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PLANT GROWTH-PROMOTION POTENTIAL OF PHOSPHATE SOLUBILIZING ACTINOMYCETES FROM WAYANAD SOILS

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Abstract

Phosphours is the second important key element after nitrogen as a mineral nutrient in terms of quantitative plant requirement. Although abundant in soils, in both organic and inorganic forms, its availability is restricted as it occurs mostly in insoluble forms. Deficiency of phosphorus in soil results in reduction in plant metabolism and growth since for all plant Phosphorus is an essential plant nutrient.

Wayanad District of Kerala depends on Agriculture for sustainability. To satisfy crop nutritional requirements, Phosphours is usually added to soil as chemical P fertilizer, however synthesis of chemical P fertilizer is expensive, moreover, plants can use only a small amount of this P since 75–90% of added P is precipitated by metal–cation complexes, and rapidly becomes fixed in soils.

In such a situation phosphate-solubilizing Actinomycetes have been seen as best eco-friendly agent for P nutrition of crop. This study focuses on the diversity of PSA, effect on plant growth, P solubilisation capacity, the present and future scenario of their use and for application of this knowledge in managing a sustainable environmental system.

Keywords: Soil phosphorus; Actinomycetes; P solubilization; Plant growth promotion.

I. INTRODUCTION

The term “Actinomyces” encompasses a wide range of bacteria. They are free living, spore forming, and chemo organotrophic Gram positive bacteria having high G+ C content in their DNA. They occur in a wide variety of natural and man-made habitats, growing a vast range of substrates and are mostly distributed in soil [You et al., 1996].

An increasing demand for low input agriculture has resulted in a greater interest in soil microorganisms which are able to enhance plant nutrition and health, and to improve soil quality (Jeffries et al., 2003). Among the microbial groups, actinomycete bacteria are known to promote activities which can improve agricultural developments (Barea et al., 2005), thus these microorganisms appear as a research target with regard to sustainability purposes (Johansson et al., 2004).

Actinomycetes are one of the major components of the microbial populations present in soil. They belong to an extensive and diverse group of Gram-positive, aerobic, mycelial bacteria that play important ecological roles in soil nutrient cycling (Ames et al., 1984; Nonomura, 1989; Halder et al., 1991; Elliot and Lynch, 1995). In addition, these bacteria are known for their economic importance as producers of biologically active substances, such as antibiotics, vitamins and enzymes (de Boer et al., 2005). Actinomycetes are also an important source of diverse antimicrobial metabolites (Lazzarini et al., 2000; Basilio et al., 2003; Terkina et al., 2006).

The most commonly described actinomycete genera have been Streptomyces. The genus Streptomyces is in fact known as one of the major sources of bioactive natural products (Bull et al.,

1992; Basilio et al., 2003; Terkina et al., 2006). Particularly, it has been estimated that approximately two-thirds of natural antibiotics have been isolated from actinomycetes, and about 75% of them are produced by members of the genus Streptomyces (Newman et al., 2003; Jimenez-Esquilin and Roane, 2005).

In the last decade research has focused on minor groups of actinomycetes, including species that are difficult to isolate and cultivate, and those that grow under extreme conditions, i.e. alkaline and acidic conditions (Lazzarini et al., 2000; Phoebe et al., 2001). However, most soil actinomycetes show their optimum growth in neutral and slightly alkaline conditions, thus their isolation procedures have been traditionally based on this neutrophilic character. Several selective isolation methods have been developed (Goodfellow and OˇıDonnell, 1989; Edwards, 1993; Sabaou et al.,1998; Zakharova et al., 2003).

Phosphorus is the most important key element in the nutrition of plants, next to nitrogen (N). It plays an important role in virtually all major metabolic processes in plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration (Khan et al. 2010) and nitrogen fixation in legumes (Saber et al. 2005). Although Phosphorus is abundant in soils in both inorganic and organic forms, it is a major limiting factor for plant growth as it is in an unavailable form for root uptake.

Inorganic Phosphorus occurs in soil, mostly in insoluble mineral complexes, some of them appearing after frequent application of chemical fertilizers. These insoluble, precipitated forms cannot be absorbed by plants (Rengel and Marschner 2005). Organic matter is also an important reservoir of immobilized Phosphorus that accounts for 20–80% of Phosphorus in soils (Richardson 1994). Only 0.1% of the total Phosphorus exists in a soluble form available for plant uptake (Zhou et al. 1992) because of its fixation into an unavailable form due to Phosphorus fixation.

Microorganisms are an integral component of the soil Phosphorus cycle and are important for the transfer of Phosphorus between different pools of soil Phosphorus. Phosphate Solubilzing Microorganisms (PSM) through various mechanisms of solubilization and mineralisation are able to convert inorganic and organic soil Phosphorus respectively (Khan et al. 2009a) into the bioavailable form facilitating uptake by plant roots.

In this study, actinomycetes were isolated from various soil samples and their phosphate solubilising capacities were analyzed. Plant growth promoting effect of isolates were studied by in vivo evaluation

II. MATERIALS AND METHODS

ISOLATION OF ACTINOMYCETES

Soil samples were collected from 3 different habitats of wayanad district (geographical coordinates: latitiiude 11.6 ^{o}N and longtitude 76 ^{o}E), kerala , India. The habitas include rubber field, forest rhizosphere and ground soil. Actinomycetes were isolated by serial dilution technique using starch casein agar plates incubated at 30^{o} c for 7-10 days.

IDENTIFICATION OF ISOLATES

The isolated Actinomycetes were characterized by morphological test including macroscopic and microscopic methods. The mycelium structure, arrangement of spores on the

mycelium and colors of colonies were observed and compared with Bergey's manual of determinative bacteriology.

SCREENING OF PHOSPHATE SOLUBILIZING ACTINOMYCETES

Actinomycetes cultures were inoculated on modified starch casein agar medium containing 2% of tricalcium phosphate as a sole phosphorous source for selectively screening the phosphate solubilizing Actinomycetes, which have the ability to release inorganic phosphate from tricalcium phosphate. The inoculated plates were incubated at 30^{o}c for 7-10 days. After the completion of the incubation period, the plates were observed for the presence of clear zone around the colonies which indicates the extent of phosphate solubilization.

INVIVO EVALUATION

All the isolated actinomycetes were evaluated under pot culture conditions using garden pea plants as the test plant.

PREPARATION OF INOCULUMS:

Selected isolates were grown in starch casein broth for 10 days at room temperature.

POT CULTURE EVALUATION: These experiments were conducted in poly bags of 15 cm diameter. Non sterile, sieved field soil was mixed with sand and cow dung in a ratio 500:500:50. The pot mixture was filled into 9 different pots used for raising the plant. The seeds were sawn in each labelled pot and different Actinomycetes (10 ml) isolates was also added accordingly. The pots were watered whenever required. Uninoculated pots served as control. The pots were harvested after 30 days and the growth was measured.

III. RESULT AND DISCUSSION

The present study was focused to analyze the phosphate solubilising and plant growth promoting effect of actinomycetes isolated from three different soil samples collected from wayanad.

ISOLATION AND IDENTIFICATION OF ACTINOMYCETES:

Total 8 Actinomycetes were isolated from different locations of wayanad such as rubber field, forest and garden soil. The isolated Actinomycetes were identified based on bergey's manual of determinate bacteriology 2009. Six were identified as streptomycetes sps., one psudonocardia and one nocardia sp.,

IDENTIFICATION OF PHOSPHATE SOLUBILISING CAPACITY:

The samples were cultured on modified starch casein agar with tricalcium phosphate as the soul phosphorous source. Phosphate solubilising capacities of the isolates were identified by measuring the diameter of the clear zone around the colony. The results are given in table-1

Table-1. Phosphate Solubulizing isolates of Actinomycetes

Actinomycetes isolate	Zone of clearance (mm in diameter	Isolate
A1	0.5	Streptomycetec sp.,
A2	0.5	Psudonocardia
A3	12	Nocardia
A4	13	Streptomycetec sp.,
A5	13	Streptomycetec sp.,
A6	16	Streptomycetec sp.,
A7	20	Streptomycetec sp.,
A8	0.6	Streptomycetec sp.,

INVIVO ANALYSIS

Growth parameters mainly plant height, root length, and the nodule number of the plant showed a variation due to different isolates. The parameters were recorded highest by application of actinomycete isolates. The lowest values in regards to these characters were recorded in control pot (uninoculated).

The isolate which showed solubilisation in the laboratory studies (A7) gave maximum plant growth responds.

The results are given in table 4 and figure- 3.

Sl.no	Sample	Plant height(cm)	Root length(cm)
1	A1	43.5	6.2
2	A2	40	6
3	A3	29.5	4.6
4	A4	30	5.2
5	A5	55	6.6
6	A6	63.5	9.6
7	A7	70.5	14.5
8	A8	32	5.5
9	CONTROL	23	3.9

The study showed that actinomycetes can be used as good bio-fertilizer for the need of plant growth promotion due to their phosphate soulbilising ability. Here we found that wayanad is a good region of biodiversity and has been adequately acceptable due to its vast floral diversity and also microbial diversity.

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