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SIDEROPHORE PRODUCED BY *BACILLUS SHACKLETONII. GN-09* AND SHOWED ITS PLANT GROWTH PROMOTING ACTIVITY

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ABSTRACT

A level of at least one micromolar iron is needed for optimum growth. These environmental restrictions and biological imperatives have required that microorganisms form specific molecules that can compete effectively with hydroxyl ion for the ferric state of iron, a nutrient which is abundant but essentially unavailable.ABacillus shackletonii.GN-09 siderophore producing bacteria was isolated from rhizospere soil of groundnut field in Solapur region, Maharashtra. The Chrome Azurol S (CAS) assay was performed to detect the siderophore production. In CAS plate assay, the dark medium produce bright zone with yellowish fluorescent color. Arnow's assay and Csaky's assay were used. Checked its phosphate solubilizing activity on Pikovaskaya medium. Moreover, Bacillus shackletonii.GN-09 isolate enhanced seed germination, root length and shoot length of soyabean under pot culture condition. These results showed that siderophore producing bacteria could increase the germination power of agricultural plants.

KEY WORDS

Siderophore, Csaky assay, Arnowassay, Seed germination, Phosphate solubilisation.

INTRODUCTION

Iron is essential for almost all life, essential for processes such as respiration and DNA synthesis. Free available iron, in aerobic environments, is present at a concentration of approximately 10-18M at pH-7 (Biedermann, G., and P. Schindler, 1957). The low solubility of external iron presents a problem for microorganisms living in an aerobic environment and having an absolute requirement for iron. To combat this low solubility, most bacterial and fungal species have high-affinity iron transport system. A siderophore (Greek - iron carrier) is an iron chelating compound secreted by microorganisms. Iron Fe^{3+} ions have a very low solubility at neutral pH and therefore cannot be utilize by organisms. Siderophore dissolve these ions by chelation as soluble Fe^{3+} complexes that can be taken up by active transport mechanisms. Siderophores produced by rhizosphere bacteria may enhance plant growth by increasing the availability of iron near the root or by inhibiting the colonization of roots by plant pathogens or other harmful bacteria. The role of these compounds is to scavenge iron from the environment and to make the mineral, which is almost always essential, available to the microbial cell (AlexanderD. B. and ZubererD. A., 1991). Siderophores production by bacteria is considered as an important component of bacterial machinery for iron sufficiency and likely to be more important for survival and growth in the competitive soil environment, which is usually deficient in soluble iron (Khan *et al.*, 2006). Free living soil and rhizosphere bacteria that are beneficial to plants are often referred to as plant growth promoting rhizobacteria (PGPR). (Glick, 1995; Kloepper, 1989). PGPR promote

growth of several annual crops by increased uptake of nitrogen (Bakker *et al.*,1991) ironthrough siderophore(Sindhu*et al.*,1997) and phosphorous (Linderman,1992) and by controlling plant disease (Glick *et al.,*1994; Xie*et al.,* 1996). Present work is focused on the detection, purification and paper chromatograhic analysis of purified siderophore from a*Bacillus shackletonii.GN-09* isolated from rhizospheric soil of groundnut field also checked its plant growth activity on soyabean seeds.

MATERIAL AND METHODS

Isolation and identification of *Bacillus shackletonii.GN-09:*

Rhizosphere soil of Ground nut field was collected from Solapur region, Maharashtra. Nutrient medium used for the isolation of bacteria. About 1gram of soil was taken and serial diluted to 10^{-6}.Then 1ml of sample from 10^{-5} dilution was transferred to sterilePetri dishes containing the nutrient agar and plates were then incubated at room temperature for 24 hrs. Siderophore producing bacteria was detected by Siderophore assay. The promising bacteria were characterized morphologically as Gram positive rods and identified by 16r RNA analysis.

Preparation of Deferrated medium:

Modified Succinic acid medium (Meyer and Abdullah,1978) was taken in a separating funnel and washed with equal volume of chloroform containing 1.5% 8-hydroxyquinoline and the traces of hydroxyquinoline was removed by washing thrice with chloroform to obtain deferrated medium. The medium was then autoclaved at 15 lbs for 15 min. This deferrated media was used for siderophore detection.

Siderophore assay:

Siderophore production was assayed by the plate method using Chrome Azurol S (CAS) (Schwyn and Neilands, 1987). Isolates were enriched on Succinate medium and enriched broth were centrifuged and supernatant was poured in the well of plate containing dye as an indicator for formation of bright yellowish orange zone in dark blue medium indicates the siderophore production.

Determination of Siderophore type:

Two specific assays, *viz.*Arnow's assay for catecholate type (Arnow, 1937**)** and Csaky's assay for hydroxymate type (Csaky, 1948) were performed to determine the type of siderophore produced by the organism.

Paper chromatography analysis:

Purified siderophore was subjected to analyse by paper chromatography using the solvent system Butanol : Acetic acid : Water (12:3:5) and these were compared with standard desferrisoxamine and 2,3di-hydroxy benzoic acid for hydroxamateand catecolate respectively

Influence of *Bacillus shackletonii.GN-09* on Soya bean germination and growth:

Soyabean seed were surface sterilized using 0.1 % (W/V) $HgCl_2$ followed by three washing with sterile distilled water. Sterilized seeds were mixed / immersed for 10 min insiderophore rich broth of *Bacillus shackletonii. GN-09* grow in Succinate medium for 48 hrs. Seeded pots were irrigated with sterile water after every 48 hrs to maintain the moisture necessary for the germination of seed. Observations like increase in root length, shoot length and rate of germination, were recorded after 15-20 days of sowing.

P-Solubilization:

Phosphate solubilization test was conducted qualitatively by plating the bacteria in agar containing precipitate tricalcium phosphate.The Pikovaskaya medium was used for this test. Promising bacterial culture was streaked on the surface of Pikovaskaya medium. The presence of clearing zone around bacterial colonies after 24 hrs incubation indicate positive Phosphate solubilization.

RESULT AND DISCUSSION

Many isolates were isolated from Groundnut field with standard isolate of *Pseudomonas spp.* Out of seven isolate, GN-09 was showed good siderophore production activity than other isolates.

Isolation and Identification:

The organism was isolated from rhizospheric soil of Groundnut and it was identified by using biochemical tests and 16s rRNA analysis technique.The result of biochemical tests showed the isolate was Catalasa positive and Oxidase positive. The isolate was reduced nitrate to nitrite and hydrolyse starch and Gelatin. The phylogenetic tree was developed by using MEGA 4.0 (**Figure 1**)

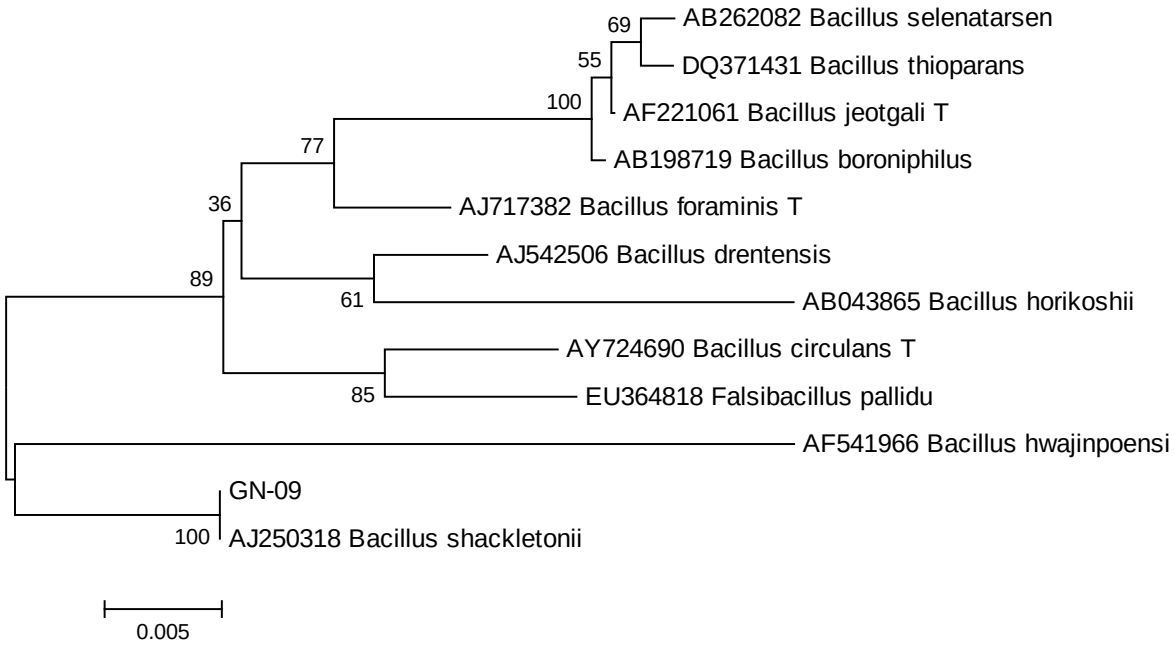

Figure 1: Phylogenetic tree of *Bacillus shackletonii GN-09.* Phylogenetic analysis of 16s rRNA gene sequence of *Bacillus shackletonii GN-09.* The present number at the nods indicate the levels of bootstrap support based on neighbor-joining analyses of 1000 replicates.The scale bar(0.005)indicates the genetic distance.

Siderophore assay:

Promising Bacteria was grown on modified succinate medium and this bacterium was plated in CAS containing medium and this bacteria showed the formation of bright zone with yellowish fluorescent colour in the dark medium was detected as production of siderophore.

Determination of Siderophore type:

There was no positive result for Arnow's assay and Csaky'sassay showed a positive result for Hydroxymates type of siderophore as the supernatant gave strong Csaky'spositive test.

Paper chromatography analysis:

The purified siderophore was subjected to analysis by paper chromatography using the solvent system Butanol : Acetic acid : Water (12:3:5) with standard (2,3 di-hydrobenzoic acid) and purified siderophore showed Hydroxymate type of siderophore, this analysis further indicated hydroxamate type nature of siderophore.

P-Solubilization:

This test was carried on Pikovaskaya medium and these bacteria after incubation showed clear zone around growth of organism.

Influence of *Bacillus shackletonii.GN-09* on Soya bean germination and growth:

It showed 20% more germination power in soyabean seeds as compared to control and also increased its root length and shoot length. **(Table: 1).**

Table 1: Influence of *Bacillus shackletonii.GN-09* inoculation on Soya bean growth

Treatment	Root length		Shoot length		%Seed germination
	(mm)	(Increased in mm)	(mm)	(Increased in mm)	
Control	35.0	-	30.7	-	30
Test	42.0	07	70.9	40.2	50

CONCLUSION

The present study deals with the siderophore producing microorganism on CAS agar plates from the rhizosphere soil of groundnut field. From these isolates *Bacillus shackletonii.GN-09*showed maximum siderophore production. Siderophore producing bacteria are good candidates to be used for plant growth promotion, especially in neutral to alkaline soil (Edi Husen, 2003). P-solubilizing bacteria have good phosphate prospect to improve plant growth especially in the soil, with large amount of precipitation. The experimental results also showed that siderophore producing bacteria are involved in plant growth promotion of soyabean. However, further research is needed to elucidate in detail the mechanism of action of this strain and their compatibility with other components in integrated management of soyabean diseases.

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