Research Paper :

Screening of Rhizospheric Bacteria as Growth Promoter and Biocontrol of Fungal pathogen of Groundnut and Soybean

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SUMMARY

Rhizospheric bacteria were isolated from soil of different crop plants and identified and characterized as a *Rhizobium, Azotobacter, Azospirillum,* and *Pseudomonas* . All isolates were able to inhibit the growth of fungal pathogens of groundnut and soybean by producing siderophore. There was also considerable increase in height, weight and germination period of seedling of groundnut and soybean by seed inoculated with rhizospheric bacteria.

Key words : Rhizospheric bacteria, Biocontrol, Soybean, Groundnut

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Rhizospheric bacteria promote the growth of crop plants, by synthesis of plant growth regulators auxin, gibberellin and ethylene etc. siderophore, HCN and antibiotics (Arshad and Frankenberger, 1992).Indole acetic acid (IAA) is one of the most physiological active auxins produced by several plant growth promoting rhizobacteria (Frankenberger and Brunner, 1983), which increase plant growth by solubilizing phosphate (DeFreitas *et al*., 1997). They suppress the growth of deleterious microorganisms by production of siderophore, antibiotics, and cyanide (Edi, 2005).Siderophore is an iron chelating compound produced by microorganisms under iron stress condition, which results in elimination of fungal pathogens by iron starvation in soil (Arora *et al.*, 2001).

The phytopathogenic fungi cause number of diseases such as charcol rot, dry rot, wilt, in crop plants. To control these phytopathogens, chemical management is not feasible, as pathogens are both seed and soil borne. Biocontrol can thus offer a very good alternative for management of the pathogens. Therefore, rhizospheric bacteria were isolated which promote growth of plant and inhibit the growth of soil and seed borne phytopathogens.

MATERIALS AND METHODS

The rhizospheric bacterial strains used in this study were isolated from rhizospheric soil of different crops and identified by growing on selective media *i.e. Azotobacter* on Ashby's agar medium, *Azospirillum* on Nitrogen free nutrient agar, *Rhizobium* on Yeast extract manitol agar and *Pseudomonas* on Citramide agar. Plant pathogenic fungi *i.e.*, *Alternaria alternata, Aspergillus flavus, Fusarium oxysporum, Macrophomina phaseolina,* were isolated from the diseased seeds of groundnut and soybean by blotter technique (DeTempe, 1963). These pathogen were identified by using standard literature of mycology (Alexopoulos, 1962).

Siderophore assay:

Siderophore production by the different rhizospheric bacteria was tested by chromo azural S (CAS) assay (Schwyan and Neilands, 1987). Siderophore production was also checked by the top layer method. The strains were spread over citramide agar and incubated for 48h at 30^0C. After incubation, a thin layer of CAS reagent in 0.7% agar was spread on the bacterial growth and plates were again incubated for 24h at 30^0C, formation of yellow orange zone around the colonies indicates siderophore production (Carson *et al*., 1992). The type of siderophore produced by rhizospheric bacteria was determined by growing on Succinate medium (Meyer and Abdullah, 1978) containing g/l succinic acid 4,

K_2HPO_4 6, KH_2PO_4 3, $(NH_4)_2SO_4$ 1, $MgSO_4$ 0.2, and PH, 7.0. These strains were inoculated and incubated on rotatory shaker incubator for 36h at 28^{0}C. A 5ml culture supernatant was harvested by centrifuging the culture at 10,000 rpm in cooling centrifuge at 4^{0}C for 10min and supernatants were used. The presence of hydroxamate siderophore in the supernatants was checked according to Casky method (Gillan *et al*., 1981) by taking hydroxamate as a standard.

Effect of siderophore on fungal plant pathogen:

The identified fungal plant pathogens were grown on Potato dextrose agar media and incubated for 8 days to get profuse growth of selected fungi, thereafter 10mm diameter dish of each fungus was obtained by cork which was placed at the centre of plate containing spread siderophore supernatant of different rhizobial strains. Then the plates were incubated for 36h at 28^{0}C and the liner growth in the form of diameter of the fungal growth was measured and compared with control. Per cent inhibition was determined by comparing reduction in fungal growth in relation to control.

Indoleacetic acid production:

To determine indoleacetic acid production, the isolate was grown on Pikovaskya's medium supplemented with 0.01% of tryptophan as a precursor of IAA (Gaur, 1990) on rotatory shaker at 180rpm for 24h at 28^{o}C. A culture supernatant was harvested by centrifuging the culture at 15000 rpm for 30 min. Indoleacetic acid produced by bacteria was assayed colorimetrically by using ferric chloride-perchloric acid reagent $FeCl_3$-$HClO_4$. (Gordon and Weber, 1951).

Phosphate solubilization:

Phosphate solubilization ability of isolate was determined by growing them on modified Pikovaskya's medium (Sundara Rao and Sinha, 1963). After 24 h of incubation, the presence of clearing zone around bacterial colonies which indicate the positive phosphate solubilization.

Effect on growth promotion of crop:

Each rhizospheric bacterial cells were harvested by cooling centrifuge and coated on the surface sterilized seeds with 0.1% $HgCl_2$ of groundnut and soybean (approximately 10^8/g) by slurry method (Samasegaran and Hoben, 1994). After drying for 90 minutes, the seeds were kept in the plates containing wet Whatmann filter paper. After 6 days of incubation period, the height, weight of seedlings and germination period of seeds was measured and compared with control.

RESULTS AND DISCUSSION

All isolated and identified rhizospheric bacteria showed siderophore production on Chromo azurols agar by developing yellow to orange coloured zone around the colonies on agar plates. The type of siderophore was determined by Casky assay where all isolates showed hydroxamate type siderophore but the amount was varied in *Pseudomonas*. This produced maximum hydroxamate type siderophore as shown in Table 1.

Table 1 : Hydroxamate type siderophore production by Rhizospheric bacteria

Bacterial strains	Hydroxamate mg/l
Azotobacter	21.0
Azospirillum	20.0
Rhizobium	38.0
Pseudomonas	51.0

These strains were then used to study for biocontrol of fungal pathogens. It was found that *Pseudomonas* showed maximum inhibition of fungal pathogen, *Azospirillum* showed least inhibition and *Rhizobium* and *Azotobacter* showed intermediate inhibition of these fungal pathogens as shown in Table 2.

The ability of IAA production and to solubilize precipitated phosphate was positive in all the isolated rhizospheric bacteria. The plant growth promoting effect of rhizospheric bacteria revealed that *Rhizobium* sp. showed faster germination of seed and increase in height, weight of seedlings of groundnut and soybean as compared to other rhizospheric bacteria shown in Table 3.

These observation are similar to the findings of Farah *et al*. (2005) in case of siderophore and IAA production

Table 2: Inhibition of fungal pathogen by Rhizospheric bacteria

Fungal pathogen	Inhibition (%)			
	Pseudomonas	*Rhizobium*	*Azotobacter*	*Azospirillum*
Alternaria alternata	70	64	61	30
Aspergillus. flavus	72	60	*60*	28
Fusarium oxysporum	58	65	61	27
Macrophomina phaseolina	55	64	62	32

Table 3: Effect on growth promotion of groundnut and soybean crop

Plant	Treatment	Germination time (hr)	Height of seedling after 6 days in (cm)	Weight of seedlings after 6 days in (mg)
Groundnut	Groundnut + *Azotobacter*	23	1.8	0.20
	Groundnut + *Azospirillum*	21	1.8	0.22
	Groundnut + *Rhizobium*	20	2.2	0.27
	Groundnut + *Pseudomonas*	30	1.7	0.20
	Control	48	1.00	0.19
Soybean	Soybean + *Azotobacter*	18	2.2	0.30
	Soybean + *Azospirillum*	16	2.5	0.32
	Soybean + *Rhizobium*	12	3.2	0.35
	Soybean + *Pseudomonas*	20	2.0	0.28
	Control	24	1.5	0.25

and phosphate solubilization (Edi, 2003). Biocontrol and growth promotion ability of rhizospheric bacteria were studied by Arora *et al.*. (2001) and Kloepper *et al*. (1980).

The isolated rhizospheric bacteria promote the plant growth by producing IAA as a phytoharmone by solubilization of phosphate. They inhibit the growth of fungal pathogen by producing hydroxamate type of siderophore in soil. Thus, rhizospheric bacteria not only act as a plant growth promoter but also proved as a good biocontrol agent.

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