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RESEARCH ARTICLE

EVALUATION OF PLANT GROWTH PROMOTING POTENTIALS EXHIBITED BY RHIZOBACTERIA ASSOCIATED WITH BEANS PLANT

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ABSTRACT

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Plant growth promoting rhizobacteria (PGPR) isolated from the rhizosphere of velvet Beans plant grown on the agricultural research farmland of Federal University of Technology, Owerri, were evaluated for their growth promoting potentials. The four isolates: *Micrococcus sp*, *Bacillus sp*, *Corynebacterium sp*, and *Enterococcus sp*, were evaluated for plant growth promoting abilities, such as phosphate solubilization, indole acetic acid (IAA), ammonia (NH3), and Hydrogen Cyanide (HCN) production. *Micrococcus sp*, *Bacillus sp*, and *Enterococcus sp* produced IAA, while *Corynebacterium sp*, and also *Bacillus sp* and *Enterococus sp* solubilized phosphate. All the isolates were able to produce HCN and NH3. Rhizobacteria associated with beans plant constitute important sources of potentially beneficial microorganisms with plant growth promoting activity or antagonistic effects against phytopathogens. The results obtained in this study suggests that these rhizobacteria possess multiple plant growth promoting attributes which can be applied as biofertilizers or as biocontrol agents in agriculture, to improve plant growth and productivity.

KEYWORDS

Biofertilizer, rhizobacteria, rhizosphere, phytopathogens.

1. INTRODUCTION

Based on a study, the rhizosphere is that region of the soil where plant roots and microorganisms interact with each other [1-3]. This zone contains different plant root exudates such as amino acids and sugars, which serve as nutrients for the colonizing bacteria [4]. The rhizosphere soil is richer in nutrients than the bulk soil, and the interactions between the plants and microorganisms help to make the soil fertile and in turn improve plant growth and yield. According to research, bean plants are leguminous plants, and are known to have symbiotic interactions with nitrogen fixing bacteria, which help to improve soil fertility [5].

Rhizobacteria, also known as plant growth promoting rhizobacteria are bacteria that colonize the roots of plants, forming symbiotic associations with them. Studies have shown that plant roots supply energy sources for these microorganisms, which in turn reciprocate by affecting plant growth and yield in several ways [6]. The different mechanisms suggested for plant growth promotion include: nitrogen fixation, production of phytohormones: auxins, gibberellins, cytokinins, ethylene production, solubilization of phosphorus, oxidation of sulfur, increase in nitrate availability, extracellular production of antibiotics, lytic enzymes, hydrocyanic acid, increases in root permeability, competition for root sites and available nutrient, suppression of diseases caused by pathogenic rhizobacteria; increased nutrient availability and absorption, etc. [7].

Several studies have identified that plant growth promoting rhizobacteria can affect plant growths by either direct or indirect mechanisms [8]. Direct plant growth promoting mechanisms include: atmospheric nitrogen fixation, insoluble phosphate solubilization, secretion of hormones such as indole acetic acid (IAA), gibberellins, and kinetins beside ACC (1-Aminocyclopropane-1-carboxylic acid) deaminase production, which regulates ethylene. Indirect plant promotion mechanisms include: induced systemic resistance (ISR), antibiosis, competition for nutrients, parasitism and secretion of metabolites such as hydrogen cyanide and siderophores, that are suppressive to pathogenic rhizobacteria.

The aim of this study was to isolate possible rhizobacteria from the rhizosphere of growing velvet bean plant and evaluate them for multiple plant growth promoting attributes.

2. MATERIALS AND METHODS

2.1 Study Area

The farmland soil of the School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State, Nigeria (5.3905°N, 6,9907°E.)

2.2 Collection of Samples

Bean plants growing on the research farmland soil of School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State, Nigeria were randomly selected and gently uprooted and the rhizosphere soils (soils around the bean plants) were collected in sterile polythene bags, bulked and taken to the laboratory immediately for analysis.

2.3 Microbiological Analysis

Based on a study, one (1) gram of rhizospheric soil of the bean plant was serially diluted and cultured on appropriate media [9]. Appropriate dilutions were plated on Nutrient Agar and incubated at 37°C for 24 hours to obtain discrete colonies and pure cultures of the organisms.

2.4 Evaluation for Plant Growth Promoting Potentials

2.4.1 Hydrogen Cyanide Production

Bacterial isolates grown on Nutrient Agar had the lids of the plates placed

with filter paper strips previously saturated in 0.5% picric acid solution and 2% sodium carbonate solution. The culture plates were incubated in inverted positions over the lids at 37°C for 24 hours. A color change of the filter paper from deep yellow to reddish or orange brown was noted.

2.4.2 Ammonia Production

Production of ammonia by the isolates was tested in peptone medium. Freshly grown pure cultures of the isolates were grown for 48 – 72 hours at 28°C, and Nesseler's (0.5ml) reagent added. Development of yellow to brown color was noted.

2.4.3 Indole Acetic Acid Production

Cultures of the isolates on Jones medium were incubated for 24 hours at 28°C. They were centrifuged at 350 rpm for 15 minutes and 2ml of Salkowski reagent added to 2ml of the supernatant, shaken and incubated in the dark at room temperature for 30 minutes. They were observed for color changes and results recorded.

2.4.4 Phosphate Solubilization

The isolates were grown on Pikovskya's medium for 48 – 72 hours at room temperature. They were observed for halo zones around the colonies which indicated phosphate solubilization and results recorded.

3. RESULTS

3.1 Microbiological Analysis

The bacteria isolated from the rhizospheric soil of velvet bean were identified as *Micrococcus* sp, *Bacillus* sp, *Corynebacterium* sp, and *Enterococcus* sp based on cellular morphology, microscopic features, biochemical characteristics and carbohydrate utilization.

3.2 Evaluation of Plant Growth Promoting Potentials

The plant growth promoting potentials of the rhizobacteria isolated from velvet bean are shown in Table 1 below

Table 1: Plant Growth Promoting Potentials of the Bacterial Isolates

Isolates	HCN	NH_3	IAA	PO_4
Micrococcus sp	++	+	+	-
Bacillus sp	++	++	+	++
Corynebacterium sp	+	++	-	+
Enterococcus sp	++	+	+	+

Key: **HCN**: Hydrogen Cyanide Production
IAA: Indole Acetic Acid Production
NH_3: Ammonia Production
PO_4: Phosphate Solubilization
+: Positive
-: Negative

Note: The positive reaction capacity is shown by the number of (+) symbols,
i.e. + = positive; (++) = strongly positive.

4. DISCUSSION

Based on a research, the four bacteria isolated from this study had multiple plant growth promoting potentials. This is in agreement with previous reports by a researcher that plant growth promoting rhizobacteria are usually nonpathogenic to the plant, and also increase the plant's yield by one or more mechanisms [10]. These mechanisms may be direct or indirect and help facilitate rooting and growth of plants generally. Direct mechanisms of plant growth promotion were observed, since all the organisms produced ammonia (NH_3); and three of the four isolates produced indole acetic acid and solubilized phosphate. *Micrococcus* sp, *Bacillus* sp and *Enterococcus* sp produced IAA, except *Corynebacterium* sp. Moreso, *Bacillus* sp, *Corynebacterium* sp and *Enterococcus* sp were able to solubilize phosphate (Table 1.0). Indirect plant growth promoting mechanism was also exhibited through the production of hydrogen cyanide which play important roles in the suppression of pathogenic rhizobacteria, thereby improving plant growth and health. All the isolates: *Micrococcus*sp, *Bacillus* sp, *Corynebacterium* sp *and Enterococcus* sp were able to produce hydrogen cyanide. *Bacillus* sp and *Enterococcus* sp had all the plant growth promoting attributes studied. This corroborates reports of a researcher in his list of some notable plant growth promoting rhizobacteria genera[10].

According to research, plant growth promoting rhizobacteria (PGPR) can be applied as biofertilizers to improve plant yield and growth [11]. According to research, *Bacillus* sp have been used previously to improve yield in non-leguminous crops such as sugar beet, sugar cane, rice, maize and wheat [12]. The production of indole acetic acid (IAA) or auxins affect the plant root by increasing its size, weight and number of branches, as well as surface area in contact with the soil. This helps in nutrient uptake, improving plant nutrition and growth capacity [13].

The production of hydrogen cyanide by all the four isolates highlights their potential for use as biocontrol agents. Cyanide is a phytotoxic agent and is a characteristic of rhizobacteria deleterious to others and helps plants in disease suppression by pathogenic microorganisms in the rhizosphere. This could also form control for weeds and reduce their negative effects on the growth of desired plants [14-16].

5. CONCLUSION

Plant growth promoting rhizobacteria (PGPR) affect plant growth and health through several mechanisms, directly or indirectly. The rhizosphere of beans plant is endowed, by implication, with valuable microbiota that have potentials as biofertilizers, as well as biocontrol agents which could be employed in sustainable agriculture, thereby improving food security and safeguarding our environment from pollution that may result from the use of inorganic fertilizers and chemical pesticides.

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