Original Research Article

Isolation And Evaluation Of Bacteria Exhibiting Multiple Plant Growth Traits In The Rhizosphere Of Yellow Bell Pepper (*Capsicum chinense*)

ABSTRACT

AIM; The study identified and evaluated bacteria exhibiting multiple plant growth traits in the rhizosphere of yellow Bell Pepper (*Capsicum chinense*)

Comment [s1]: Use :

Study Design; Seeds of *Capsicum chinense* were planted in a soil and allowed to grow. After some weeks of planting, soil samples from the rhizosphere were collected and the bacterial community present in the rhizosphere soil of *Capsicum chinense* was studied. The isolated organisms were assessed for their ability to produce plant growth promoting traits.

Comment [s2]: Mention exact number

Place and duration of study: This study was carried out at an agricultural research farmland in the Federal University of Technology, Owerri, Nigeria.

Methodology: Seeds of *Capiscum chinense* were planted in the soil samples in a greenhouse. Rhizosphere soil was collected for analysis to identify the bacterial composition of the rhizosphere soil.

Results: In this study the presence of *Bacillus cereus*, *Staphylococcus aureus*, *Corynebacterium sp*, *Enterococcus feacalis* and *Bacillus polymyxa* were evident in the rhizosphere samples collected. All isolates showed multiple plant growth promoting traits except Staphylococcus aureus which was positive for hydrogen cyanide production only.

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Conclusion: The results from this study showed that the bacterial community present in the soil can be used to effect significant vegetative crop yield and agricultural production. The isolated rhizobacteria can be formulated as bio-fertilizers or bioinnoculants, etc.

1. INTRODUCTION

Peppers are an important source of nutrients in human diet [1,2]. The Capsicum peppers, including sweet peppers, bell peppers, hot peppers like jalapeño cayenne, serrano, cherry and many others [3] are the most worldwide cultivated [4] and are widely appreciated in the culinary as spice. These peppers are characterized by their high levels of vitamin C (ascorbic acid), provitamin A (carotene) and calcium. Ingestion of 50-100 g fresh pepper fruits can provide about 100% and 60% of the recommended daily amounts of vitamin C and A, respectively. Ripe fruits of pepper are also rich in compounds with antioxidant and anticancer activity[5].

A major factor influencing plant growth and health is soil fertility which also determines the microbial population living both in the rhizosphere and as endophytes within healthy plant tissues. Soil fertility refers to the amount of nutrients present in the soil capable of supporting plant life [6] and largely depends on micro and macro nutrients and micro and macro organisms.

Soil microorganisms are very important in almost every chemical transformation taking place in the soil. They play an active role in maintaining soil fertility as a result of their involvement in the nutrient synthesis and circulation. The presence of these microorganisms in the rhizosphere of the soil largely counts for the microbial community present in the soil.

Microbial population in and around the roots includes bacteria, fungi, yeast etc. Some are free living while others form symbiotic relationships with the various plants [7]. The community structure of soil microorganisms in the rhizosphere differs from that in the non-rhizosphere soil largely due to the biological interactions between the microorganisms and the roots of the plants [8].

These biological interactions accounts for plant growth and improved soil fertility. The bacterial community can be seen to synthesize nutrients and compounds that can be used to enhance plant growth. Plant Growth Promoting Bacteria characterized with their fast metabolism and growth are always readily colonising the root surface [7]. This makes them suitable as biofertilizers, seed treatments and as biocontrol agents.

This study was aimed at evaluating the plant growth promoting traits of bacteria isolated in the rhizosphere of *Capsicum chinense*.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the Research Farmland of the School of Agriculture and Agricultural Technology, Federal University of Technology, Owerri, Imo State, Nigeria.

2.2 Collection of Samples.

2.2.1 Soil sample

Soil samples were randomly collected from an uncultivated portion of the farmland to a depth of 15-30cm below the surface. The collected soil samples were bulked to form a composite sample and 5 kg each was measured and stored in separate polythene bags in which the pot planting experiment was carried out.

2.2.2. Collection of yellow bell pepper seeds

The yellow pepper seeds were obtained in sealed plastic bags from Imo Agricultural Development Program [ADP] Centre, Owerri, Imo state, Nigeria.

2.3 Planting of Seed

Capsicum chinense seeds (5 seeds per bag) were planted in the bags containing soil samples collected at random from the farmland and allowed to grow for five weeks.

2.4. Isolation of microorganisms

The rhizopheric soil samples of growing yellow bell pepper seeds were aseptically collected and introduced into different sterile test tubes, properly labelled and taken to the laboratory for Microbiological investigation. Isolation of microorganisms was carried out by using spread plates method according to Cheesbrough [8] .The nutrient agar plates were incubated at $37^{o}C$ for 24 hours. The culture plates were observed for the growth of microorganisms.

2.5. Identification of Microorganisms

The bacterial isolates were identified by using cultural, morphological and biochemical characteristics as described by Cheesbrough [9]

2.6. Evaluation of plant growth promoting traits

IAA production: IAA production by the isolates was estimated by using Salkowaskis reagent. Appearance of pink color was indicating IAA production which can be read at 535nm [10].

Phosphate solubilization activity: All bacterial isolates were then screened for inorganic phosphate solubilization. Qualitative estimation was done by using Pikovskaye medium containing tri-calcium phosphate, iron phosphate. Positive results can be recorded by formation of clear halo zone around the culture [11]

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Hydrogen cyanide production

The production of HCN was detected by spreading 1 ml of 24 h old broth culture on the King's B medium supplemented with 4.4g/l glycine and incubated with the Whatmann filter paper flooded with the solution containing 0.5% picric acid in 2% sodium carbonate. After 24-48 h, yellow to brown change in the color of the filter paper was observed [12]

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Ammonia production

All the bacterial isolates were tested for the production of ammonia using Nessler's reagent. Production of ammonia can be detected by formation of faint yellow to dark brown color [13]

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3. RESULTS AND DISCUSSION

The increasing importance of beneficial bacteria in agriculture has resulted in many efforts to isolate and identify bacteria associated with the soil and rhizosphere of plants, in order to identify their roles in plant growth promotion and protection against pathogens. The application of PGPR is a potentially attractive approach to disease management and improved crop productivity in sustainable agriculture.

Bacterial analysis of the rhizosphere soil showed the presence of mostly Gram-positive organisms. Results in Tables 1 and 2 reveals the morphological and biochemical characteristics of the bacterial isolates. The bacterial isolates were *Bacillus cereus*, *Bacillus polymyxa*, *Enterococcus faecalis*, *Corynebacterium* sp., and *Staphylococcus aureus*.

This implies that the microorganisms that were isolated from the plant rhizosphere are pathogenic and potentially toxin-producing microorganisms which can lower the quality of Yellow pepper plants and can also be responsible for causing pepper diseases.

Similar work by Hanna et al. [14] also revealed the isolation of *Pseudomonas* spp., and *Bacillus* spp. *Bacillus* spp., and *Pseudomonas* spp., are the most frequently reported genera of PGPR [15,16,17].

The plant growth promoting characteristics viz., IAA production and ARA were examined with the ten selected PGPR isolates. Table 3 shows the plant growth promoting potentials of the bacterial isolates. All isolates showed multiple Plant Growth Promoting (PGP) trait except *Staphylococcus aureus* which was positive for the production of Hydrogen Cyanide only.

Hydrogen cyanide production was found to be the most frequent trait exhibited by *Bacillus cereus* and *Enterococcus feacalis* while Ammonia production was exhibited mostly by *Bacillus cereus, Bacillus polymyxa and then Corynebacterium* sp. Bacterial plant growth promotion is a well-established and complex phenomenon that is often achieved by the activities of more than one PGP trait exhibited by plant isolated bacteria [18].In this study, 80% of the isolates exhibited more than two PGP traits which may promote plant growth directly, indirectly or synergistically. Similar to these findings, multiple PGP activities among Plant Growth Promoting Rhizobacteria have been found in some bacteria including species of *Pseudomonas, Azospirillum* sp,, *Azotobacter* sp, *Serratia* sp etc. and have been reported to enhance plant growth [19]. Hartmann et al. [18] had reported that some studies suggest that PGPR enhances the growth, seed emergence, crop yield and contribute to the protection of plant against certain pests and pathogens as well as nutrient availability.

Indole Acetic Acid is effective in root growth and development, fruit growth and development, apical dominance and flowering [7]. Similar studies have shown that IAA production is very common among PGPR [20,21,22,23,24,17]. The production of producing hydrogen cyanide by some of the isolated rhizobacteria, which several studies have attributed a disease protective effect, is a very strong indication of the biocontrol potentials of these organisms. This is similar to how phosphorus solubilizing bacteria like *Bacillus cereus* and *Enterococcus faecalis* are effective in increasing the plaint available phosphorus in the soil as well as the growth yield of crops [25]. A review by Kucey et al. [26] had emphasized on the ability of some phosphorus solubilizing microbes to stimulate phytopathogen biocontrol that affect plant growth via the production of siderophores, hydrolytic enzymes and HCN. Most of the isolates from this study tested positive to the production of ammonia. Ammonia production has been reported as another key trait that significantly increases the crop vegetative growth and yield [27].

Table 1 Showing cell morphology and microscopic characteristics of bacterial isolates.

Colony code	Cell morphology	Mot	Gram Stain	Spore	Flagellum	Capsule	Probable identity
YPB1	Dull, dry serrated cream colonies	+	+R	+	+	-	*Bacillus cereus*
YPB2	Serrated with medusa head	-	+R	+	-	-	*Bacillus polymyxa*
YPB3	Moist and shiny cream colonies	-	+S	-	-	-	*Enterococcus sp.*
YPB4	Dull, dry umbonate cream colonies	-	+R	-	-	-	*Corynebacterium sp.*
YPB5	Golden yellow colonies	-	+S	-	-	-	*Staphylococcus sp.*

Key: Mot= Motility, - = Negative, += Positive, +R= Positive Rod, +S= Positive Spherical, YPB= Yellow Pepper Bacterial Isolate

Table 2 : Biochemical and carbohydrate fermentation test of bacterial isolates

Colony	Cat	Oxi	Coag	Ind	MR	VP	Cit	TSI	NO_3	Ure	Glu	Suc	Lac	Fru	Mal	Mann	Identity 0f Isolates

Code																	
YPB1	+	-	-	-	-	+	+	-	+	+	+	-	-	-	-	-	*Bacillus cereus*
YPB2	+	-	-	-	-	-	+	-	-	-	+	+	-	-	+	-	*Bacillus polymyxa*
YPB3	-	-	-	-	+	-	+	-	-	-	+	+	+	+	-	+	*Enterococcus feacalis*
YPB4	+	-	-	-	-	+	+	-	+	-	+	-	-	-	+	-	*Corynebacterium sp.*
YPB5	+	-	+	-	-	+	-	-	+	+	+	+	+	+	+	+	*Staphylococcus aureus*

Key; Cat=Catalase, Coag= Coagulase, Oxi=Oxidase, Ind=Indole, MR=Methyl Red, VP=Voges Proskauer, Cit = Citrate Utilization, Ure= Urease Production, NO_3=Nitrate Production, TSI= Triple Sugar Iron Test, Glu= Glucose, Suc=Sucrose, Mal=Maltose, Lac=Lactose, Mann=Mannitol, Fru=Fructose.

Table 3 showing plant growth promoting potentials of the bacterial isolates

Isolates	HCN	NH_3	IAA	PO_4
Bacillus cereus	++	++	+	++
Bacillus polymyxa	+	++	+	+
Enterococcus feacalis	++	+	+	+
Corynebacterium sp.	+	++	-	+
Staphylococcus aureus	+	-	-	-

Key; HCN= Hydrogen Cyanide Production, NH_3= Ammonia Production, IAA= Production of Indole Acetic Acid, PO_4= Phosphate Solubilisation,

+ = Positive, - = Negative

Note: The positive reaction intensity is indicated by the number of (+) symbols.

5. CONCLUSION

The use of PGPR inoculants to improve agricultural production is a dynamic process and one with a wide range of capabilities. This study isolated bacterial isolates that demonstrated PGPR traits. These soil microbes are active elements for soil development and in the long run pushes for sustainable agricultural practices. Taken together, these results suggest that PGPR are able of inducing the production of IAA, solubilization of phosphorus and resistance to fungal pathogens, thereby improving growth of plants. The potentials of these strains may be applied to enhance the growth and yield of yellow bell pepper. Due to the diverse nature of the PGPR strains, instead of using one strain, two or more strains with multiple PGP traits can be used as biofertilizer which is an efficient approach to replace chemical fertilizers and pesticides for sustainable pepper cultivation. Further investigations, including efficiency test under field conditions, are needed to ascertain the role of PGPR as biofertilizers that exert beneficial effects on the plant growth and development.

COMPETING INTERESTS. There are no competing interests

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