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Detection and Identification of Plant Growth Promotion Using Phosphate Solubilizing Bacteria.

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ABSTRACT:

Different types of microbes promote plant growth and there are different products which stimulate plant growth and development. There are some specialized microbes which are called as plant growth promoting bacteria called as Rhizobacteria. These are the soil bacteria. There are few free-livingmicroorganisms in the soil which have the capacity to produce enzymes like phosphatase. Phosphatase enzyme is able to break down organic phosphate into inorganic phosphate which is used to produce high phosphorous to the plants. There are few bacteria which are named as phosphate solubilizing bacteria (PSB) whose main action is to breakdown both organic phosphate as well as inorganic phosphate into simple form of phosphorous which can be taken up by the plants very easily. The present study is conducted in order to check the potential of phosphate enzyme in plant growth promotion. The screening of the phosphatase enzyme is done by the preparation of Pikovskayas broth medium because phosphorous helps stimulation of root development, improve flower production and seed formation as well as improvement in crop quality. Hence in this present study we see about different aspects like collection of the soil, preparation of Pikovskayas broth medium in a sterilized condition, screening of phosphatase enzyme and the potrail study of the plants like by calculating the length of the root as wells shoot length and also the length of the whole plant and the weight of the whole plant in a comparative mode.

KEY WORDS:

Pikovskayas broth medium, Phosphorous, Phosphate solubilizing bacteria, Rhizobacteria, Screening

INTRODUCTION:

Based on our study as our study is mainly regarding the different types of minerals as well as nutrients which should be given to the plant in order to increase their productivity and remaining major aspects of a plant life. Hence coming to the point firstly we talk about the fertilizer, a fertilizer is nothing but the chemical substance or an organic substance which is sprayed or mixed up with soil in order to increase the soils fertility. So hence the fertilizer is very important for a plant to increase its productivity and many other aspects. There are different types of fertilizers we see in everyday life. Normally the three main primary macronutrients which are required for the plants were Nitrogen, Potassium and phosphorous. There are three secondary macronutrients for the plants which are required for the plant growth and development. Those are Calcium, Magnesium and Sulphur. And the macronutrients which are required by the plant are Copper, Iron, Manganese, Molybdenum,

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Zinc, Boron. Silicon, Cobalt and Vanadium. Now let us discuss about the different types of fertilizers. Firstly, based on the nature the fertilizers are divided into three types. They are as follows 1. Organic fertilizers which are nothing but the fertilizers which are naturally obtained and which consists of living matter like compost, debris of different fruits, vegetables and so on. Hence organic fertilizers won't do any damage to the plant as they are naturally driven from the environment. Coming to the second type of fertilizer it is called 2. Inorganic fertilizer which are defined as the fertilizers which are made up of chemically and in brief the inorganic fertilizers are those which are manufactured industrially by using different types of chemicals in order to promote the plant growth and yield. Hence inorganic fertilizers are some what dangerous and harmful to the crop. Finally coming to the last one 3. Biofertilizer which are defined as the fertilizers which are mainly composed of different types of living micro-organisms which helps the plant to increase its productivity. As we talked earlier the bio fertilizers are the rhizobacteria which are soil borne and help the soil to retain its fertility. Hence coming to the next classification of the fertilizers based on the form which can be divided as 1. Solid fertilizers and 2. Liquid fertilizers. Now coming to the third classification which is based on the complexity of fertilizers. They are as follows 1. Single nutrient or straight fertilizer and 2. Multiple nutrient or complex fertilizer. The multiple nutrients or the complex fertilizer is again divided into two types they are as follows binary fertilizers and NPK. So, coming to our study we had also used NPK fertilizer in our study in order to check the plant growth and development in a comparative study in potrail method. Using fertilizer to the soil is really a good idea in order to enhance the soil fertility. But in case if the fertilizer is used in a heavy dose, then it might completely destroy the plant. Normally the fertilizers which are if nature oriented are very good for each and every plant and there won't be any destruction in the plant. But incase if the fertilizer is of inorganic which is industrially synthesized or that contains different types of chemicals in it to promote the plant growth and development then there is really a big problem as the soil completely loses its fertility after a single use and never retains its fertility back and also, they create air pollution as well as soil pollution. So in order to overcome this problem we had completed our project based upon this fertilizers by promoting the growth of phosphate solubilizing bacteria and screening of phosphatase enzyme in order to check the potential of plant growth promotion with these phosphorous enzyme by avoiding inorganic fertilizers.

OBJECTIVES OF THE STUDY:

- Collection of samples from Rhizosphere soil
- Isolation of bacteria by using serial dilution method
- Bacterial colony isolation
- Screening of phosphatase enzyme
- Plate Assay
- Chemical Assay
- Optimization studies
- Plant growth study by using Enzyme and commercial fertilizer
- Above all objectives were carried out using standard protocols.

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Soil sample collection:

Six different samples of the soil were randomly collected from leguminous rhizosphere soil in Chennai district, Tamil Nadu, India. The samples were brought to laboratory and taken for the experimental study.

Preparation of Nutrient Agar Media: Using standard method Isolation of bacteria:

0.1ml aliquots of sample from appropriate dilution let we say 10^{-5} to 10^{-9} was taken and then it was inoculated onto the sterilized as well as solidified nutrient agar media by a specialized technique called spread plate method using "L" shaped rod. The cultures were incubated at 30°C for 24 hours using the incubator. For this spread plate we had used 5 Petri plates for the growth of cultures.

Obtaining of pure culture and Preservation of pure culture:

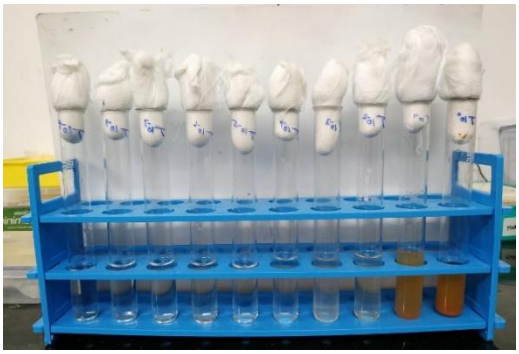
Screening of Phosphatase enzyme:

The bacterial species which were isolatedare screened for the production of extra cellular phosphatase using Pikovskayas screening medium. Then there is a streaking method going on with the pure cultures at the center of the sterile PVK plates and the plates were incubated at 37°C for 3 days. The phosphate solubilization zone was absorbed around the colony.

RESULTS:

1. Collection of samples:

2. Serial dilution: The sample was serially diluted



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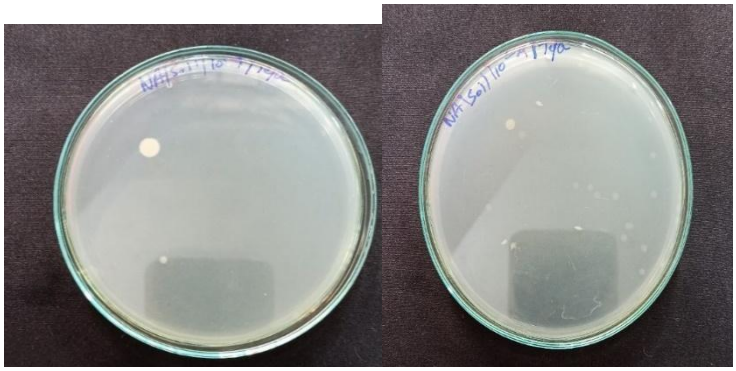
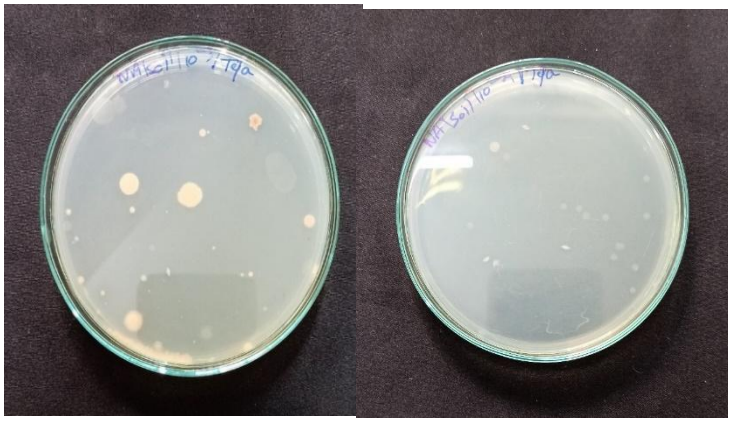
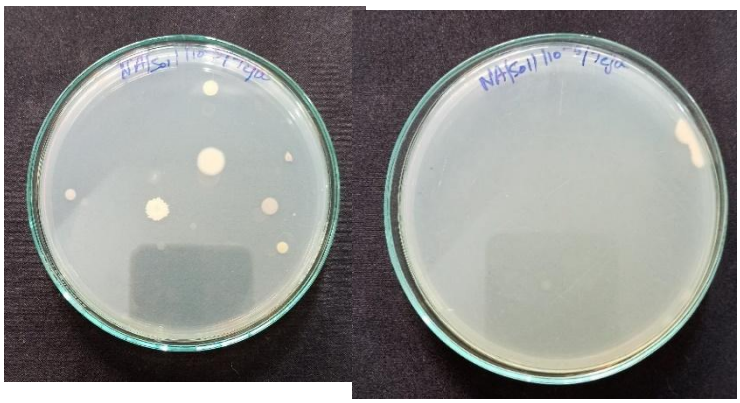
3.Spread plate method:

Spread plate method was performed using nutrient agar media from the dilutions 10 to 10 and incubated for 24 hours. After 24 hours we can see the colonies of bacteria grown.



(a) (b)





4.Purification and preservation of pure cultures:

The bacterial cultures which were taken from the spread plate were now streaked on different freshly prepared media. Six different cultures were taken and streaked by giving the number 1 to 6.

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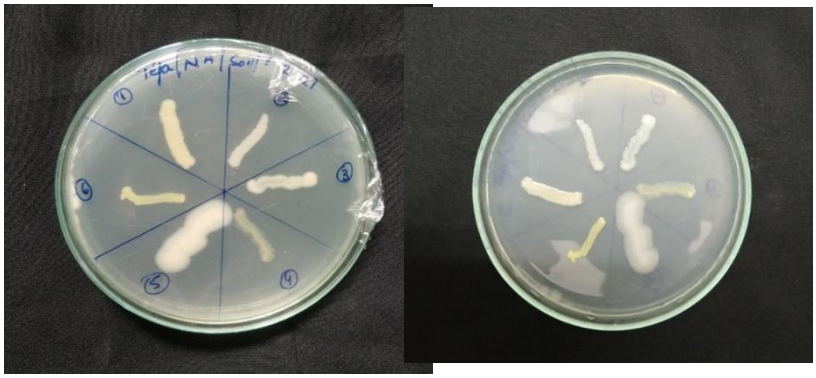
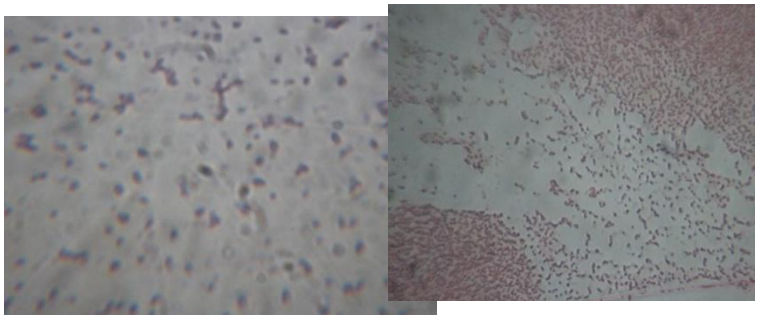


5.Screening of phosphatase enzyme:

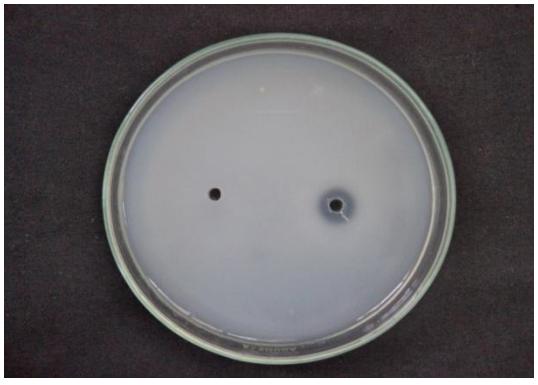
From this pure culture the phosphatase enzyme was screened by streaking these cultures on Pikovskayas broth medium which is prepared with specific composition.

6. Gram's staining:

The Gram staining technique was done by the above said and discussed method. And the result of Gram staining was observed.



7. Plate Assay: The result of plate assay for phosphatase activity



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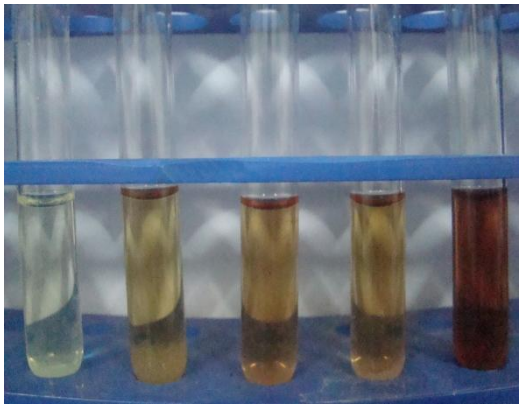
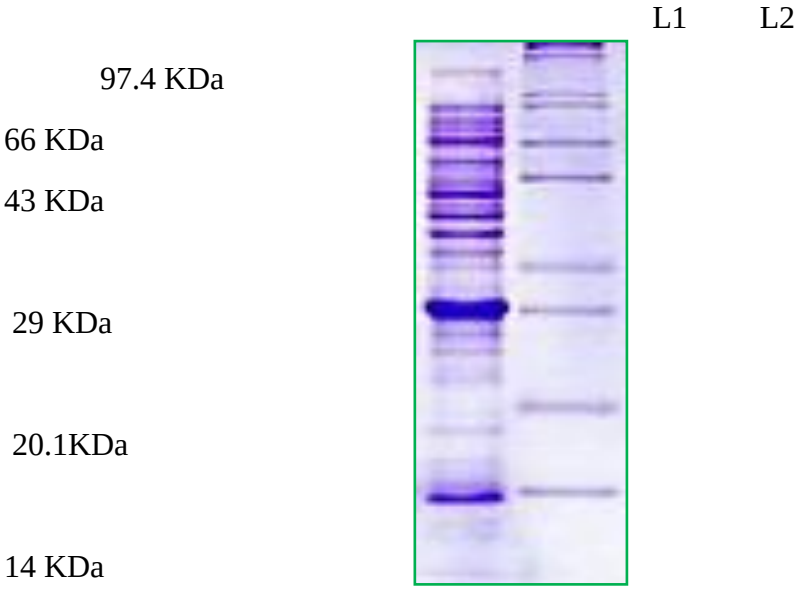
8. Chemical Assay: The result of the chemical assay for phosphatase activity



9. SDS PAGE: The protein profile in SDS PAGE was observed as follows



Lane 1. Marker Lane 2. Sample

10. Potrail study:

The potrail study was carried out as follows. The first image (a) shows us that the two pots are combinedly tied soon after sowing the seeds and also the required materials. The (b) image can also be seen in the same way with two pots tied combinedly.

The image (a) contains two pots in which one of the pots contain control and the second pot contains the chemical fertilizer.

Whereas coming to the image (b) with two pots one of the pots contain only the enzyme itself and the other pot contain the enzyme along with chemical fertilizer in it.

The difference in the length of the shoot and root in each pot can be observed.

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(Control – NPK) (Enzyme – Enzyme + NPK)

11. Roots and Shoots growth:

Test 1: CONTROL

Test 2: NPK

Test 3: PHOSPHATASE ENZYME

Test 4: PHOSPHATASE ENZYME + NPK

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Table 8: Root and shoot growth in pot trail:

TEST NO	SHOOT SYSTEM				ROOT SYSTEM			
	Total length cm	Shoot length cm	Shoot weight gm	No of leaves	Root's length cm	Root's weight gm	No. of leaves	Total weight gm
Test 1	51.6	35.4	1.95	5	16.2	1.57	8	3.52
Test 2	55.9	37.2	2.47	5	18.7	1.72	11	4.19
Test 3	62.4	42.6	3.37	6	19.8	2.37	16	5.74
Test 4	92.5	63.8	6.08	7	28.7	6.12	21	12.20

DISCUSSION:

In this present study about the phosphate solubilizing bacterial species were isolated from leguminous plant rhizosphere soil of Chennai district, Tamil Nadu, India. From the soil samples, Phosphate dissolving microbial consortium were developed in Pikovskayas liquid medium. On screening the consortium numerous colonies were found on the plates, which gave zone of clearing. Six different bacterial colonies were picked up from the plates of Pikovskayas agar showing the maximum zone of the clearing around these colonies. From the 6 bacterial species, based on solubilization efficiency test better halo zone formed bacterial strain was used for further study. The *Pseudomonas* species were able to solubilize phosphate effectively. The isolated positive strain was identified as *Pseudomonas species* based on Manual of Determinative Bacteriology. The growth study of the organism is essential for the production of enzyme because most of the extracellular enzymes are produced during log phase of the organisms. Generally, during growth study, the biomass of the cells will be estimated in plate assay (Qualitative analysis) the enzyme activity was identified by a clear zone. Since it is an enzyme production, the modified growth study was carried out.

The protein profile was analysed in SDS-PAGE; it showed the presence of multiple bands. Obviously, because the medium contains protein source, so unutilized protein also may be present in the exhausted medium. Moreover, along with phosphatase some other

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proteins can be produced by the organisms. But the presence of protein band nearing the molecular weight 68kd believed to be the presence of enzyme. In zymogram of phosphatase was studied in SDS PAGE and native gel the stained area with malachite-green solution appears as green band, this confirmed the presence of alkaline phosphatase in the crude enzyme. And finally, the 20^{th} day old plants were taken for the experimental parameter records the shoot and root length, number of leaves, number of roots. The recorded result in application part of in this present study was presented as follows.

The control pot plant growth showed normal whereas compared with rest of the experimental group Test 4. Altogether, the test 4 showed exponential growth than the all-other groups. Among experimental group, the total length of shoot system is gradually increased towards the order T1 to T4. In the case of total weight of shoot system highest observed in T4, and followed by T3, T2 and T1. Highest shoot length was observed in T4 and followed by T4 and followed by T3, T2 and T1. The experimental group T4 shows high shoot weight in T4 and followed by T3, T2 and T1. The experimental group T4 shows highest number of leaves, and followed by T3, T2 and T1. In root system, the T4 shows highest root length, root weight and number of roots. In root length T2, T3 and T1 total root weight is least was observed in T1. The number of roots least in T2 and T3 and highest in T4. The number of leaves least in T2 and T1 and highest in T3 and T4.

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