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Short Communication

Plant growth promoting properties of endosymbionts isolated from root nodule of legumes

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The symbiotic rhizobia-legume association is one of the most useful plant-microbe interactions. The symbiotic association has by tradition been used in agricultural practices to provide nitrogen to plants and thereby enhance plant growth. The plant microbiome is a largely unexplored resource of beneficial microorganisms with diverse properties and a hidden potential to manipulate plant growth and success in stressful environments. The Rhizobial symbiosis plays an important role in nitrogen (N) nutrition. This symbiosis involves legumes and a specific group of soil bacteria, collectively known as rhizobia, which are able to form root nodules and fix atmospheric nitrogenwhen associated with legumes. The most widely reported phylum Bacteroidetes, Firmicutes, and Proteobacteria, of which the most commonlystudiedare *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, *Gluconacetobacter*, *Enterobacter*, *Serratia*, *Paenibacillus* and *Rhizobium* are well known PGPR (Bhattacharyya and Jha, 2012).Many plant growth promoting rhizobacteria secrete phytohormones, such as cytokinins, gibberellins, auxin, and ACC deaminase (Glick, 2014) and influence plant growth functions. This study is aimed to isolate plant growth promoting endosymbionts of legumes and to evaluate their plant growth promoting traits.

Five different root nodule producing leguminous plants were collected around the village of Thanjavur district and identified. Herbarium was submitted at Botany Department, Saint Joshep College, Tiruchirapalli for identification. Nodules were washed under running tap water and then treated with 0.1% $HgCl_2$ for 2 min and successively washed three times with sterile distilled water under aseptic conditionfor 1 min each. Then the nodules were crushed with the help of sterile forceps and the 100 µL contents were spread on CYEMA plate. All the plates were incubated at 28 ± 2°C for 5 days. The cultures were maintained on YEMA slants and all the isolates were subjected to biochemical tests followed by morphological characteristics. Differentiation of Agrobacterium and Rhizobium done by growth at the Hofer‟s alkaline medium (pH 11) as described by Singha *et al.* (2016). Furthers isolates were subjected to production of siderophore, organic acid, ammonia and phosphate solubilization (Pulawska *et al.*, 2012). The phosphate Solubilization Index was calculated as PSI= zone diameter (cm) -colony diameter (cm)/ colony diameter (cm).

Among the five plants, *V.mungo* (PS3) showed $54X10^{7}$CFU followed by $43X10^{-7}$ from *Aeschynomene indica* (PS2). The CFU of *Mimosa pudica, Crotalaria albida* and *Vigna trilobata* showed 36, 37 and $38X\ 10^{7}$CFU, respectively on YEMA plates. Most of the colonies were mucoid, raised with smooth edges and musky odour of the colony was observed under low power microscope. The mucoid colonies would represent a mechanism involved in the process of adaptation and survival of Rhizobium in adverse conditions. More than 40 percentage of isolates were fast-growing rhizobia and formed single colonies with diameter of 2–3 mm within 3 days on YEMA. Based on colony morphology and biochemical characters isolates were identified as *Agrobacterium sp* (1)*, Bacillus sp* (6)*, Bradyrhizobium* sp (2), *Blastobacter* sp (1), *Enterobacter* sp (3), *Ensifer* sp(1)*, Methylobacterium* sp (1), *Pseudomonas* sp (3), *Streptomyces* sp(1), *Rhizobium* sp (3), *Mesorhizobium* sp (1). Rhizobium sp strains failed to absorb congo red stain where as Agrobacterium absorbed and appeared as red and showed positive growth on Hofer‟s alkaline medium. Occurrence of aquatic bud- and cluster-forming freshwater bacterium *Blastobacter* sp was reported by Van Berkum *et al.* (2006)

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Table 1: Physiological characteristics of root nodule associated isolates

Isolates	Phosphate solubilisation	Siderophore production	IAA	Organic acid	Ammonia
Methylobacterium sp	-	-	Positive	-	-
Bacillus sp	-	-	Positive	-	-
Pseudomonas sp	-	positive	Positive	-	-
Mesorhizobiumsp	Positive	Positive	Positive		
Bacillus sp	-	-	-	-	-
Enterobacter sp	-	-	-	-	-
R.nepotum	-	-	Positive	Positive	Positive
Blastobacter sp	-	positive	-	-	positive
Bacillus sp	-	-	-	-	-
Agrobacterium sp	-	-	Positive	-	-
Pseudomonas sp	positive	-	-	Positive	-
Bacillus sp	-	-	Positive	-	-
Bradyrhizobium sp		-	Positive	-	-
Rhizobium sp	Positive	-	Positive	-	-
Pseudomonas sp	-	-	-	Positive	-
Bacillus sp	Positive	-	-	-	-
Enterobacter sp	-	-	-	-	-
Streptomyces	Positive	-	Positive	Positive	-
Enterobacter sp	-	-	-	-	-
Methylobacterium sp	-	-	Positive	-	-
Bradyrhizobium sp	-	positive	Positive	-	-
Ensifer sp	-	-	Positive	-	-
Bacillus sp	Positive	-	-	-	-

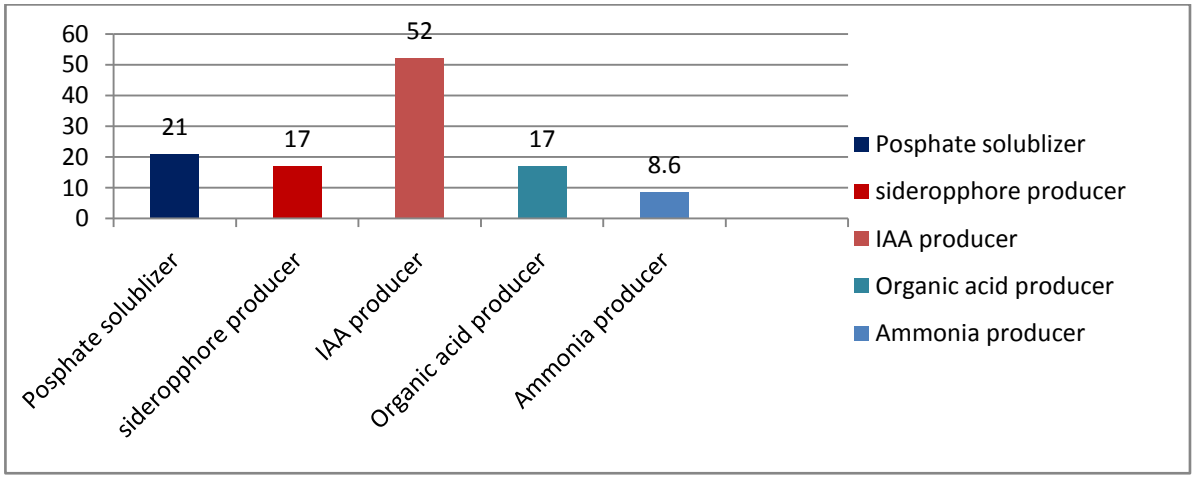

Figure 1: Frequency of Plant growth promoting trait of isolates

Totally 23 isolates were subjected to *In vitro* plant growth promoting (PGP) activities. Data showed that 21% were phosphate solubilisers, 17% organic acid and siderophore producers, 52% isolates produced IAA and 8.6% were ammonia producing in nature. Production of IAA by non rhizobial and rhizobial isolates was frequently reported. Phosphate solubilization activity was noted in *Rhizobium* sp and *Bacillus* sp isolated from *V.trilobata.* Similarly *Bradyrhizobium* sp, *Mesorhizobium sp, Pseudomonas* spand *Blastobacter* spisolates were the only four who gave positive results on siderophore. Among phosphate-solubilizing, *Bacillus* sp from *Calbida* showed 72 % phosphate Solubilization index followed by Pseudomonas (68%). Gaonkar and Bhosle *et al.*, (2013) studied siderophore producing *Bacillus* sp. and *Pseudomonas aeruginosa* produced a yellowish fluorescent siderophore identified as pyoverdine. Nodule colonization of selected leguminous plants showed presence of rhizobial and coexistence of non rhizobials with plant growth promoting physiological traits confirmed by *in vitro* analysis

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