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Isolation, Screening and Optimization of Phosphate Solubilizing Bacteria and it Effective Role on Plant Growth Promotion

Anuradha Thallapaka

PG and Research Department of Microbiology, Thanthai Hans Roever College (Autonomous), Perambalur, Tamil Nadu, India.

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Abstract

In this present study, phosphate solubilizing bacterial (PSB) strain was isolated from Rhizosphere beans plant soil sample from Srikalahasthi A.P, India and used for screening and production alkaline phosphatase in Luria broth medium. The parameters like Time, Temperature, pH, Carbon sources and Nitrogen sources were optimized for better production of alkaline phosphatase. In consideration of time parameter at 48 hrs incubation of specific strain showed maximum alkaline phosphatse production. Among different temperatures the maximum alkaline phosphatse was recorded at 37 °C temperature. In pH parameter, pH 8 showed maximum production of alkaline phosphatase activity in isolated species. In the case of carbon source lactose and in nitrogen source Ammonium nitrate were showed the higher production of alkaline phosphatase. The partial purification of alkaline phosphatase was employed by ammonium sulphate precipitation method, dialyzed and characterized. The protein profile was analyzed in SDS-PAGE was carried out. The phosphatase enzyme helps the growth of Maize plant.

Keywords: Phosphatse enzyme, Carbon source, SDS-PAGE, Maize plant.

Introduction

Phosphorus (P) is one of the major essential macronutrients for plants and is applied to soil in the form of phosphatic fertilizers. However, a large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plants (Goldstein, 1986). Microorganisms are involved in a range of processes that affect the transformation of soil P and are thus an integral part of the soil P cycle. In particular, soil microorganisms are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization (Frances et al., 1963). Currently, the main purpose in managing soil phosphorus is to optimize crop production and minimize P loss from soils. Plants require major nutrients for growth. It includes nitrogen (N), potassium (K) and phosphorous (P).

Phosphorus is a major growth-limiting nutrient, and unlike the case for nitrogen, there is no large atmospheric source that can be made biologically available. Root development, stalk and stem strength, flower and seed formation, crop maturity and production, N-fixation in legumes, crop quality, and resistance to plant diseases are the attributes associated with phosphorus nutrition. Phosphatase enzymes are responsible for soil organic phosphorus mineralization and the release of inorganic phosphorus needed by microorganisms and plants. Phosphatases are collective name for enzymes (extracellular) that cleave phosphate from organic compound (e.g. phospholipids, nucleic acids).

Biofertilizers are a substance which contains living microorganisms, which promotes growth by increasing the supply or availability of primary nutrients to the host plant. Phosphate solubilization is a complex phenomenon which depends on many factors such as nutritional, physiological and growth conditions of the culture. Solubilizing activity increases the availability of phosphorous to vegetation and improves plant growth. Bacteria that convert non-available inorganic phosphorous (Pi) present in soil into an available from utilizable by plants are called phosphate solubilizing bacteria (PSB). These are a group of beneficial bacteria capable of hydrolyzing organic and inorganic phosphorous from insoluble compounds. The bacteria that are capable for phosphate solubilizing can be applied for their use as biofertilizer. The use of PSB as inoculants increased P uptake and crop yield (Han et al., 2006). Recently PSM have attracted the attention of agriculturists as soil inoculums to improve the plant growth and yield (Henri Fankem et al., 2006).

The present study aims to isolation and identification of alkaline phosphatase producing bacteria from rhizosphere soil sample and also partial purification and molecular analysis of alkaline phosphatase. Further, the PSB used as biofertilizer (phosphate solubilizer) on

Correspondence
Anuradha Thallapaka
E-mail: thallapaka.anuradha@gmail.com,Ph.+9178932 98491

maize (*Zea mays* L) to study its effectiveness on plant shoot and root growth.

Materials and Methods

The soil samples were collected from depth of 6-15 cm from the agricultural land of Srikalahasthi, Andhra Pradesh. Soil samples were collected from rhizosphere of beans plant soil. Collected soil samples were stored in polythene bags aseptically and maintained at the laboratory for further study. The samples were subjected into serial dilution and pure culture techniques. The different culture were selected and stored in refrigerator at 4 ºC.

Screening for the phosphatase activity

The isolated pure bacterial species were screened for the production of extra cellular phosphatase using Pikovskaya screening medium. The pure cultures were streaked at the center of the Sterile PVK plates and the plates were incubated at 37ºC for 3 days. The phosphate solubilization zone was absorbed around the colony and calculated the solubilization efficiency (SE).

SE = Solubilization of diameter / Growth of diameter x 100

Phosphatase enzyme assay

Plate assay (Qualitative analysis)

The plate assay was performed using Pikovskaya agar medium (Pikovskaya, 1948). After solidification of the agar plates, around 10 mm diameter of well was cut out aseptically with the help of a cork borer. The well was filled with the culture filtrate (test) and other was filled with non-culture filtrate (control) and incubated at 37°C for 3 days in humid chamber. The observation was made to see the phosphate solubilizing zone around the well (Qureshi *et al*., 2009).

Chemical assay (Quantitative analysis)

Alkaline phosphatase activity was measured spectrophotometrically by monitoring the release of *p*-nitrophenol from *p*-nitrophenyl phosphate (*p*NPP) at 400 nm. A typical reaction mixture contained 100 µl of enzyme diluted in 2ml of 200 mM Tris buffer (pH 8.5), 100 µl of 5mM $CaCl_2$, 100 µl of 500 µmol *p*NPP and the final volume was made up to 3 ml using distilled water. Blank was prepared by mixing 2 ml of 200 mM Tris buffer (pH 8.5) and 1ml distilled water. The reaction was performed at 37^{o}C for 30 min and stopped by addition of 500 µl of 4 M sodium hydroxide. One unit of phosphatase is the amount, which hydrolyses 1 µmol of substrate per min. The standard curve obtained by absorbance of *p*-nitrophenol (0 - 500 µmol) at 400 nm was used for quantification of enzyme activity (Ramkumar *et al*., 2005).

Estimation of total protein

The chemical assay for the total protein content from the sample was determined using Bradford method.

Determination of phosphatase protein activity

Based on the P – solubilization studies, an isolate showing maximum P solibilizing potential were selected for phosphatase enzyme studies using Pikovskaya broth medium. The supernatant was used as a crude enzyme extract for determining protein content in culture filtrate was estimated according to the method described by Bradford (1976) using Coomassie Brilliant Blue G-250.

Preparation of Bradford reagent (Coomassie brilliant blue)

100 mg of Coomassie brilliant blue dye G-250 was dissolved in 50ml of 95% ethanol. 100 ml of 85% (w/v) phosphoric acid was added and the mixture was makeup to 1000 ml with double distilled water. The dye was filtered through Whatmann No.1 filter paper and stored in amber bottle in refrigerator. The 1 ml of culture filtrate was taken and 5 ml of Bradford reagent was added. The tube was gently tilted once for mixing and the absorbance was taken at 595 nm in UV- VIS spectrometer. The blank was prepared by mixing 1 ml of distilled water with 5 ml of reagent. The protein concentration was determined by comparing the value with standard graph prepared using Bovine serum albumin.

Optimization Studies

Effect of various parameters on phosphatase production

Time

250 ml of sterile production medium was prepared and 5% inoculum was added aseptically. The inoculated medium was incubated at 37°C with shaking around 150 rpm. The culture was periodically drowned at 8 hrs intervals up to 64 hrs (0hrs, 8hrs, 16hrs, 24hrs, 32hrs, 40hrs, 48hrs, 56hrs and 64hrs). After incubation the culture filtrate was examined for the total protein content and phosphatase activity.

Temperature

250 ml of sterile production medium was prepared in different conical flask and inoculated with 5% inoculum. Each flask was incubated at different temperatures such as 27ºC, 32ºC, 35ºC, 37ºC, and 45ºC for 48 hours. The protein estimation and enzyme activity were estimated.

pH

250 ml of sterile production medium was prepared in different conical flasks and each flask was adjusted to different pH such as 5, 6, 7, 8 and 9 using 0.1N NaOH and 0.1N HCl. After sterilization flasks were inoculated with 5% inoculum. The flasks were incubated at 37 ºC at shaker around 150 rpm for 48hrs. The protein estimation and enzyme activity were estimated.

Carbon source

250 ml of sterile production based medium

(pH 8) was prepared in different conical flasks. Each flask was amended with different carbon sources such as sucrose, galactose, lactose, maltose, dextrose and starch. The flasks were inoculated with 5% inoculum and incubated at 37ºC and kept on shaker around 150 rpm for 48 hrs. The culture filtrate was collected and protein estimation, enzyme activity was determined.

Nitrogen source

250 ml of sterile production based medium (pH 8.0) was prepared in different conical flasks each flasks were amended with different nitrogen sources (0.5%) such as casein, peptone, yeast extract, ammonium chloride, urea and ammonium nitrate. The flasks were inoculated with 5% inoculum and incubated at 37ºC and kept on shaker around 150 rpm for 48 hrs. The culture filtrate was collected and Protein estimation, Enzyme activity was determined.

Partial purification of phosphatase

Ammonium sulphate fractionation of proteins

The solubility of proteins is markedly affected by the ionic strength of the medium. As the ionic strength is increased, protein solubility also increases. This is referred to as 'salting in'. However, beyond a certain point the solubility begins to decrease and the proteins precipitate out as the salt concentration increases. This is known as 'salting out'. At low ionic strengths the activity coefficients of the ionizable groups of the proteins are decreased so that their effective concentration is decreased. This is because the ionizable groups become surrounded by counter ions, which prevent interaction between the ionizable groups. Thus protein-protein interactions are decreased and the solubility is increased. At high ionic strengths much water becomes bound by the added ions that not enough remains to properly hydrate the proteins. As a result, protein-protein interactions exceed protein-water interactions and the solubility decreases. Because of differences in structure and amino acid sequence, proteins differ in their salting in and salting out behavior. This forms the basis for the fractional precipitation of proteins by means of salt.

Ammonium sulphate is a particularly useful salt for the fractional precipitation of proteins. It is available in highly purified form and has great solubility allowing for significant changes in the ionic strength and is inexpensive. Changes in the ammonium sulphate concentration of a solution can be brought about either by adding solid substance or by adding a solution of known saturation, generally a fully saturated (100%) solution. The enzyme separation from the exhausted medium was done by Ammonium sulphate precipitation technique. Enzyme extraction was made from culture filtrate using 70% w/v of ammonium sulphate saturation**.** The mixture was then stored in cold room for 24 hrs to precipitate all the proteins. Then the precipitated solution was separated by centrifugation around 10,000 rpm for 10 mins. Then carefully the supernatant was discarded and the remaining precipitate was dissolved with 2 ml of 50 mM Tris HCl (pH 8) buffer. Then the mixture was subjected to dialysis.

Dialysis

Dialysis is carried out to obtain a pure form of a protein. The binded ammonium sulphate will be discharged through the dialysis membrane. While the crude pure form protein will be remain inside the dialysis membrane. The dialysis tube of required length was boiled once in distilled water, containing a pinch of sodium azide and EDTA. Sodium azide serves as an antimicrobial agent and EDTA helps to remove metal ions by chelation. Then the bag is thrice washed in distilled water. The dialysis tube was then put in 50 mM Tris HCl (pH 8) buffer. One end of the membrane tube was closed tightly by fixing the clip. The precipitated protein was then transferred in the dialysis tube and other end was tied with a thread. The pack was suspended freely into a large beaker, which contains around 500 ml of 50 mM Tris HCl (pH 8) buffer. The buffer was stirred slowly using magnetic stirrer. The entire setup was placed in the cold room for 48 hrs. Every 12 hrs the buffer was changed periodically for better dialysis. After dialysis the clip from one end of the membrane was removed and the sample was transferred in to the clean lyophilization flask.

SDS PAGE analysis

SDS-PAGE, officially sodium dodecyl sulfate polyacrylamide gel electrophoresis, is a technique used in biochemistry, genetics and molecular biology to separate proteins according to their electrophoretic mobility (a function of length of polypeptide chain or molecular weight as well as higher order protein folding, posttranslational modifications and other factors).

Evaluation of phosphate solubilizing bacteria and its growth of shoot and root in maize (*Zea mays*)

Pot Trial

Preparation of phosphate source

Vermicompost – 2 g of vermicompost dissolved in 100 ml of tap water.

NPK - 0.5g of phosphate dissolved in 100 ml of tap water.

Posphatase enzyme - 20 ml of bacterial culture filtrate containing enzyme dissolved in 100 ml of tap water.

A pot experiment for phosphate utilization in maize (*Zea mays*) was made in surface sterilized pots (Plastic pots of about 6 X 10 inches). 8 kg soil was prepared with 1% Tricalcium phosphate and pH was adjusted by adding calcium carbonate to 8. The soil was divided into seven equal parts and sterilized. After sterilization the 1kgof soil was filled in eight pots, labeled as control, phosphate, Vermicompost, Phosphatase Enzyme, phosphate + Phosphatase Enzyme, Vermicompost+ Phosphatase Enzyme, phosphate + Vermicompost + Phosphatase Enzyme. The 10 g seeds

were soaked overnight and sowed in each pot 10 seeds. Sprinkle a thin layer of soil on the Black gram grains. The pots kept in glass house and 75% of moisture was maintained by pouring 9ml of source solution directly on seeded area. Growth was observed every day (Table 1).

Data collection and analysis

The whole plant were pulled out from the experimental pot and used for experimental observation. Before placing the roots in the bucket, a sieve was placed at the bottom of bucket. Then by gradual movement of the roots in water, the roots were separated from the soil. Roots were further cleaned in gently running tap water and peat masses were removed with forceps. Any broken root portion collected in the sieve was carefully washed out. The root portion was separated from shoot. The length of the shoot was measured from the base of the stem to the growing point of the youngest leaf. The length of root was measured from the growing point of root to the longest available lateral root apex. For fresh weight of shoot and root the portion was blotted dry and the weight was recorded before the materials could get desiccated. The number of primary and secondary branching was also counted by eye estimation.

Results and Discussions

Naturally microorganisms are having ability to produce the variety of industrial enzymes like amylase, protease, cellulase, lipase, pectinase, phytase, etc and most of the enzymes are industrially important and human welfare. Phosphatase is one of the important enzymes, which can be produced from various microorganisms like fungi, actinomycetes and bacteria. Phosphate-solubilizing bacteria are known to improve solubilization of fixed soil phosphorus and applied phosphates, resulting in higher crop yield. Phosphate solubilizing microbes have been routinely isolated from rhizosphere soil of various plants such as wheat, soybean, mustard, Aubergine, and chili.

There are many microorganisms have been isolated form rhizosphere and they are cabaple to solublize the organic phosphate to bioavailable form. The bacteria, *Bacillus subtilis, B.simplex, B.pumilis, B.licheniformis, B.lentimorbus, B.megaterium, B.marinus, B.firmus, Pseudomonas putida, P.chlororaphis, P. fluroscenes, P.cepacia, P.liquifaciens, P.striata, P. aeruginosa. Azatobacter chrococcum, Enterobacter cloaeceae, E.aerogenes, E. agglomerans, Lupinus albescens, Klebsilla terrigena,Burkholderia capacia,Arthrobacter globiformis, Rhizobium melioti, R.lupine, R. leguminosarum, R. japonicum* are able to solbulize the phosphate (Qureshi *et al*., 2009; Ramkumar *et al*., 2005).

In this present investigation about the phosphate solubilizing bacterial species were isolated from Rhizosphere beans plant soil of Srikalahasthi A.P, India **(**Plate 1.A). From the soil samples, Phosphate dissolving microbial consortium were developed in Pikovskaya liquid medium. On screening the consortium numerous colonies were found on the plates, which gave zone of clearing. Four different bacterial colonies were picked up from the plates of Pikovskaya agar showing the maximum zone of the clearing around these colonies. From the 4 bacterial species, based on solubilization efficiency test better halo zone formed bacterial strain was used for further study. The solibilization by the tested bacterial strains on Pikovskaya medium. The isolate 1 were able to solubilize phosphate effectively, and recorded higher solubilization efficiency up to 275 **(**Figure 1) (Nguyen *et al*,. 1992). 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 **(**Figure 2a**).** Since it is an enzyme production, the modified growth study was carried out.

Effect of Various Parameters on Phosphatase Production

The effect of various parameters are listed in figure 3 – 7.

Effect of time

The bacterial culture was withdrawn and checked for enzyme activity at every 8 hrs and the enzyme activity was investigated. The result revealed that least amount has been produced during 8^{th} (19 U/ml) and high amount of production was found at 48^{th} hrs (127 U/ml) **(**Table 2). In the case of total protein of production time experimental group, the high amount recorded at (148 µg/ml) and least at (29 µg/ml). After 48^{th} hrs, the production level of enzyme has reduced significantly reduced. These results clearly indicated that the isolate has maintained its lag phase before 8^{th} hrs of its growth and should have maintained its log phase from around 8^{th} hrs to 56^{th} hrs, because the considerable and peak production has occurred in this duration. Moreover, it is believed that the higher production of phosphates has occurred in mid log phase because the higher production has occurred in 48^{th} hrs. This variation of log phase duration and production of enzyme is based on the nutrient present in the medium and the cultural condition of the organism. The environmental parameter also influences the maintenance time of the Bacteria (William et al., 1968).

Effect of temperature

The environmental parameters are showing great influence in the growth of the organism and the production of enzyme. The main parameters like temperature, pH, carbon source and nitrogen sources are very essential parameters of the production. To optimize the optimum temperature for the better yielding, Productions were made in various temperatures. It was found that the isolate has shown higher phosphatase activity (122 U/ml) at 37 ºC (Table 3). In the case of total

protein of temperature experimental group, the high amount recorded at (146 µg/ml) and least at (37 µg/ml). Many thermophilic bacterium like *Thermotoga neopolitana* needs 70ºC for better production of thermo stable phosphatase and a pshychrophilic *Arthrobacter* has produced at 25ºC (Paloma, 1997). The current isolate *Pseudomonas* species produced maximum phosphatase activity when incubated at 37ºC. The temperature requirement of the organism is based on the nature of environment where they grown.

Effect of pH

Next to the temperature, the pH is the important parameter which determines the growth of the organism and phosphatase production. Usually most of the bacteria require medium pH for its growth. The requirement of the pH for its better growth is also based on the environmental pH where they grow. The genus of *Bacillus subtilis* isolated from coastal region has produced the alkaline phosphatase at pH 9 (Marion hulett *et al.,* 1990) and in contrast, The *Bacillus subtilis* isolated from the soil sample has shown higher production at pH 8. In the present study results showed that the higher production have been recorded at optimum pH range from 8 (112 U/ml), so this bacteria need an alkaline pH for their maximum enzyme production (Table 4). In the case of total protein of pH experimental group, the high amount recorded at (145 µg/ml) and least at (38 µg/ml).

Effect of carbon source

The carbohydrates are essence energy source for most of heterotrophic organisms. These shows great influence on the production of many enzymes. In many of the other enzyme, the production will be carried out by medium amended with glucose as a general carbon source for better growth and production. But the isolate has produced higher quantity of phosphatase from lactose (107 µg/ml) (Table 5) and considerable amount has been produced from Sucrose, Galactose, Maltose, Dextrose and Starch. Production of higher quantity of phosphates has been produced from the medium amended with maltose and **i**n the case of total protein of carban experimental group, the high amount recorded at (131 µg/ml) and least at (63 µg/ml).

Effect of nitrogen source

The nitrogen sources are of secondary energy sources for the organisms, which play an important role in the growth of the organism and the production. The nature of the compound and the concentration that we are using may stimulate or down modulate the production of enzymes. The inorganic nitrogen source Ammonium nitrate (93 U/ml) **(**Table 6) was found to be a better nitrogen source for this isolated bacterial culture and in the case of total protein of pH8 experimental group, the high amount recorded at (104µg/ml) and least at (46 µg/ml) (Qureshi *et al.*,2010).

Even though many phosphates sources are available, not all the phosphates will induce the microbes to produce the extra cellular phosphatase. Among the several phosphate sources, tricalcium phosphate was found being suitable to produce phosphatase from this isolate. As for as phosphatase enzyme concern, the concentration of inorganic phosphate is a limiting factor for the production of extracellular alkaline phosphatase, usually the high concentration of inorganic phosphate in the medium drop the production. The higher concentration of Pi induces the nutrient arrest and halted secretion of enzyme (William, 1968). Finally, the protein profile was analyzed in SDS-PAGE; it showed (Figure 2b) the presence of multiple bands. Obviously, because the medium contain protein source, so unutilized protein also may be present in the exhausted medium. Moreover, along with phosphatase some other proteins can be produced by the organisms. But the Presence of protein band nearing the molecular weight 68.54kd believed to be presence of enzyme (Paloma *et al*., 1997).

Evaluation of phosphate solubilizing bacteria and its growth of shoot and root in maize (*Zea mays*)

The 15th day old plants wares taken for the experimental parameter record the shoot and root length, number of leaves, number of roots. The recorded results in application part of in this present investigation presented following. The control pot plant growth showed normal whereas compared with rest of the experimental group Test 2 to Test 7. All together, the test 7 showed exponential growth than the all other group. Among experimental group, the total length of shoot system is gradually increased towards the order T1 to T7. In the case of total weight of shoot system highest observed in T7, and followed by T6, T5, T4, T3, T2 and T1. Highest shoot length was observed in T7 and followed by T6, T5, T4, T3, T2 and T1. The experimental group T7 shows high shoot weight in T7 and followed by T6, T5, T4, T3, T2 and T1. Number of leaves are equal in all experimental groups was recorded. In root system, the T7 shows highest root length, root weight and secondary root. In root length T6, T5, T4, T3, T2 and T1 followed from T7. Total root weight is least was observed in T2. The number of roots least in T2 and T1 and highest in T7 **(**Table 7) (Figure 8).

Conclusion

The PSB was isolated from the soil sample and the phosphatase enzyme production was analyzed. The isolated PSB was optimized to produce more alkaline phosphatase with using of different parameters like pH, temperature, carbon, nitrogen and etc. The maximum production of alkaline phosphatase was observed at pH 8 and using of carbon source (lactose) and nitrogen source (ammonium nitrate). The protein profile of alkaline phosphatase was examined by SDS-PAGE analysis. The pot trial of PSB on maize plant gave a moderate results which indicate that the PSB support the growth of shoot and root of plants.

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Table I. Treatment procedure

S.No	Experimental group	Composition	Treatment volume
1.	Test 1	Control	9 ml of tap water
2.	Test 2	NPK	9 ml of dissolved Phosphate
3.	Test 3	Vermicompost	9 ml of dissolved Vermicompost
4.	Test 4	Enzyme	9 ml of Phosphatase enzyme
5.	Test 5	NPK+ Enzyme	4.5ml NPK + 4.5ml Phosphatase enzyme
6.	Test 6	Vermicompost + Enzyme	4.5ml Vermicompost + 4.5ml Phosphatase enzyme
7.	Test 7	NPK+ Vermicompost +Enzyme	3ml NPK + 3ml Vermicompost + 3ml Phosphatase enzyme

Table II. Phosphatase production on different incubation times

S.No	Time of culture withdrawal (hr)	Total Protein (µg/ml)	Total Enzyme Activity (U/ml)
1	0	0	0
2	8	29	17
3	16	57	43
4	24	82	67
5	32	108	81
6	40	132	98
7	48	148	127
8	56	135	106

Table III. Effect of temperature on phosphatase production

S.No	Temperature (ºC)	Total Protein (µg/ml)	Total Enzyme Activity (U/ml)
1.	27	37	21
2.	32	64	34
3.	35	125	92
4.	37	146	122
5.	42	74	58
6.	47	56	39

Table IV. Influence of pH on phosphatase production

S.No	pH	Total Protein (µg/ml)	Total Enzyme Activity(U/ml)
1.	5	46	23
2.	6	69	52
3.	7	97	75
4.	8	126	107
5.	9	102	87

Table V. Carbon source optimization for the production of phosphatase

S.No	Carbon sources	Total Protein (µg/ml)	Total Enzyme Activity (U/ml)
1.	Sucrose	63	62
2.	Galactose	127	74
3.	Lactose	131	107
4.	Maltose	97	78
5.	Dextrose	112	96
6.	Starch	87	57

Table VI. Effect of organic and inorganic nitrogen source on phosphatase production

S.No	Organic Nitrogen Source	Total Protein (µg/ml)	Total Enzyme Activity (U/ml)
1.	Casein	68	41
2.	Peptone	46	37
3.	Yeast extract	74	58
4.	Ammonium chloride	98	76
5.	Ammonium nitrate	104	93
6	Urea	57	43

Table VII. Maize (*Zea mays. L*) roots and shoot growth in pot trail (20 Days old Plants)

Test no	SHOOT SYSTEM				ROOTS SYSTEMS			
	Total length **cm**	Shoot Length **Cm**	Shoot weight **gm**	No of leaves	Roots length **cm**	Roots weight **gm**	No of Roots	Total weight **gm**
Test 1	21.6	15.1	1.03	5	7.4	1.07	14	5.52
Test 2	35.9	17.8	2.17	4	18.4	1.22	11	4.16
Test 3	42.4	23.6	3.39	5	19.2	2.37	16	6.74
Test 4	49.2	32.3	5.08	6	20.2	6.12	19	8.26
Test 5	56.9	36.2	7.47	7	21.7	8.72	19	9.19
Test 6	63.4	41.6	8.09	8	23.8	10.38	21	11.74
Test 7	92.8	63.8	11.42	8	28.7	14.17	21	12.76

Test 1: control, **Test 2:** NPK, **Test 3:** Vermicompost, **Test 4:** Phosphatase Enzyme,
Test 5: NPK + Phosphatase Enzyme, **Test 6:** Vermicompost + Phosphatase Enzyme,
Test 7: phosphate + vermicompost + Phosphatase Enzyme,

Figure I. a) Pure culture of bacterial isolate; b) Screening of phosphatase activity

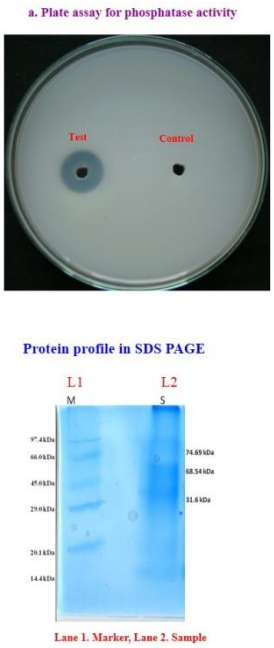

Figure II. a) Phosphatase activity ; b) Protein profile analysis by SDS-PAGE study

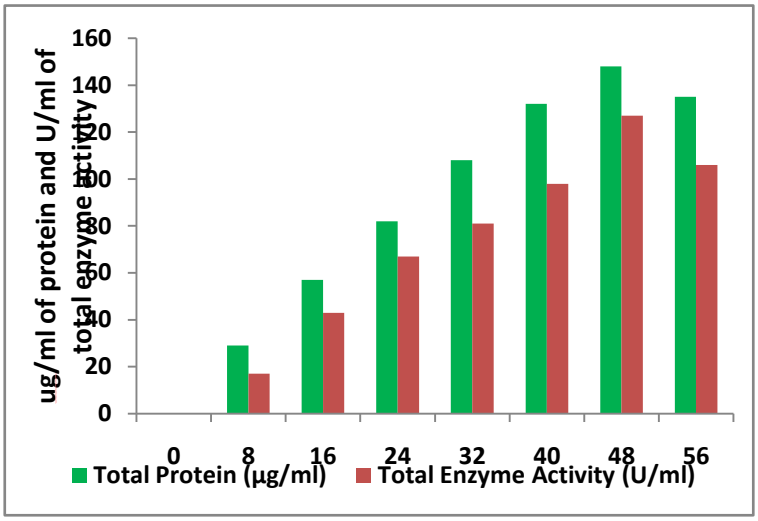

Figure III. Effect of time on *Bacteria* total protein and Total enzyme activity

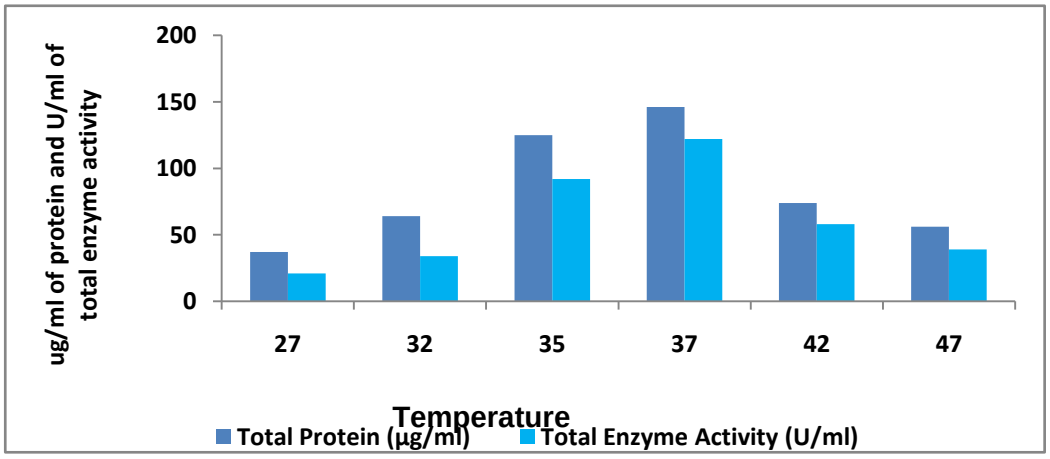

Figure IV. Effect of temperature on total protein and phosphatase enzyme production

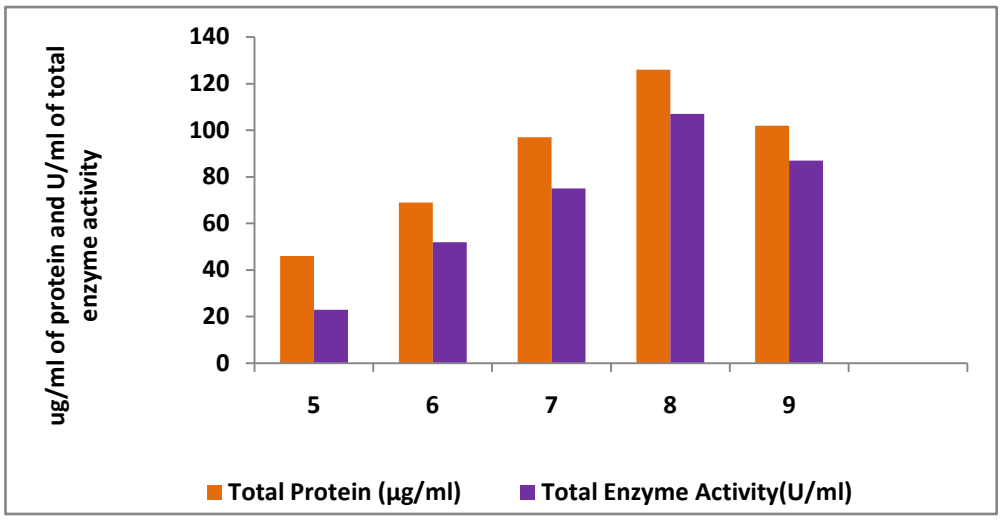

Figure V. Effect of pH on total protein and Total enzyme activity

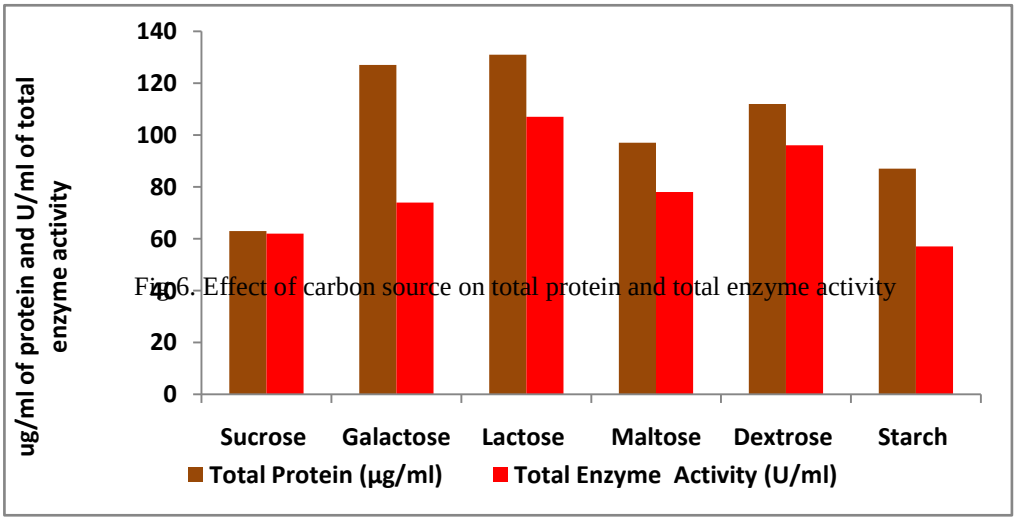

Fig 6. Effect of carbon source on total protein and total enzyme activity

Figure VI. Effect of carbon source on total protein and total enzyme activity

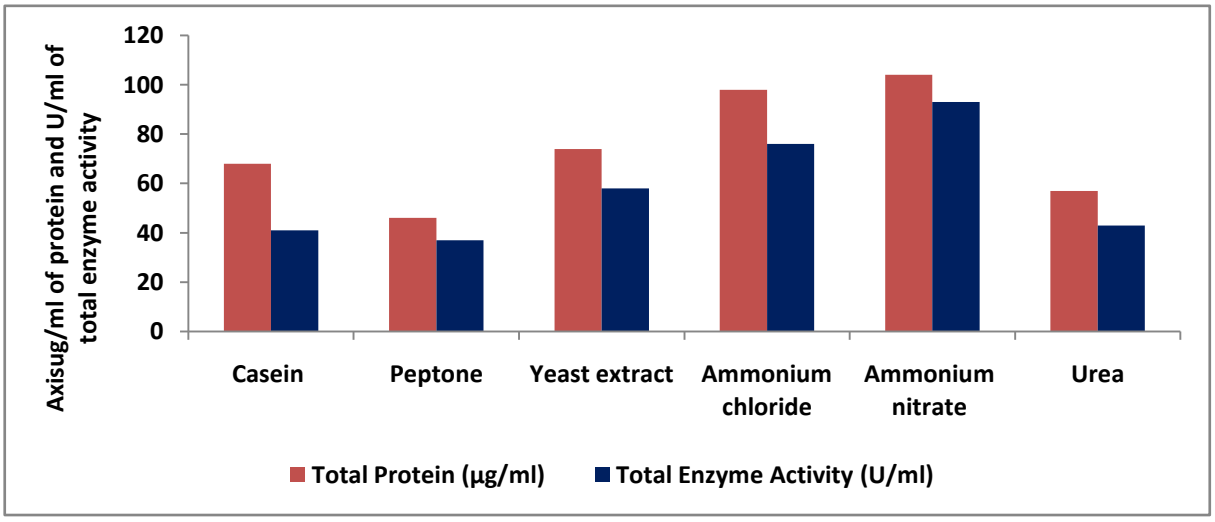

Figure VII. Effect of nitrogen source on total protein and total enzyme activity

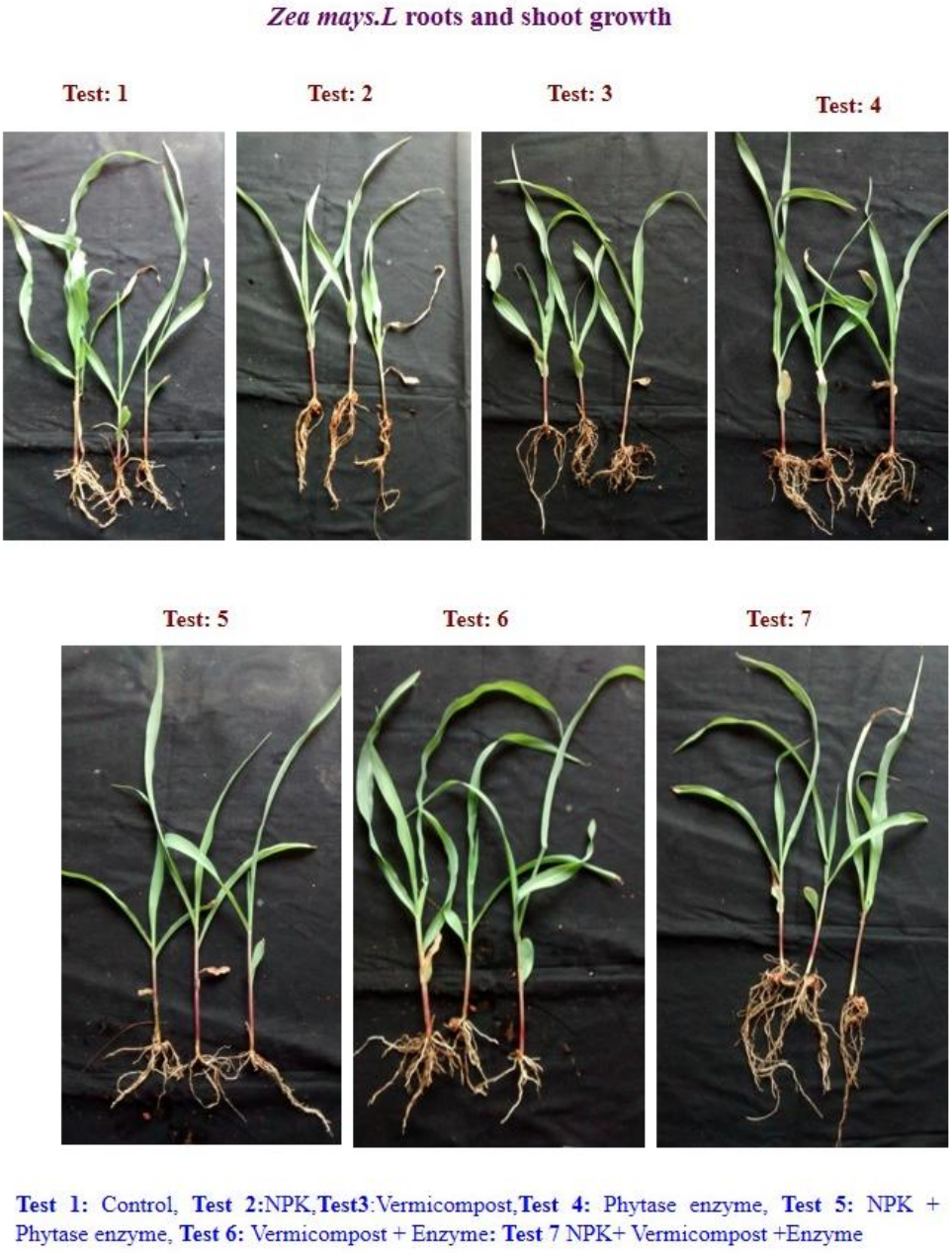

Figure VIII. Shoot and root growth in pot trial experiment