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Research Article

USE OF *RHIZOBIUM* AND *BACILLUS* AS BIOFERTILIZER

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ABSTRACT

Rhizobium and ***Bacillus sp.*** **isolated from agricultural soil were used to prepare biofertilizer and when applied to the seeds, seed germination was improved to a considerable extent and also protected from plant diseases due to antifungal substances produced by them.**

Key words: Biofertilizer, *Rhizobium*, *Bacillus.*

INTRODUCTION

Biofertilizer or microbial inoculants are live microorganisms used for improving plant growth. Rhizobia fix atmospheric nitrogen increasing the production of inoculated crops and also leave a good amount of nitrogen in the soil thus benefiting the subsequent crops (Crozat *et al*., 1982; Tiwari, 2010*)*. Rhizobia are able to colonize the rhizosphere, infect legume roots and biologically fix nitrogen in the soil through symbiotic process. *Rhizobium* bacteria stimulate the growth of leguminous plants and fix atmospheric nitrogen into the soil by interacting symbiotically with leguminous plants, using the nitrogenase enzyme complex. In addition, *Rhizobium* strain secrete growth hormones like indole acetic acid and other enzymes which shows positive influence on plant growth and also plays an important role in the formation and development of root nodules (Nutman, 1965; Kiers *et al*., 2003*)*.

Bacillus species are found in soil. Phosphate-solubilizing bacteria solubilize inorganic soil phosphates, such as Ca3(PO4)2, FePO4, and $AlPO_4$, through the production of organic acids and siderophores (Jones, 1998; Chen *et al.*, 2006; Rodríguez *et al.*, 2006, 2007; Sharma *et al.*, 2007) and promote plant growth (Ahmad *et al*., 2009). Singh *et al*. (2014) evaluated phytase producing bacteria for their plant growth promoting activities. The present work was undertaken to evaluate these two microbes for their biofertilizer abilities.

MATERIAL AND METHODS

Isolation of *Rhizobium* and *Bacillus* from soil

Soil samples were collected from the field and kept in sterilized and air tight condition. The media used for growth were

YEMA media, Pikovaskaya's media and Nutrient agar/broth. The isolate were purified through streak plate technique.

Characterization of Rhizobium and bacillus isolates

Biochemical tests like IMViC, catalase, amylase, urease and various sugar fermentation tests-lactose, glucose and sucrose were done as per Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Plant growth promoting activity

The plant growth promoting activities of recovered isolates were studied by qualitative screening of their phosphorous solubilization, IAA and HCN production.

Phosphate Solubilization

Bacterial culture was inoculated on Pikovaskaya's agar plate and incubated at 30˚C for 3-4 days and then observed for a clear zone around the colony of isolates which is indicative of positive test result.

Indole acetic acid (IAA) production

Bacterial culture (*Rhizobia* and *Bacillus*) was inoculated in 5 ml of nutrient broth containing 100µgml^{-1} and 200µgml^{-1} of L-tryptophan and incubated at 30˚C for 48 h; fully grown cultures were centrifuged at 8000 rpm for 10 min. The supernatant (2ml) was mixed with two drops of orthophosphoric acid and 4 ml Salkowski reagent (50ml, 35% of perchloric acid, 1ml 0.5 M $FeCl_3$ of solution). Presence of pink color indicates IAA production and yellow color indicates negative reaction.

HCN Production

Rhizobia and *Bacillus* was streaked on plates containing YEMA and Pikovaskaya media with glycine (1.4 gL^{-1}). Whatman No. 1 Filter paper strips was soaked in 0.5% picric acid and 2% sodium carbonate and placed in the lid of each Petri plates and then Petri plates were sealed with parafilm and incubated at 30˚C for 1-4 days. Uninoculated control was kept for comparison of results. Plates were observed for change of color of filter paper from yellow to dark brown.

Seed Germination Test

Seeds were soaked in sulphuric acid for 5 min washed 3 times with sterile distilled water and then were treated with bacterial strain (*Rhizobium* and *Bacillus* species) for 30 min while control was not treated. Untreated were placed on agar in sterilized condition then kept for germination in dark at 27˚C and after 7 days result were recorded.

Antibacterial activity

Rhizobium and *Bacillus* culture was spread over the surface of agar plate and bacterial cultures were poured in wells followed by overnight incubation at 37˚C. The plates were examined for growth inhibition.

Antifungal activity

Bacterial culture was spread over the surface of potato dextrose agar plates and after 2-4 days incubation at 30˚C, the plates were inoculated with PDA plugs cut from 48h fungal cultures. Control plates were inoculated only by fungi. Plates were incubated at 25˚C and examined for evidence of fungal growth inhibition.

Biofertilizer production

Submerged culture technique was employed for growing bacteria on mass scale. The carrier was mixed with calcium carbonate to neutralize pH (6.5-7.0) and mixed with 10% water before sterilization.

Coconut shell powder, coal powder, leaves and cow dung was added into the soil sample, mixed properly using sterilized gloves and then *Rhizobium* and *Bacillus* was added, mixed properly and stored at a cool place for 4-7 days. It was the applied into the earthen pots. Two pots each for two plants, soybean and maize which favor *Rhizobium* and *Bacillus* to grow in their roots were maintained. Uninoculated biofertilizer pots served as control.

RESULTS AND DISCUSSION

On YEMA, the colonies of *Rhizobium* were white circular, round structure (Figure 1) and all members of *Bacillus* species produced smooth, pin pointed white to creamish colonies (Figure 2).

Table 1. Effect of biofertilizer on growth (cm) of maize and soybean plants.

Plant	Biofertilizer	Group	Days after seeding			
			5	10	20	30
Maize	*Bacillus*	treated	-	19.5	55	63
		control	-	17	52	60
Maize	*Rhizobium*	treated	-	19.5	30	59
		control	-	16	27	54
Soybean	*Bacillus*	treated	-	35	42.5	75.5
		control	-	30	37	59.5
Soybean	*Rhizobium*	treated	-	8.4	29	70
		control	-	7	27	52

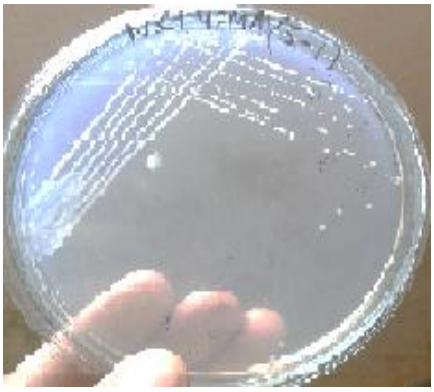

Figure 1. Pure culture of *Rhizobium*.

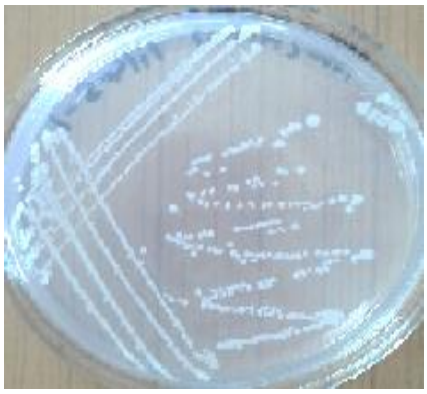

Figure 2. Pure culture of *Bacillus*.

Biofertilizer as a biocontrol agent

In the present study, *Rhizobium* and *Bacillus* were successfully isolated from the soil sample. *Rhizobium* and *Bacillus* colonies grown on YEMA and Pikovaskaya media produced non capsulated cultures as observed by Ahmad and Ahmad (2008). *Rhizobium* and *Bacillus* were recovered and characterized by various biochemical tests like IMViC, catalase, amylase, urease test

etc. They were also subjected to various sugar fermentation tests and were found positive to lactose, glucose and sucrose confirming the bacterial species which corroborate the results of Geetha *et al* (2014).

The present work confirmed the occurrence of nitrogen-fixing bacteria in the soil sample. In plant roots, a large variety of materials are released to their surrounding soil, including various sugars, amino and organic acids, alcohol and vitamins. However, it was observed that these species were able to grow on glucose, galatose, mannitol, lactose and sucrose. Phosphorus solubilizing bacteria are reported to dissolve insoluble phosphates by production of inorganic or organic acids and/or by the decrease of the pH. Most of the previous reports stated that calcium phosphates are dissolved by acidification. The isolates were found to produce siderophore, indole, ammonia, solubilize phosphate and also able to grow on nitrogen free media indicating that it may have ability to fix atmospheric nitrogen.

It is well known that Phosphate Solubilising Bacteria in soil solubilize insoluble phosphates mainly by secreting acids into the medium (Chung *et al*, 2005). *Rhizobium* and *Bacillus* produce IAA in various amounts (Barbieri *et al*., 1986). Our results showed that isolated strains were efficient IAA producers. Our results indicated that the inoculation of *Rhizobium* and *Bacillus* increased the plant growth, which is in conformity of the report of Ahmad *et al*., (2009).

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