Vigna mungo* plant

particular sample. The rhizospheric effect is the estimation of rhizosphere to soil bacteria (R: S ratios) and generally determines the relative stimulation of the microorganisms in the rhizosphere of *Vigna mungo* plant.

Rhizosphere to soil ratio ranged from 0.85 to 3.25. Ample studies have been documented regarding magnitude of the rhizospheric effect is affected by many biotic and abiotic factors like amount and nature of root exudates secreted by individual plant species in particular climatic conditions[18, 19]. The R: S values obtained from different sites were found above 1 which indicates positive rhizospheric effect or absence of antagonistic metabolites in the rhizosphere of selected plant that causes suppressive effect on rhizobacterial population (Fig. 2). During the course of investigation, a total of sixteen bacteria were isolated from three different sites. Eight bacterial isolates were from Chandpur, five from KVK and four isolates were from Sarokhanpur from selected crop.

In the present study, the most abundant bacterial species were isolated from rhizospheric soil of KVK site (VM10) and non rhizospheric soil of *Vigna mungo* plant of Sarokhanpur region (VM17) with occurrence percentage of 92.85% and 88.28% respectively. The percentage frequency of occurrence of dominant bacteria is given in (Fig. 3, 4 and 5). Out of seventeen bacteria isolates, over sixty four percent of the isolates were found to be gram negative rods, seventeen percent gram positive cocci and eighteen percent were found to be gram positive rods in soil samples collected from different sites.

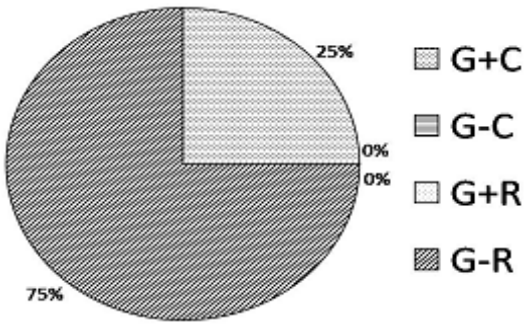

Fig. 3. Occurrence percentage of bacteria in Chandpur region

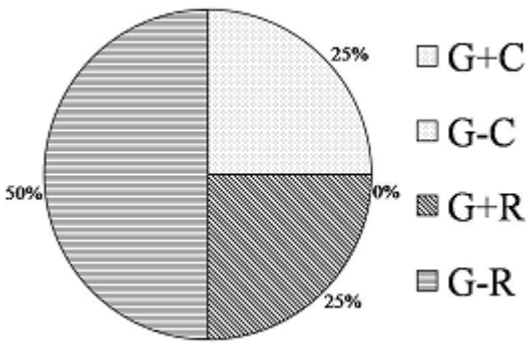

Fig. 4. Occurrence percentage of bacteria in KVK Buxa region

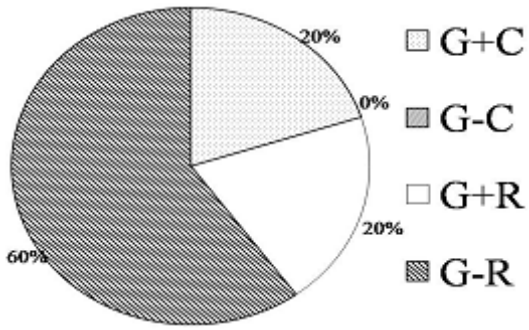

Fig. 5. Occurrence percentage of bacteria in Sarokhanpur region

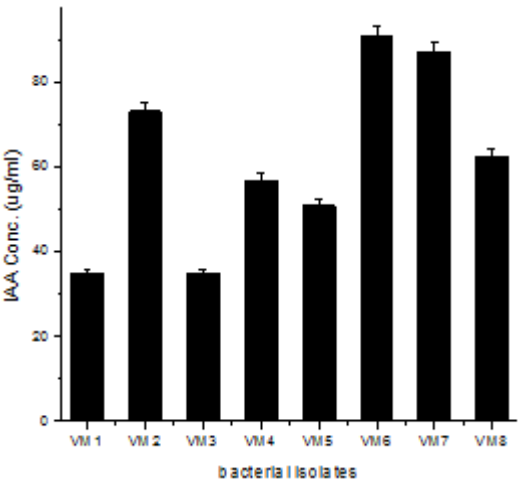

Fig. 6. IAA production by bacterial isolates in the soil of Chandpur region

IAA is one of the important plant growth promoting trait of rhizobacteria that directly enhance the growth of plant in the presence of physiological precursor tryptophan[4, 5]. Production of variety of plant hormones such as, auxins (IAA), cytokines and gibberellins by naturally occurring microorganisms present in the soil environment have been reported by several researchers over the last 20 years[20]. Among all these, Indole-3 Acetic Acid (IAA) is the most important principle auxin found in higher plants which regulate various physiological processes including root initiation, cell enlargement, cell division stimulation and resulting in increase the root surface area enabling the plant to acquire significantly more nutrients from soil[11, 21]. Therefore, all the bacterial isolate isolated from rhizosphere of selected crop were further subjected for Indole-3 acetic acid production colorimetrically using Salkowski reagent. Ample studies have been documented that 80% of soil microbial communities associated with rhizosphere of various crops i.e. wheat, maize and sugarcane possess the ability to produce plant hormones i.e. IAA[22]. In our study, majority of bacterial isolates were able to produce IAA in the presence of tryptophan (Fig. 6, 7 and 8). A comparatively highest level of IAA was found in the bacterial isolate IGV6 (90.64 µg/ml) followed by IGV7 (87.15 ug/ml) compared to other bacterial isolates.

In our findings, two isolates IGV6 and IGV7 showed best positive result for indole-3-acetic acid production. Therefore, these promising isolates can be considered for as possible phytostimulator, bioenhancer and microbial

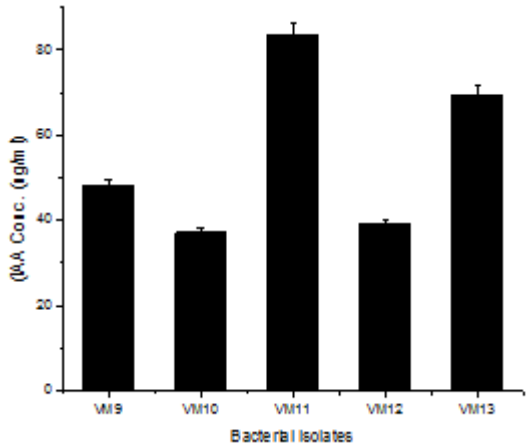

Fig. 7. IAA production by bacterial isolates in the soil of KVK Buxa region

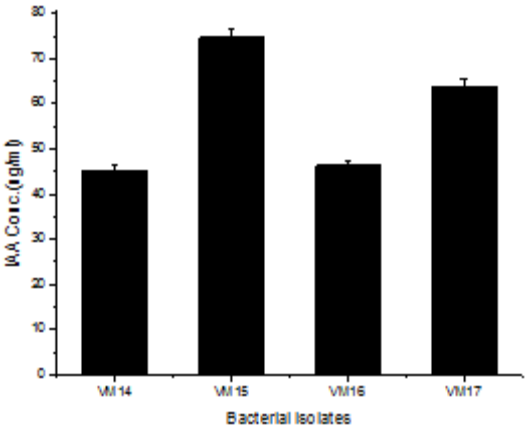

Fig. 8. IAA production by bacterial isolates in the soil of Sarokhanpur region

inoculant in rhizosphere of *Vigna mungo* plant. Apart from this, bacterial communities associated with rhizosphere of *Vigna mungo* plant can be further explored for their bioprospecting potential and application in agriculture and industry.

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