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Characterization of Coconut Rhizobacteria for Plant Growth Promoting Traits

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Abstract

This study aims to identify the plant growth promoting rhizobacteria from coconut rhizosphere for utilizing under coconut based intercropping system as potential bioinoculants. Twenty bacterial strains isolated from different organically grown coconut rhizosphere soils were tested for plant growth promoting (PGP) traits and extracellular enzyme activities. In PGP properties tested, the results revealed that 9(45%) strains were found to produce indole acetic acid (IAA), 14 strains (70%) were rich in siderophore production and 16 strains (80%) could solubilize inorganic phosphate. In extracellular enzyme production, 10 strains (50%) were found to have the ability to produce protease, 15 strains (75%) produced ammonia and 16 strains (80%) produced cellulase and catalase enzymes. The overall results revealed that the five *Bacillus* strains collected (CSA01, CSA09, CSC03, CSD09 and CSE01) from rhizosphere regions of organically grown coconut plantations of Andaman Islands were found efficient in all the PGP properties tested.

Key words: Rhizobacteria, coconut rhizosphere, plant growth promotion, *Bacillus* and Andaman islands.

Introduction

Soil borne micro-organisms belonging to various groups plays important role in plant-soil interactions (Mantelin and Touraine, 2004). Among them, few bacterial groups could colonize the plant rhizosphere region which are beneficial for plant growth and development are called as Plant Growth Promoting Rhizobacterias (PGPRs) (Kloepper, 1993). PGPR activity has been reported in strains of bacteria belonging to several genera, such as *Azotobacter, Azospirillum, Pseudomonas, Acetobacter, Burkholderia* and *Bacillus* (Kloepper 1993; Glick and Bashan 1997). Among which *Bacillus* spp were well reported for their positive influence on plant growth promoting abilities and antimicrobial traits (Wu et al., 2005). PGPR can alter the stimulation of plant growth through its direct or indirect mechanisms which includes the ability to synthesize hormones like indole acetic acid (IAA) (Patten and Glick, 2002), gibberellic acid (Mahmoud et al., 1984) and cytokinins (Tien et al., 1979); the ability to produce ACC deaminase (Glick, 1995); asymbiotic nitrogen fixation (Kennedy et al., 1997); antagonism against phytopathogenic microorganisms by producing hydrolytic enzymes like proteases, celluloses, chitinases etc., (Catellan et al., 1999; Pal et al., 2001) and solubilization of mineral phosphates and mineralization of nutrients (Idriss et al., 2002). The productivity of most of the crops are very low in Andaman and Nicobar islands, India which is mainly due to adoption of primitive methods of cultivation techniques and therefore, increase in production is one of the major concerns in the islands. Coconut plantations cover the 50% of area (~ 22000 ha) under island cultivation and in recent days the intercropping with red gram, green and black gram, cow pea, ground nut, ginger, elephant foot yam, legume vegetables and fodder crops under coconut cultivation is being promoted for effective land utilization and to increase the island agricultural production. By considering the fragile nature and uniqueness of island, the use of chemical fertilizers is generally discouraged and at the same time the organic method of improving crop production is highly recommended. Therefore, the present work was aimed to characterize the *Bacillus* strains from the organically cultivated coconut rhizosphere soils and to study their plant growth promoting traits in order to utilize the efficient strains as potential bioinoculant in coconut based intercropping systems.

Materials and methods

Sampling site and isolation

Rhizosphere soil samples were collected from different organically grown coconut fields (Sippighat, Wandoor, Manjery, Calicut and Guptapara) of South Andaman Islands, India and selective isolation of *Bacillus* spp were carried out *according* to Travers et al. (1987) with little modifications. Briefly, One gram of soil sample was treated at 80° C for 30 min and serial dilutions were performed using sterilized water up to the concentration of $10^{-6,}$ from which 0.5ml of solution was plated on LB media. All the plates were subjected to incubation at 28°C for 2 days. The dominating and well separated colonies were purified on NA plates and maintained in 20% glycerol stock at -20 C for further studies.

Screening for plant growth promoting properties

Siderophore production

Freshly grown bacterial cultures were streaked on King's B medium amended with an indicator dye. The tertiary complex Chrome Azural S (CAS) / Fe^{3+}/ Hexadecyl trimethyl ammonium bromide served as an indicator. Change of blue color of the medium surrounding the bacterial growth to fluorescent yellow indicated the production of siderophore. The reaction of each bacterial strain was scored either positive or negative to the assay (Schwyn and Neilands 1987).

Phosphate solubilization

All bacterial strains were screened for inorganic phosphate solubilization according to Verma et al. (2001). A loopful of fresh bacterial culture was streaked onto Pikovaskaya's medium amended with inorganic phosphate and the plates were incubated at 28 ± 2ºC for 3-4 days. A clear halo around the bacterial colony indicated solubilization of mineral phosphate.

Indole Acetic Acid production

Production of IAA was determined qualitatively by following the standard method (Brick et al. 1991). Briefly, overnight grown single colony was streaked onto LB plates amended with 5mM L-tryptophan. Plates were overlaid with sterile Whatman no. 1 filter paper (82 mm diameter) and bacterial strains were allowed to grow at 28º ± 2º C for 3 days. After incubation, the paper was removed and treated with Salkowski's reagent (Gordon and Paleg 1957) at room temperature for 60 min. IAA production of the strains were confirmed by the formation of characteristic red halo on the filter paper.

Screening for production of extra cellular enzymes

Protease and Cellulase assays

Proteolytic activities of the cultures were screened quantitatively in a medium containing skimmed milk (Mahanta et al. 2008). Zone formation around the colonies (appearing over the next 48 h) was taken as evidence of proteolytic activity. Cellulase production was determined by using the method suggested by Samanta et al. (1989). M9 agar medium with yeast extract plates were inoculated with individual bacterial strains and incubated for 3-5 days at 28C. Bacterial growth surrounded by clear halos was considered as positive indication of cellulase production.

Ammonia production

For ammonia production, freshly grown cultures were inoculated in 30ml peptone water in sterile tubes. Suspension was incubated at 38º ± 2º C for 48-72 h and then Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman, 1992).

Results and Discussion

A total of 20 bacteria with white/ dull color, with well distinguished irregular/ serrated margins representing *Bacillus* were recovered from rhizospheric soil of organically cultivated coconut plants of Andaman & Nicobar Islands were screened for Plant Growth Promoting (PGP) traits and for production of extracellular enzymes. In PGP properties tested, the results revealed that 9(45%) strains were found to produce indole acetic acid (IAA), 14 strains (70%) were rich in siderophore production and 16 strains (80%) could solubilize inorganic phosphate. In extracellular enzyme production, 10 strains (50%) were found to have the ability to produce protease, 15 strains (75%) produced ammonia and 16 strains (80%) produced cellulase and catalase (Table 1).

Table2. Bacterial strains showing the PGP and extra cellular activity

Strains name	Phosphate solubilization	Ammonia	Catalase	Amylase	Cellulase	IAA	Protease	Siderophore
CSA01	+ 0.1cm	+	+	+++ 1.9cm	+++ 1.2cm	-	+++ 1.1cm	-
CSA06	+ 0.2cm	+	+	+ 0.4cm	+ 0.2cm	-	+++ 1.0cm	+
CSA09	+ 0.1cm	+	+	+++ 1.2cm	++ 0.9cm	+	+++ 1.3cm	+
CSA21	+ 0.4cm	-	+	-	++ 0.5cm	-	-	-
CSB01	+ 0.2cm	+	-	+++1.0cm	+++ 1.0cm	-	+++ 1.1cm	-
CSB06	-	+	+	-	+ 0.2cm	+	-	+
CSB18	++ 0.7cm	+	+	+ 0.1cm	-	+	-	+
CSB22	++ 0.7cm	+	+	-	-	-	-	+
CSC03	+ 0.1cm	+	+	+ 0.1cm	+ 0.4cm	+	++ 0.9cm	+
CSC04	+ 0.3cm	-	-	+ 0.1cm	+ 0.3cm	-	-	-
CSC12	++ 0.6cm	-	+	-	-	-	-	+
CSC14	-	-	+	+ 0.3cm	+++ 1.2cm	+	-	+
CSD01	-	+	-	++ 0.9cm	+ 0.1cm	-	++ 0.5cm	+
CSD06	+++ 1.5cm	+	+	-	-	-	-	-
CSD09	+ 0.3cm	+	+	+ 0.2cm	+ 0.2cm	+	++ 0.7cm	+
CSD17	+ 0.1cm	+	+	+ 0.1cm	+ 0.1cm	-	++ 0.7cm	+
CSE01	+ 0.3cm	+	+	++ 0.5cm	+ 0.1cm	+	++ 0.9cm	+
CSE04	+ 0.2cm	+	+	+ 0.3cm	+ 0.2cm	+	-	+
CSE05	-	+	+	+ 0.4cm	+ 0.4cm	+	-	+
CSE08	+ 0.2cm	-	-	++ 0.7cm	+++ 1.1cm	-	++ 0.9cm	-

- no active, + (0.3-0.5cm), ++ (0.6-0.9cm), +++ (>1cm)

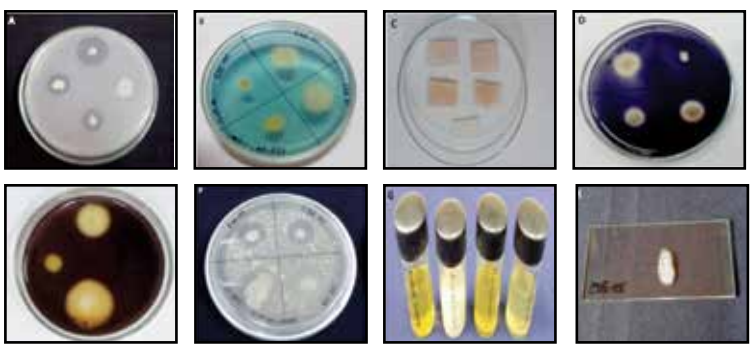

Figure: A. Phosphate solubilization; B. Siderophore production; C. Indole acetic acid production (IAA); D. Amylase production (Starch hydrolysis); E. Cellulase production; F. Protease production; G. Ammonia production; H. Catalase production.

Phosphorus is one of the most essential nutrients required by plants. But the amount of phosphorus available to the plants is very less due to availability of phosphorous in insoluble form in most of the soils. Few rhizobacteria have the potential to convert the insoluble form of phosphorous to soluble form which helps the plants to intake in the required form. Our results revealed that 16 out of 20 strains could solubilize the ticalcium phosphate on Pikovskaya's agar among which four bacterial strains (CSD06, CSB18, CSB22 and CSC12) found highly efficient in P solubilization as they produces zone of above 1 cm.

PGPR can enhance the root architecture and promote plant development with the production of different phytohormones like IAA, gibberellic acid and cytokinnins (Kloepper et al. 2007). Those bacterial strains could produce IAA in the rhizospheric soil plays a significant role in increasing the root surface area and number of root tips in many plants (Han et al. 2009). From our studies it is revealed that 9 strains (CSA09, CSB06, CSB18, CSC03, CSC14, CSD09, CSE01, CSE04 and CSE05) out of 20 strains could produce IAA.

In aerobic environments, iron occurs principally as Fe3+ which plant cannot utilized directly. But bacteria could acquire iron by the secretion of low-molecular mass iron chelators referred to as siderophores which have high association constants for complexing iron. Plants assimilate iron from bacterial siderophores by means of different mechanisms, for instance, chelate and release of iron, the direct uptake of siderophore-Fe complexes, or by a ligand exchange reaction (Schmidt, 1999). From our studies it is revealed that 14 strains (CSA06, CSA09, CSB06, CSB18, CSB22, CSC03, CSC12, CSC14, CSD01, CSD09, CSD17, CSE01, CSE04 and CSE05) could produce siderophore production.

The production of extra cellular enzymes is another important criterion for the plant growth promoting rhizobacteria (Pal et al., 2001). These enzymes directly acts on deleterious plant pathogens or acts as an precursor for the induction of systemic resistance inside plant cells against pathogens thereby indirectly helps in increase of plant growth and yield. Our results revealed that, the strains CSA01, CSA09 and CSB01 could produce amylase followed by the strains CSD01, CSE01 and CSE08. For cellulose production, the strains CSA01and CSC14 were found best further followed by the strains CSE08, CSB01, CSA09 and CSA21. Similarly the zone formation in skim milk agar tests revealed the strains CSA09, CSB01, CSA01 and CSA06 were found best in protease production.

The overall results revealed that the five *Bacillus* strains collected (CSA01, CSA09, CSC03, CSD09 and CSE01) from rhizosphere regions of organically grown coconut plantations of Andaman Islands were found efficient in all the PGP properties tested. Hence this multi potential PGP Bacilli from coconut rhizosphere could be utilized as potential bioinoculants with suitable formulations and delivery systems under coconut based organic intercropping.

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