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OPTIMIZATION STUDIES ON PLANT GROWTH PROMOTING BACTERIAL ISOLATES FROM CHILLI RHIZOSPHERE

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Abstract:

Plant growth promoting bacteria are known to influence the plant growth by various direct or indirect mechanisms. In search of efficient PGPR strains with multiple activities, a total of 10 bacterial isolates belonging to *Bacillus* species isolated from chilli rhizosphere, Guntur district of Andhra Pradesh, India. Out of ten only one isolate *Bacillus* sp. PB-3 showed potential activity of producing IAA, Gibberellic acid production, Siderophore production. *Bacillus* sp. PB-3 showed the maximum IAA (85 µg/ml) and Gibberellic acid productions 52 µg/ml were observed. An orange halo appears around the *Bacillus* species on CAS agar media indicates the siderophore production. The optimum conditions for siderophore production in *Bacillus* sp. PB-3 was selected for this study. CAS medium was supplemented with mannitol (18.6 µg/ml) and Urea (18.4 µg/ml) was used as carbon and nitrogen sources showed maximum amount of siderophores. These plant growth promoting abilities can make this isolate a potential PGPR candidate for its application in sustainable agriculture.

Keywords: Gibberellic acid, Indole Acetic Acid, Siderophore

Introduction:

In agriculture plant growth promoters play a major role for sustainable production. Bacteria and fungi also play a key role in agriculture. The genus *Bacillus* sp. stand out as one of the main genus of PGP used to promote of plant growth. Rhizospheric microorganisms produce vitamins, antibiotics, plant hormones, synthesis of vitamins, amino acids, auxins, cytokinins and gibberellins which stimulate plant growth and antagonism with potential plant pathogens and communication molecules that all encourage plant growth. Plant growth hormones (IAA and

Gibberellic acid) were produced in chemically defined medium using the bacterial strains Bacillus cereus, *Bacillus Licheniformis, B. pumilus and B. subtilis.* Bacterial species like *Bacillus pumilus* and *Bacillus licheniformis*, isolated from the rhizosphere of *Alnus glutinosa* L. Gaertn., both have strong growth promoting activity (Probanza *et al*., 1996). Although it was soon found that both were auxin producers (Gutierrez and Manero *et al*., 1996), the characteristics of the induced growth are also suggestive of GA-like promotion.

More than 80% of the bacteria isolated from the rhizosphere can produce (IAA) Indole Acetic Acid (Khalid *et al*., 2004) as secondary metabolite by obtaining tryptophan either through root exudates or from the proteins released by the dead bacteria cells (Patten and Glick, 1996). The metabolites obtained from microorganisms. Indole-3-acetic acid (IAA), a plant hormone compound, is a natural auxin produced by plants, bacteria, fungi and a diverse group of organisms. Indole Acetic acid is a metabolite derived from tryptophan by many tryptophan dependant and tryptophan independent mechanism in plants and bacteria. There is more than one mechanism could be present in a bacteria (Pattern and Glick, 2002). Siderophores are broadly grouped into two main categories, viz. phenolates and hydroxamates. Bradyrhizobia and rhizobia infecting Cicer, Cajanus, Vigna, Leucrena, Medicago, cluster bean, peanut and Acacia are known to produce species-specifics siderophores of hydroxamate, catechols and organic acid type as well as some unknown types of siderophores (Dudeja *et al*. 1997).

Materials and methods

Isolation

One gram representative soil sample was suspended in 10 ml of sterile distilled water and shaken thoroughly for 10 minutes. Microorganisms were isolated from collected samples by the serial dilution plate technique using Nutrient Agar Medium (NAM). Serial dilutions up to 10^{-5} of each sample were prepared by using sterilized water (Sneath, 1986). Sample dilutions were plated (in triplicates) on NAM and incubated at 35° C for 24 to 48 h. Pure Colonies were picked and maintained on NAM slants at 4° C and further assessed for enzyme production in liquid medium.

Indole Acetic acid production

To determine the production of IAA, culture suspension of the strain was inoculated in starch tryptone broth supplemented with 0.5% L-Tryptophan, adjusted to initial pH 7.0 and incubated for 48 h, at 30°C. After incubation, culture broth was centrifuged at 10,000 rpm for 20 min. The supernatant (5 ml) was mixed with 1ml of IN HC1 and 4 ml of Salkowski reagent and observed for the development of pink colour. Optical density was read at 530 nm in a spectrophotometer (Sinha and Basu, 1991). Standard Graph was prepared by using increasing concentration of authentic IAA (Gordon and Weber 1951).

Gibberellic acid production:

To determine the production gibberellic acid, the culture suspension was inoculated into starch broth supplemented with 60 mM concentration of mevalonic acid, adjusted with initial ph 7.0 and incubated at 30° C for 48 h. After incubation culture broth was centrifused 10000 rpm for 20 min. The supernatant was acidified to pH 2.5 with HCl and extracted using liquid-liquid (Ethyl acetate/NaHCO3) extraction (Cho *et al*., 1979). Gibberellic acid in the ethyl acetate phase was measured by UV spectrophotometer at 254 nm (Bruckner Blechschmidt, 1991).

Siderophore production:

Siderophore production by the plant growth promoting bacteria was estimated by the method described by Schwyn and Neilands (1987). Siderophore production was indicated by orange halos around the colonies after the incubation.

Siderophore assays

For the detection of siderophore production in Bacillus sp. PB-3 was grown on the medium containing 0.5 µM of iron, and incubated for 24 h on rotary shaker at 200 rpm at room temperature. A clear orange hallo zone around the colonies appears on Chrome Azurol S (CAS) agar medium which indicate the siderophore positive.

Chrome Azurol S (CAS) Agar medium

For the detection of siderophore, Bacillus sp. PB-3 isolate was grown in synthetic medium, containing 0.5 µM of iron and incubated for 24 h on a rotary shaker at 200 at 30° C. Culture supernatant was added to the wells made on the CAS agar plates and incubated at room temperature for 24 h. Formation of yellow to orange coloured zone around the well indicates siderophore production (Schwyn and Neiland's, 1987). All glassware used to store stock solution of the medium were treated with concentrated HNO_3 and left to overnight. After 24 h, the acid was removed and the glassware was rinsed thoroughly with double distilled water.

Optimization for Siderophore production

Various factors like effect of iron concentration, effect of carbon and nitrogen sources influence the siderophore production. Quantitative estimation of siderophore production was done by using spectrophotometer (480 nm).

Effect of incubation period

For the production of siderophore different incubation periods (24, 48, 72, 96, 120, 144 and 168 h) were carried out in this study.

Effect of iron concentration on Siderophore production

In this experiment Different concentrations (10, 20, 30, 40 and 50 µM) of iron (Fecl3) was determined by growing the rhizobacteria in the basal medium upto 168 h of incubation for siderophore production.

Effect of carbon sources on Siderophore production

To study the siderophore production by using different carbon sources (Mannitol, glucose, sucrose, Succinate and citrate) were studied. The rhizobacteria were inoculated in the basal medium for 168 h of incubation and estimated the siderophore production.

Effect of nitrogen sources on Siderophore production

To study the siderophore production by using different nitrogen sources (ammonium sulphate, sodium nitrate, urea, glutamine and glycine) were replaced with 0.1% yeast extract. The rhizobacteria were inoculated in the basal medium for 144 h of incubation and estimated the siderophore production.

Results and discussion

Total of 10 isolates were obtained from chilli rhizosphere in the vicinity of Guntur, Andhra Pradesh. The preliminary characterization like cultural and biochemical characteristics of rhizobia was done by Bergey's manual of systemic bacteriology. All the isolates belong to Bacillus species according to their preliminary and biochemical studies. . All the isolates were designated as Bacillus sp. PB-1 to Bacillus sp. PB-10 and tested for plant growth promoting study (Table-1). All the isolated showed that the IAA, gibberellic acid and Siderophore productions. Isolate Bacillus sp. PB-3 showed maximum IAA and gibberellic acid productions. Tryptophan is the main precursor of IAA biosynthesis (Patten and Glick, 1996). Lee *et al*., (2004) have reported that L- tryptophan was more active for IAA production, though bacteria were able to produce IAA in absence of tryptophan (Jayaprakashvel *et al*., 2014).

Table-1 Plant growth promoting characteristics of Bacillus species isolated from chilli rhizosphere

Isolates	Indole Acetic Acid production (µg/ml)	Gibberellic acid production (µg/ml)	Siderophore production
Bacillus sp. PB-1	-	**22.4**	-
Bacillus sp. PB-2	-	**13.8**	-
Bacillus sp. PB-3	**85.0**	**52**	+
Bacillus sp. PB-4	**38.2**	-	-
Bacillus sp. PB-5	**45.0**	-	-
Bacillus sp. PB-6	**33.8**	-	-
Bacillus sp. PB-7	-	-	+
Bacillus sp. PB-8	-	**16.7**	+
Bacillus sp. PB-9	-	-	+
Bacillus sp. PB-10	**35.1**	**22.8**	-

Effect of incubation period on siderophore production

Siderophore production started after 24 h of incubation time showed by Bacillus sp. PB-1. Maximum zone was observed after 168 h of incubation (Table-2). Maximum production was obtained at 144 h of incubation. Further increase in incubation period there is no change in the production.

Table 2: Effect of incubation period on siderophore production by Bacillus sp. PB-3

Incubation periods	Siderophore production µg/ml
24	**2.40**
48	**5.20**
72	**11.8**
96	**14.4**
120	**19.6**
144	**23.0**
168	**23.0**

* The overall model is significant with $p<0.05$

Effect of iron concentration

Iron concentration influences the siderophore production by bacillus species. Siderophore production was increase with increasing iron concentrations 10 µM to 50 µM (Table-3). Generally the siderophore production was observed in the iron restricted medium. Iron stressed conditions lead to production of strong iron-chelating agents such as siderophores (Diaz *et al*., 2002).

Table - 3.Effect of iron concentration hydroxamate type on Siderophore production

Iron concentrations (µM)	Siderophore production µg/ml
10	**2.40**
20	**5.20**
30	**11.8**
40	**19.6**
50	**13.0**

* The overall model is significant with $p<0.05$

Effect of carbon sources

Among the 5 carbon sources tested, maximum siderophore concentration was observed in Glucose containing medium, Bacillus sp. PB-3 showed the maximum siderophore production with mannitol containing the medium (18.6 µg/ml) (Table-4). The amount and the type of the siderophore produced by an organism depend on the availability of organic and inorganic nutrients (Neilands 1982; Abd-Alla, 1998). Glucose and Mannitol proved the most suitable carbon source for hydroxamate type of siderophores in *Pseudomonas aeruginosa*, *Aspergillus nidulans*, *Pseudomonas chrysogenum* and *Bradyrhizobium japonicum* (Mahmoud and Abd- Alla, 2001).

Table 4: Effect of carbon sources on siderophore production by Bacillus sp. PB-3

Carbon sources	Siderophore production µg/ml
Control	**1.20**
Mannitol	**18.6**
Glucose	**14.0**
Sucrose	**16.8**
Succinate	**11.4**
Citrate	**8.20**

* The overall model is significant with $p<0.05$

Effect of Nitrogen sources

Siderophore production by the tested microorganisms was affected by different nitrogen sources (Table -5). According to this Urea proved to be the most suitable nitrogen source for Bacillus sp. PB-3.

Table 5: Effect of nitrogen sources on siderophore production by Bacillus sp. PB-3

Nitrogen sources	Siderophore production µg/ml
Control	**3.0**
Ammonium sulphate	**14.2**
Sodium nitrate	**14.6**
Urea	**18.4**
Glutamine	**12.0**
Glycine	**13.2**

* The overall model is significant with $p<0.05$

CONCLUSION

The present strains *Bacillus* sp. PB-3 isolated from chilli rhizospheres showed that indole acetic acid, Gibberellic acid and siderophore production. For Optimization studies CAS medium was supplemented with mannitol and urea as carbon and nitrogen sources showed maximum siderophore production. This isolate may useful for biocontrol, and is evaluated to improve of siderophore production in agriculture fields.

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