RESEARCH PAPER

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Study on direct mechanism of growth promotion of soybean

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Nine isolates of soil bacteria were tested *in vitro*. for direct mechanism for the growth promotion of soybean. Parameters assessed was siderophore production. Siderophores production was tested by CAS assay as well as types of siderophores were also determined wether it was hydroxymate or catachole type.. Five isolates, NM/S1/CA, NM/S4/NA, NM/S5/NA, NM/R2/RA, NM/R3/RA showed siderophore production.

Key words : PGPR, Growth promotion, Siderophores, IAA, Soybean

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INTRODUCTION

The microbe-plant interaction in the rhizosphere can be beneficial, neutral, variable or deleterious for plant. Rhizobacteria that can exert beneficial affects on plant development are termed plant growth promoting rhizobacteria (PGPR) (Kloepper and Schroth 1978). The term rhizobacteria is used for bacteria that aggressively colonize the rhizosphere (Subbarao, 1999).It is evidenced from the previous studies conducted in different laboratories that rhizobacteria are attached to seed and root exudates by chemotaxis which rightly said as first step in seed and root colonization.

Although the mechanisms by which PGPR promote plant growth are not yet fully understood, many different traits of these bacteria are responsible for growth promotion activities (Cattelan *et al.,* 1999). Initially, *Azotobacter* and *Azospirillum* were believed to promote plant growth due to their ability to fix dinitrogen. Later it was known that other plant growth stimulating hormones such as IAA was also involved (Kennedy, 1998). The use of p-solubilizing bacteria was reported to increase plant growth in some cases, but in other cases it was not. It indicated that other mechanisms may involve in growth response (De Freitas, *et al.* 1997).

Plant growth promoting rhizobacteria enhance plant growth by direct or indirect mechanisms (Glick *et al.,* 1995). Plant growth promoting rhizobacteria enhance plant growth by producing different metabolites which are responsible for the growth promotion of plants by direct mechanism, such as production of plant hormones like indoleacetic acid (IAA), gibberellic acid, cytokines, ethylene and production of siderophores. Production of various acids which can solubilize phosphate and make it available to the plants (Edi Husen, 2003).

Present Investigation was aimed to assess the potential of bacterial isolates to promotes the growth of soybean by direct mechanism.

RESEARCH METHODOLOGY

All the chemicals used for the present research work were procured from Glaxo, Mumbai and Hi Media Pvt. Ltd. Mumbai. The glassware used here of Borosil make and were cleaned with 6 N HCl, rinsed with distilled water and oven dried before use.

Microorganisms NM/S1/CA, NM/S3/NA, NM/S4/NA, NM/S5/NA, NM/S6/NA, NM/S7/NA, NM/S8/CA, NM/R2/RA, NM/R3/RA were isolated from rhizospheric soil of soybean from different locations and were tested for their ability to produce siderophores.

Study of direct mechanism for plant growth promotion :

Siderophore production :

For siderophore production, Iron restricted succinate

medium (Meyer and Abdullah, 1978) was taken in (100 ml) flask and inoculated with previousely developed inoculums at 5 per cent (V/V) level. This flask was incubated on shaker at 120 rpm for 48 hrs. After 48 hours of incubation a 5 per cent (V/V) from this culture was inoculated in fresh sterilized succinate medium and incubated on shaker at room temperature as stated above. Like this 3-4 transfers were made to ensure that the secondary metabolism is triggered. Further the culture was centrifuged at 8000-10,000 rpm for 20 min. The cell free supernatant was analyzed for presence of siderophore by universal CAS assay (Meyar and Abdallh, 1978). The supernatant was analyzed for qualitative as well as quantitative assays.

Qualitative analysis :

For qualitative analysis equal amount of cell free supernatant (1ml) and CAS reagent was mixed in a acid cleaned and oven dried test tubes. Along with test, a control was also kept where equal amount of uninoculated succinate medium (0.5-1ml) and CAS reagent was reacted and observed for decolorization in colour of CAS reagent.

Quantification of siderophore production :

One ml of culture supernatent was mixed with equal amount of CAS assay solution. Uninoculated medium was used as reference. The optical density at 630nm was measured for loss of blue colour resulting from siderophore production. Siderophore produced was calculated by using formula, (Bendale *et al*., 2010)

per cent decolorization units = [(Ar-As)/Ar] × 100

where,

Ar - Absorbance of reference at 630 nm

As – Absorbance of sample at 630 nm

Detection of siderophore type

Type of siderophores (*i.e*. either hydroxamate or catecholate) was determined by Csaky assay (For hydroxamates) and Arnows's test (catecholate/ phenolates).

Csaky's assay :

For detection of hydroxamates 1 ml supernatant of culture was hydrolysed with 1 ml of 6N H2SO4 in a boiling water bath/6hrs or130^{0}C/30mins. To this, 3ml Na-acetate for buffering, 1 ml sulfanilic acid and then 0.5 ml iodine soln were added. After 3- 5mins, excess iodine was destroyed with 1ml of Na-arsenite soln. 1 ml of alpha napthalamine was then added and water was used to make up volume to 10 ml. Colour was allowed to develop for 20-30mins. Absorbance was measured with the help of uv-vis spectrophotometer at 526nm. (Meyar and Abdallh, 1978).

Arnow's assay :

For detection of catheclates 1 ml culture supernatant was mixed after each orderly addition,1 ml HCl followed by 1 ml nitrite-molybdate (catechols produces yellow colour) and then 1 ml NaOH (color changes to red). Colour was stable for 1hour and absorbance was measured at 510 nm using a uv-vis spectrophotometer (Meyar and Abdallh, 1978).

RESULTS AND ANALYSIS

In present investigation five isolates, NM/S1/CA, NM/S4/NA, NM/S5/NA, NM/R2/RA and NM/R3/RA showed siderophores production. In CAS assay the colour was changed from blue to orange. The intensity of colour can be related to the siderophores concentration. The two major groups of siderophores, hydroxymate and catachol yielded highly coloured complexes (Table 1).

Table 1: Qualitative analysis for siderophore production

Culture name	siderophore production
NM/S1/CA	+ ve
NM/S3/NA	-ve
NM/S4/NA	+ve
NM/S5/NA	+ve
NM/S6/NA	-ve
NM/S7/NA	-ve
NM/S8/NA	-ve
NM/R2/RA	+ve
NM/R3/RA	+ve

In quantitative analysis for siderophore NM/S1/CA, NM/R2/RA, NM/R3/RA showed higher production of siderophore. Siderophore production was calculated by using the method of Bendale *et al*., (2010). In CAS assay, NM/ R2/RA, NM/R3/RA have been produced highest per cent of decolourization units. 70.58 per cent. NM/S5/NA also showed good production 68.62 per cent (Table 2).

Table 2:Quantitative analysis for the siderophore production in succinate medium incubated at room tempereture

Culture name	Reference optical density(620nm)	Optical density (620nm)	%decolo-rizing units
NM/S1/CA	0.51	0.12	76.470
NM/S4/NA	0.51	0.24	52.941
NM/S5/NA	0.51	0.16	68.627
NM/R2/RA	0.51	0.15	70.588
NM/R3/RA	0.51	0.15	70.588

Table 3 : Quantitative analysis of siderophores by Csaky assay

Sr.No.	Culture name	O. D. at 526 nm	Concentration in µg/ml according to std. curve
1.	NM/S1/CA	0.27	73 µg/ml
2.	NM/S3/NA	-	-
3.	NM/S4/NA	-	-
4.	NM/S5/NA	-	-
5.	NM/S6/NA	0.26	63 µg/ml
6.	NM/S7/NA	0.29	90 µg/ml
7.	NM/S8/NA	0.30	100 µg/ml
8.	NM/R2/RA	-	-
9.	NM/R3/RA	0.21	20 µg/ml

Type of siderophores (*i.e.* either hydroxamate or catecholate) was determined by Csaky assay (for hydroxamates) and Arnows's test (catecholate/ phenolates). In Csaky assay five isolates showed positive result. Highest concentration of siderophores production that is 100µg/ml was produced by NM/S8/NA. NM/S7/ NA (90µg/ml) NM/S1/CA (73 µg/ml)(Table 3). In Arnows assay two isolates showed positive results (Table 4).

Table 4 : Quantitative analysis of siderophores by arnows assay

Sr.No.	Culture name	O. D. at 510 nm	Concentration in µg/ml according to std. curve
1.	NM/S1/CA	-	-
2.	NM/S3/NA	-	-
3.	NM/S4/NA	-	-
4.	NM/S5/NA	-	-
5.	NM/S6/NA	-	-
6.	NM/S7/NA	0.028	16µg/ml
7.	NM/S8/NA	-	-
8.	NM/R2/RA	-	-
9.	NM/R3/RA	0.032	20 µg/ml

Coclusion :

From the above results and discussion, it is concluded that microorganisms NM/S1/CA, NM/S3/NA, NM/S4/ NA, NM/S5/NA, NM/S6/NA, NM/S7/NA, NM/S8/CA, NM/R2/RA, NM/R3/RA, isolated from rhizospheric soil of soybean are efficient for the production of the metabolites such as IAA, phosphate solubilizers, siderophore.

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