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ORIGINAL ARTICLE

ISOLATION, BIOCHEMICAL CHARACTERIZATION AND IDENTIFICATION OF PHOSPHATE SOLUBILIZING PSEUDOMONAS FROM RHIZOSPHERE OF SOYBEAN AND STUDY OF ITS ANTIMICROBIAL ACTIVITY

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Abstract:

Rhizosphere is the region where soil & roots makes contact. The microbial growth is enhanced by nutritional substances released from plant tissues. It is highly favorable habitat for proliferation and metabolism of numerous microbial types. Next to nitrogen phosphorus is most important plant nutrient. Microorganisms influence phosphorus availability to plant through the process of mineralization & immobilization. Pseudomonas species live in a commensal relationship with plants. Pseudomonas species have ability to produce different types of metabolites like antibiotics which are harmful to plant pathogens. This practice is known as bio-control. In the present work screening of phosphate solubilizing Pseudomonas from rhizosphere of soybean was done by using selective media. Further study was done on Pikovaskys medium. After incubation at room temperature for 4-6 days clear zone (Zone of solubilizzation) were observed around the colony. The quantification of phosphorus solubilizing ability has been evaluated as PSE. Based on morphological, physiological & biochemical tests, Pseudomonas species were identified. It was observed that two isolates of Pseudomonas PSPF-02 & PSPF -05 shows high PSE. The isolates of Pseudomonas were further tested for antimicrobial activity. It was found that Bacillus subtilis NCIM2010; E .coli NCIM2064; Klebsiella pneumoniae NCIM 2719 were highly sensitive while C. albicans NCIM3557; Fusarium oxysporium (soil isolate) Xanthomonas compestris NCIM2956 were less sensitive to the isolates of Pseudomonas.

KEYWORDS:

Phosphate solubilizer, rhizosphere, Pseudomonas, antimicrobial activity.

INTRODUCTION

The term rhizosphere was introduced by German scientist Hilther (1904). It is the region where soil and roots makes contact. It is a unique subterranean habitat for microorganisms. The microbial growth is enhanced by nutritional substances released from plant tissues e.g. amino acids, vitamins and other nutrients. The growth of plant is also influenced by the products of microbial metabolism that are released into the soil. The rhizosphere represents a tremendously complex biological system. There are several beneficial microorganisms in the rhizosphere, which can improve soil quality, enhance crop production and protection, conserve natural resources and ultimately create more sustainable agricultural production and safe environment .Some microbes can produce metabolites that enter plant tissues and induce defense against other plant pathogen. Rhizobacteria may present a front line of defense for plants against pathogens, biotic and a biotic stresses. The nature and practice strategies for detection and characterizing systems for biological control of plant and soil born pathogen have been elucidated earlier (Cook & Baker, 1983; Hoitink et al 1983; Downer et al 2001). They are becoming increasingly important component in the

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management of nutrients and harmful microorganisms in sustainable crop production. Phosphorus is the most important plant nutrient. The organic phosphorus in soil is unavailable to plant. The inorganic phosphorus which is unavailable to plants is solubilized by many microorganisms into solution .Such bacteria are abundant on root surfaces of plants & account for the proliferation & metabolisms of numerous types of microorganisms. Bacteria, fungi & actinomycetes play an important role in solubilizzation of phosphates. Microbial involvement in the solubilization of inorganic phosphate was shown by Stalstrom (1903), who observed solubilizzation of tricalcium phosphate when it was inoculated with milk cultures & soil infusions

Biocontrol of plant pathogen is an attractive alternative to chemical pesticides, which cause environmental pollution and development of resistant strain. With increasing awareness of environmental hazards bio based technologies for sustainable agriculture & bio- pesticide can provide long lasting effective solution (Mc Envoy, 1989). Chemical control of soil borne plant diseases is frequently ineffective because of the physical & chemical heterogeneity of the soil, which may prevent effective concentrations of the chemical from reaching the pathogen. Biological control agents colonize the rhizosphere, the site requiring protection & leave no toxic residues, as opposed to chemicals. Micro organisms have been used extensively for the biological control of soil borne plant diseases as well as for promoting plant growth. Chemical fertilizers & herbicide application in conventional agriculture also affect negatively the potent ional for tap-down control of above ground pests possibly due to reduced microbial diversity. They also change interactions within & between below & above ground components, ultimately promoting negative environmental impacts of agriculture by reducing internal biological cycles & pest control. On the contrary organic farming fosters microbial & faunal decomposers and thus propagates into the above ground system via generalist predators, thereby increasing biological control. Today's main problem is the development of antibiotic resistance among pathogens (both human & plants).So there is continuous need of new antibiotics producing strains. In the present study screening of Pseudomonas species from rhizosphere soil of soybean cultivated in Latur area was done. Then its phosphate solubilizing efficiency & antimicrobial activity was studied.

MATERIALS & METHODS

Collection of rhizosphere soil:

10 soil samples were collected from different rhizosphere region of soybean cultivated in Latur area in polythene bags and bought to laboratory within 12 hours & stored at low temperature until microbiological analysis.

Isolation & identification of Pseudomonas species:

1.0 gm of soil sample was added in 99 ml of sterile distilled water. Then different dilutions of it were prepared. 1 ml suspension from each dilution (1/10000) was plated on Kings B agar medium (Peptone -20gm, glycerol -10ml, K2HPO4-1.5gm, MgSO4.7H2O -1.5 gm, Agar- 25 gm) and plates were incubated at 25-30 ºC for 24-48 hrs. Pseudomonas was identified by morphological, cultural & biochemical properties.

Study of Phosphate solubilization activity:

The 10 isolates of Pseudomonas were spot inoculated on Pikovskaya's agar (M520 Hi-media) plates containing: Glucose 10.0 gram, Tri calcium phosphate – 5.0 gm, Ammonium sulphate-0.50 gm, Potassium chloride-0.20 gm, Magnesium sulphate - 0.10 gm, Manganese sulphate – 0.0001 gm, Ferrous sulphate- 0.0001 gm , Yeast extract- 0.50 gm, Agar-25gms , in 01 liter of distilled water. The plates were incubated at 32 + or – 2.0 ºC for 4-6 days. After incubation phosphate solubilizing bacteria were detected by the formation of clear zone around the colony. The zone diameter around the colony is measured and phosphate solubilizing efficiency was calculated by using following formula.

$$\text{Phosphate Solubilizzation Efficiency} = \frac{\text{Solubilizzation diameter}}{\text{Colony diameter}} \times 100 \quad \text{(Gothwal et al., 2006)}$$

Detection of antimicrobial activity:

The antimicrobial activity of isolates of Pseudomonas was determined by agar well diffusion method. Each isolate of Pseudomonas was inoculated in NB & incubated at 37º C for 18-24 hrs. Then each broth culture is centrifuged at 10000 rpm for 10 minutes. About 50 µ ml of supernatant was loaded into the well bored and test organism seeded agar plates. For Bacteria nutrient agar & for fungi PDA was used. The test organisms used were Bacillus subtilis NCIM2010; C. albicans NCIM3557 E .coli NCIM2064;Fusarium oxysporium (soil isolate);Klebsiella pneumoniae NCIM 2719 & Xanthomonas compestris NCIM2956 . The plates were kept in freeze for 30 minutes (for diffusion) and then incubated at 37ºC for 24 hours for bacteria & 30 ºC for 3-4days for fungi. The diameter of zone of inhibition was measured & noted in the table.

RESULT & DISCUSSION

10 different soil samples were collected from different rhizosphere region of soybean cultivated in Latur area. From this 10 isolates of Pseudomonas were isolated. Pseudomonas species were confirmed on the basis of morphological, cultural & biochemical characters (Table -1).

Table -1: Morphological & Biochemical characterization of Isolates of Pseudomonas:

Sr. no.	Test	Result
1	Gram nature	Gram Negative
2	Motility	Motile
3	Spore	-
4	Catalase	+
5	Citrate	+
6	Gelatinase	+
7	Indole	-
8	Lecithinase	+
9	Methyl Red	-
10	Nitrate reduction	+
11	Oxidase	+
12	Starch hydrolysis	-
13	Voges Proskauer	-
14	L Arabinose	+
15	Lactose	-
16	Maltose	-
17	Mannitol	-
18	Sucrose	-
19	D xylose	+
20	Urease	+
21	Growth in centrimide agar	+

(+ Positive; - Negative)

The isolates of Pseudomonas were then subjected for detection PSE by using Pikovskaya's agar (Table- 2).

Table -2: Phosphate solubilizzation efficiency of isolates of Pseudomonas:

Sr.no.	Bacterial Isolates	PSE
1	PSPF01	120
2	PSPF02	320
3	PSPF03	280
4	PSPF04	225
5	PSPF05	400
6	PSPF06	200
7	PSPF07	260
8	PSPF08	---
9	PSPF09	100
10	PSPF10	250

The isolates of phosphate solubilizing Pseudomonas (PSPF) were then subjected for the study of antimicrobial activity (Table -3).

Table -3: Antibacterial activity of isolates of phosphate solubilizing Pseudomonas:

ISOLATE	*Bacillus subtilis* NCIM 2010	*Candida albicans* NCIM 3537	*Escherichia coli* NCIM 2064	*Fusarium oxysporium* (soil isolate)	*Klebsiella pneumoniae* NCIM2719	*Xanthomonas compestris* NCIM 2956
PSPF01	16	10	14	-	15	-
PSPF02	14	13	20	13	11	-
PSPF03	15	-	16	-	17	11
PSPF04	20	-	19	12	13	10
PSPF05	23	14	21	14	18	13
PSPF06	18	12	-	09	16	-
PSPF07	19	-	18	-	14	08
PSPF08	13	13	15	11	-	-
PSPF09	14	-	17	10	12	09
PSPF10	17	10	13	-	-	13

Value represents diameter of zone of inhibition (mm).

Bergey's Manual of Systematic Bacteriology was used for identification of Pseudomonas. It was found that all isolates of Pseudomonas were highly diverse with respect to solubilizzation of phosphorus and antimicrobial activity against test organisms. It was observed out of 10, two isolates PSPF -2 & PSPF -5

shows high PSE. The isolates of Pseudomonas were further tested for antimicrobial activity. . It was found that Bacillus subtilis NCIM2010; E .coli NCIM2064; Klebsiella pneumoniae NCIM 2719 were highly sensitive while C. albicans NCIM3557; Fusarium oxysporium (soil isolate) ;Xanthomonas compestris NCIM2956 were less sensitive to isolates of Pseudomonas. The time required for phosphate solubilizaton is high which indicates the solubilizzation of phosphate is a slow process (Alexander 1983). The colonies of all isolates of Pseudomonas showed fluorescence when exposed to UV light. On the basis of morphology along with cultural and biochemical test suggested that these isolates were belonged to the Pseudomonas fluorescens. Similar work of isolation of phosphate solubilizing Pseudomonas as fungicide was done by Shrivastav et.al (2004). The isolate of Pseudomonas fluorescens PSPF -5 showed high PSE & antimicrobial activity, hence it could be exploited as biofertilizer as well as biocontrol agent for control of plant & human diseases. Bio-control of plant pathogen is an attractive alternative to chemical pesticides which causes environmental pollution & development of resistant strain.

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