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Phosphate solubilizing capability of actinomycetes isolated from Rhizosphere soil

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Abstract

Phosphorous has a high importance in the growth of plants and in the biology of soil. Phosphorous is available in soluble and insoluble form. Actinomycetes are one of the most important microbial groups in soil with the ability to solubilise insoluble organic and inorganic phosphates in soil. They are abundant in alkaline areas especially in dry areas and can solubilise insoluble phosphate making it available for plants. Thus crop yield can be increased a lot. Effect of different sources such as carbon, nitrogen and potash on phosphate solubilisation by actinomycetes isolated from rhizosphere soil was studied. The present study revealed that among the carbon sources offered, incorporation of glucose followed by lactose increased solubilisation of phosphate and enhanced acid production. The Actinomycetes preferred ammonium sulphate to solubilise phosphorous than the other nitrogen sources offered. Among the three potassium sources offered all the strains preferred potassium chloride followed by potassium nitrate and released maximum phosphate. Quantitative analysis of organic acids produced by phosphate solubilizing Actinomycetes revealed that they produced acetic acid, succinic acid, and lactic acid which is vital for phosphate solubilisation. Thus the Actinomycetes capable of dissolving phosphates can be isolated from native soil and can be well exploited as Phosphatic Biofertilisers.

Keywords: actinomycetes, biofertilizer, phosphate, rhizosphere, soil microbes

INTRODUCTION

Phosphorous is found in soils, plants and microorganism in a number of organic and inorganic compounds. It is second only to nitrogen as inorganic nutrient required by plants and microorganisms. It is essential in the accumulation and release of energy during cellular metabolism. It may be added to soil in the form of chemical fertilizers, plant and animal residues. Phosphate form of phosphorous is one of the least soluble mineral nutrients in the soil. Insoluble inorganic compounds of phosphorous are present in large quantity in soil. Tricalcium phosphate exists in several forms and is dominant in neutral and alkaline soils. Actinomycetes are abundant in alkaline areas. They play active role in cycling of nitrogen, sulphur, phosphorous (Goodfellow and Cross, 1974). They produce enzyme phosphates and also release metabolic acid (Alexander, 1977) which dissolve inorganic phosphate in soil and make it available to plants. Actinomycetes are the most important microbial group in soil with ability to solubilise insoluble phosphate either inorganic or organic phosphates (Banik and Dey 1981, 1982, 1983).

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MATERIALS AND METHODS

Soil samples from rhizosphere soil was collected and the soil sample was serially diluted. One ml of the dilution was plated on to soil extract agar with cyclohexamide(150µg/ml). Actinomycete colonies producing clear zones were selected and purified on starch nitrate agar. The organism was stained and confirmed as *Streptomyces sp*.

Quantitative measurement of phosphate solubilization in culture medium

Selected cultures were grown in 50 ml of Pikovskayas liquid medium and filtered. Activated charcoal was added to the filtrate. The filtrate became colourless. 2.5 ml of Barton's reagent was added to the filtrate and made up to 50 ml. After 10 minutes the colour developed was read in spectrometer at 430 nm. The OD values were extrapolated on the standard graph and the amount of phosphate solubilised was determined

Effect of different sources on phosphate solubilisation

a) Effect of carbon sources

Five carbon sources such as glucose, sucrose, lactose, mannitol and sodium acetate were substituted into Pikovskayas medium. The media was inoculated with the isolates. They were incubated for 10 days and phosphate solubilisation was determined.

b) Effect of Nitrogen sources

Nitrogen sources such as diammonium sulphate, ammonium nitrate, sodium nitrate and sodium nitrite were substituted on nitrogen basis into Pikovskayas medium. The media was inoculated with the isolates. They were incubated for 10 days and phosphate solubilisation was determined.

c) Effect of potash sources

Different potash sources such as potassium chloride, potassium nitrate, dipotassium sulphate were substituted on nitrogen basis into Pikovskayas medium. The medium was inoculated with the isolates. They were incubated for 10 days and phosphate solubilisation was determined.

Quantitative analysis of organic acids produced by phosphate solubilizing Actinomycetes

The test organisms were grown in 50 ml Pikovskayas medium (Nordman and Nordman., 1960) for 10 days. The supernatant was centrifuged at 10,000 rpm for 15 minutes.The supernatant was concentrated at low temperature to 1/40 th of its original volume. The fraction was spotted on to chromatographic paper and was separated by chromatographic technique using n-Butanol, acetic acid and water solvent in 12:3:5 ratio.

RESULTS AND DISCUSSION

The phosphate solubilising Actinomycetes appeared on soil extract agar showing zone of solubilisation. They were selected for further works. Studies on different carbon sources like glucose, sucrose, lactose, mannitol and sodium acetate on phosphate solubilisation revealed that incorporation of Glucose (Table 1) followed by lactose increased solubilisation of phosphate and enhanced acid production efficiently(Gerretsen,1948). There is less phosphate solubilisation by the other sugars.

Table 1. Phosphate solubilsation by Actinomycetes of Rhizosphere soil by utilizing different carbon sources (µg/ml)

Strain no	Glucose	Lactose	Sucrose	Mannitol	Sodium acetate
1	11	7	7.5	7	7
2	8.5	4	2.7	4.5	5
3	11	10.5	7	6	9
4	15	8	4.5	9	14.5
5	12	7	6.5	12	12
6	11	9	2	4.5	4
7	13	5	8	12	5

Among the four nitrogen sources tested, the Actinomycetes preferred ammonium sulphate to solubilise the phosphorous than other nitrogen sources (Table 2). The present study revealed that among the three potassium sources all the strains preferred potassium chloride followed by potassium nitrate and released maximum phosphate (Table 3). This perhaps might be due to the presence of chloride and nitrate which would have enhanced the growth of organisms.

Table 2. Phosphate solubilsation by Actinomycetes of Rhizosphere soil by utilizing different Nitrogen (0.5 %) sources (µg/ml)

Strain no	Ammonium sulphate	Ammonium chloride	Sodium nitrate	Ammonium nitrate
1	11	6.5	7	5
2	9	8.5	6	9.5
3	9.5	7	8.5	9
4	14	13	9	13
5	11	6.7	10	9
6	13	5	12	11
7	14	7	13	11

Quantitative analysis of organic acids produced by phosphate solubilizing Actinomycetes revealed that they produced acetic acid, succinic acid, and lactic acid (Table 4) (Guar,1972). It is well documented that organic

Table 3. Phosphate solubilsation byActinomycetes of Rhizosphere soil by utilizing different Phosphate (0.2 %) sources (µg/ml)

Strain no	Potassium chloride	Potassium nitrate	Potassium sulphate
1	11	6.5	7
2	9	8.5	6
3	9.5	7	8.5
4	14	13	9
5	11	67	10
6	13	5	12
7	14	7	13

acid produced by these organisms play a major role in solubilisation of insoluble phosphates. There is correlation between the pH and amount of soluble phosphates in the medium (Ibrahim and El-Aziz, 1977). Phosphate solubilizing Actinomycetes in soil could persist during adverse conditions like drought and high temperature. They are thermostable and can enhance plant growth by solubilising phosphate in soil. Chemical fertilizers are predominantly used to enhance the growth of the crops. They enhance crop yield but continuous use causes various draw backs like sterility of soil. On the other hand, the use of biofertilisers avoids side effects and retains the fertility of the soil and can be well exploited as Phosphatic Biofertilizers.

Table 4. Organic acids produced by Phosphate solubilising Actinomycetes from Rhizosphere soil

Strain no	Names of Standard acids	Rf value	Rf Values of unknown acids from culture filtrate of different Actinomycetes Strains.						
			1	2	3	4	5	6	7
1	Acetic	0.92	0.918	0.918	0.918	0.918	0.918	0.918	0.918
2	Citric	0.32	0.319	0.319	----	0.319	0.319	----	----
3	Formic	0.89	----	----	0.887	0.887	----	----	0.887
4	Lactic	0.65	0.639	0.639	0.639	0.639	0.639	0.639	0.639
5	Malic	0.45	0.449	----	-----	----	0.449	0.449	0.449
6	Succinic	0.81	0.809	0.809	0.809	0.809	0.809	0.809	0.809

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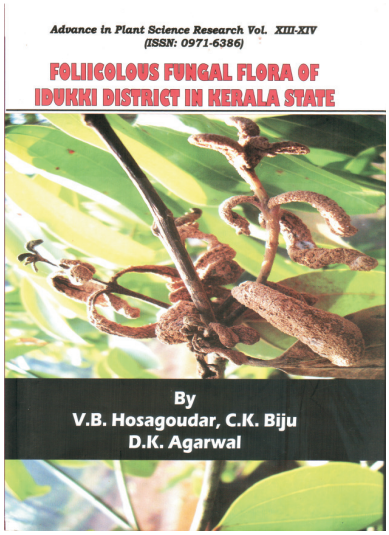
BOOK REVIEWS



Foliicolous Fungal Flora of Idukki District in Kerala State, India.
V.B. Hosagoudar, C.K. Biju, D. K. Agarwal
International Book Distributors, Dehradun - 248001, India. 2011. 436 pp. Price:Rs. .

Foliicolous fungal flora of Idukki district in Kerala state by V.B. Hosagoudar, C.K. Biju and D.K. Agarwal, Published as XII-XIV volumes of the Journal Advances in Plant Science Research, comprises 436 pages with several coloured plates. Authors have dedicated this book to Dr. Roger D. Goos who inspired the senior author. This is the work initiated in the year 1981 in and around Idukki hydroelectric project area and the second phase of this work was carried out in Munnar and its neighboring areas comprising 337 foliicolous fungal species and infraspecific taxa infected 289 host plants belonging to 228 genera of flowering plants represent 73 fungal genera. This study forms a type locality for 119 fungi (35%)! It is really amazing. All these are higher fungi belonging to Ascomycetes, Badidiomycetes and fungi imperfecti. Key to the groups, genera and species is provided. Each taxon is described and illustrated in detail and convincing the identity of the individual fungal taxa. Introduction drags the attention in introducing the subject; methods used in mounting fungi for the microscopic study are simple. This is the original work of the authors who have collected, processed, identified and placed the studied materials in both HCIO, Nelhi and TBGT, Palode. Science needs such original works which depict the actual field experience, herbarium techniques, interpretation of the individual taxa based on their observation in the natural condition are of immense value to science. I congratulate the authors for their tremendous effort in donating to the scientific community. I strongly believe that this novel book will shine in the hands of foresters, researchers, teachers, students, libraries, etc. This book is published by International book distributors, 9/3, Rajpur Road, Ist Floor, Dehradun 248 001. Price not known.

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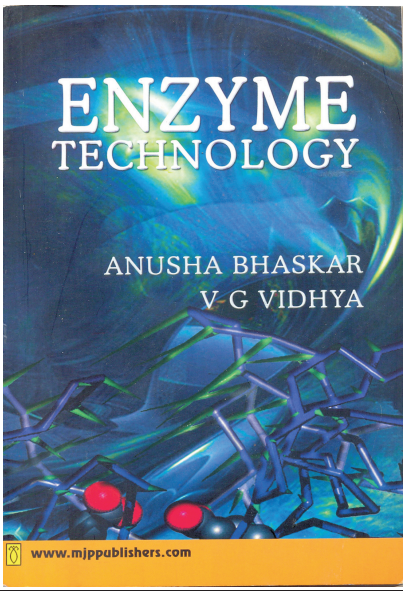

Enzyme Technology
Anusha Bhaskar, V. G. Vidhya
MJP Publishers, 44& 47, Nallathambi Street, Triplicane, Chennai - 600 005
2010. 656 pp. Price: Rs. 300.

The book "**Enzyme Technology**" written by Anusha Baskar and V.G. Vidhya deals elaborately with almost all aspects of enzyme technology that will be highly useful to all the students of biological sciences. The book provides detailed accounts on the historical perspectives of enzymes, enzyme nomenclature, enzyme characteristics, isozymes and multi enzyme complexes, coenzymes, enzyme catalysis, enzyme kinetics, isolation, fractionation and purification of enzymes, applications of immobilized enzymes, bioreactor designs and reaction engineering. In addition it covers current technologies such as biosensor technology, ribozymes, artificial enzymes and industrial enzymes and their applications. The book also deals with the applications of bioinformatics in enzymology including the active sites and the determination of amino acids in the active sites. In short its contents fulfill the needs of teachers, students and research scholars in the filed of enzyme technology. The authors have done an excellent job in bringing out such and elaborate excellent piece of work and deserve full appreciation. All the chapters are written in a scientific way and include current information. The book will be a good reference book for both teachers and students of Zoology, Botany, Microbiology, Chemistry, Bioinformatics and Bio-technology. It will be a valuable book for every library, especially in the colleges and universities and deserves to adorn the cupboards of a library.

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