Isolation and Characterization of Plant Growth Promoting Bacteria From Rhizosphere Soil

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Abstract- *PGPR were formerly recognized through different researchers the usage of laboratory screening strategies. A massive variety of micro organism which includes species of Pseudomonas, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus, Rhizobium and Serratia have suggested to induce plant growth. In this study, banana and hibiscus rhizospheric soil samples have been taken. The micro organism have been remoted and in vitro screening became accomplished for one of a kind plant boom promoting pastime i.e. phosphate solublization. PGPR were formerly recognized through different researchers the usage of laboratory screening strategies. In our study, the Vigna mungo plant has been grown in plate and pot strategies through the assist of PGPR. The PGPR is used as a biofertilizer.*

Keywords- Abiotic stresses, agriculture, biofertilizer, phosphate solubilizing micro organism, PGPR.

I. INTRODUCTION

Plants today are continuously uncovered to a huge variety of biotic and abiotic stresses which restrict plant productivity. PGPR promote growth without delay with the aid of using generating siderophores, phytohormones (inclusive of auxins), solubilizing phosphate and circuitously with the aid of using inducing systemic resistance. The PSB isolates have validated efficient in solubilizing insoluble phosphate in soils (Musa and Ikhajiagbe, 2020a; Addendum 1 and 2). Our study is to isolate and display a few widely known PGPR strains from rhizosphere of hibiscus plant. Then it is used to promote the growth of some plants. Considering the effectiveness of biopriming as a method of inoculation with PGPR microorganisms, this study ambitions to analyze the possibilities of *Vigna mungo* seeds under bio conditions with PGPR microorganisms possessing PSB competencies to enhance the growth of *Vigna mungo*.

METHODOLOGY

Cleaning of Glasswares:

Glasswares are soaked in H_2CrO_4 solution and washed thoroughly with detergent solution and faucet water. Then dried inside the hot air oven.

Sterilization:

Prepared media and glassware's were sterilized by autoclave. All the sugars were filter sterilized by 0.45µm micro pore sized filters.

Saline Blanks:

The saline solution was prepared using 1.5% NaCl. 1.5g of NaCl was dissolved in 150ml of distilled water. 9ml of saline solution was autoclaved for further process.

For 150ml,

= (1*150)%100
=1.5gNaCl

Collection of soil samples:

Rhizospheric soil samples have been amassed from *Musa paradisiaca* and *Hibiscus rosa-sinensis* plants. Plants have been decided on from agriculture fields displaying good, healthful plant boom. Intact root system became dug out and the rhizospheric soil samples have been cautiously taken in sterile collection bags and became taken into lab for in addition study.

Isolation of bacteria from rhizospheric soils:

The P- solubilizers have been remoted from the rhizospheric soil samples through serial dilution approach.

Preparation of Soil Dilution, Inoculation and Incubation:

10 g of soil became dissolved in a hundred ml of sterilized distilled water and shaken for 15 mints. Subsequent dilution became made following the serial dilution approach of Alexander (1965). 10^{-9} sample became the usage of for test and sterile saline as a clean and the diluted samples have been

plated into the sterile nutrient agar plates the usage of spread plate approach. The plates have been incubated at 37ºC for twenty-four hours. The cultured bacterial colonies have been in addition purified through streak plate approach the usage of sterile nutrient agar medium. The oxygenic mono cultures have been subculture into sterile nutrient agar slants and nutrient broth for in addition use.

Media Prepration:

Nutrient agar medium for 100ml (@pH)

INGREDIENTS	VOLUME (100ml)
Peptone	0.5g
Yeast Extract	0.3g
Beef Extract	0.3g
NaCl	0.3g
Agar Agar	0.25g

Composition of Pikovskaya's Media:

For 100ml @pH 7

INGREDIENTS	VOLUME (100ml)
Glucose	1g
Ammonium Sulphate	0.05g
Sodium Chloride	0.02g
Magnesium sulphate	0.01g
Potassium chloride	0.02g
Tricalcium phosphate $CaCl_2$ 5% KH_2PO_4 5%	 5ml 5ml
Manganese sulphate	0.002g
Ferrous sulphate	0.002g
Yeast extract	0.5g
Agar	2.5g

Screening for the Phosphatase Activity:

The isolated natural traces have been screened for the manufacturing of extra cellular phosphate manufacturing the usage of particular phosphate medium Basal spider media (Basal spider, 1958) as a screening medium. The pure cultures have been streaked on the middle of the plate and the locations have been incubated at 37degree celsius for sixty two hours. The observation became made to look the solubilization of phosphatase region across the colony. Only tremendous and higher region formed one became taken for in addition study.

Solubilizing Index:

E = (Solubilization of diameter/Growth of diameter)*100

Subculture:

The tremendous and higher region forming bacterial species became sub cultured in Luria Bertani agar plates. The natural cultures have been retrieved each week and saved at 4°C for in addition test study.

Identification of Bacteria:

The positive bacteria which produces maximum phosphatase was selected and was given for identification of ***Pseudomonas species*** by **Bergey's Manual Method.**

Preparation of Inoculum:

For similarly manufacturing of enzyme and different parameters, the inoculum became prepared the usage of Luria Bertani (LB) broth (Zolta´n Pra´Gai, 2001). The natural traces became inject into aseptic broth and became grow at 37°C in a rotary shaker for overnight. The overnight traces became used as an inoculum for the mass manufacturing of enzyme.

Composition of LB Broth (50ml):

For 100ml (@pH)

INGREDIENTS	VOLUME (50ml)
Peptone	0.25g
Yeast Extract	0.25g
Beef Extract	0.25g
NaCl	0.15g

Phosphatase Production Medium:

The enzyme production was carried out by Shake Flask Fermentation using Pikovskaya production media. 250 ml of sterile production broth was prepared and 5% inoculums were transferred aseptically in to the production medium. The inoculated medium was incubated at 37°C for 2 days and placed in a shaker at 200 rpm rotation for better aeration and growth of the organism.

Production Media composition:

For 150ml (@ pH 7)

INGREDIENTS	VOLUME (150ml)
Glucose	1.5g
Ammonium Sulphate	0.075g
Sodium Chloride	0.03g
Magnesium sulphate	0.015g
Potassium chloride	0.03g
Tricalcium phosphate $CaCl_2$ 5% KH_2PO_4 5%	 7.5ml 7.5ml
Manganese sulphate	0.003g
Ferrous sulphate	0.003g
Yeast extract	0.75g

Phosphatase Enzyme Assay :

Chemical Assay:

- The activity of phosphatase was measured in UV Spectrophotometer by monitoring the discharge of p-nitrophenyl (pNPP) at 400nm. A ordinary response combination contained 100 µL of enzyme diluted in 500 µL Tris buffer (200mM, pH 8.5), 300 µL CaCl2 (5mM), 100 µL pNPP (500 µmol), in a final volume of 1ml. The response become executed at 37degree celcius for 30min and stopped by addition of 100 µL 0f 4 M sodium hydroxide. One unit of the enzyme is the amount, which hydrolyses 1µmol of substrate according to min.
- The curve acquired by absorbance of p-nitrophenol (0-500 mol) at 400nm become used for quantification of enzyme activity.

Protein Assay :

Estimation of Total Protein:

- The chemical assay for the total protein content from the sample was determined using Bradford method (Bradford, 1976)

Preparation of Bradford reagent (Coomassic brillant blue):

- 100mg of Bradford reagent G-250 was dissolved in 50ml of 95% ethanol. 100ml of 85% (w/v) phosphoric acid was added and the mixture was made up to 1000ml with double distilled water. The dye was filtered through Whatmann No.1 filter paper and stored in dark bottles under refrigeration.

Estimation:

- 1000µl of Bradford reagent have become brought to 500µl of culture filtrate. The tube have become gently inclined only one time for mixing and the absorbance have become taken at 595nm with using UV-VIS spectrophotometer. A blank have become prepared via mixing 500µl of distilled water with 1ml of reagent. The protein concentration has become determined by standard graph.

Optimization of Phosphate Solubilizing Bacteria:

Growing of bacteria in different parameters

- Different time
- Different pH
- Different Temperature
- Different carbon source
- Different nitrogen source

Time:

150 ml of sterile production medium was prepared and 5% inoculum was added aseptically. The inoculated medium was incubated at 37°C with shaking around 150rpm. The culture was periodically drown at 12 hrs intervals up to 60 hrs. After incubation the culture filtrate was examined for the total protein content and phosphatase activity by UV Visible Spectrophotometer.

pH:

50 ml of aseptic production medium became organized in specific conical flasks and every flask became adjusted to 5,6,7,8 and 9 pH respectively. Then it has been inoculated with 5% inoculum. The flasks have been incubated at 37ºC at shaker round a hundred and fifty rpm for forty eight hrs. The protein estimation and enzyme activity have been predicted with the aid of using UV Visible Spectrophotometer.

Temperature:

50 ml of aseptic manufacturing medium became prepared in distinct conical flask and inoculated with 5% inoculum. Then it became incubated at 25ºC, 30ºC, 35ºC, 40ºC, and 45ºC for 60 hours. The protein concentration had been predicted by UV Visible Spectrophotometer.

Carbon source:

50 ml of aseptic production based medium (pH 7) was prepared in different conical flasks. Each flask was amended with different carbon sources such as sucrose, Glucose, lactose, maltose and Fructose. Add 5% inoculum and

incubated at thirty seven degree Celsius and kept on shaker around 150 rpm for 48 hrs. The culture filtrate was collected and Protein estimation, Enzyme activity was determined by UV Visible Spectrophotometer.

Nitrogen source:

50 ml of sterile production based medium (pH 7.0) was prepared in different conical flasks each flasks were amended with different nitrogen sources (0.5%) such as casein, peptone, yeast extract, ammonium chloride, and ammonium molybdate. Add 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 by UV Visible Spectrophotometer.

Accessment of Phosphate Solubilizing Bacteria and its Growth of Shoot and Root in BLACK GRAM *Vigna mungo*:

Plate Trial:

- Take Black Gram seeds and soak it for 2 (or) 3 hrs. After Sprouting grow in 10 Petri Plates.
- Mark it as
    1. Control - 2
    2. PSB - 2
    3. Vermicompost - 2
    4. Phosphate - 2
    5. PSB +Vermicompost- 2

Pot Trial:

Materials required:

- Chemical fertilizer
- Organic fertilizer
- PSB
- Soil taken from garden
- Pots
- Red soil
- Faucet water

Preparation of source:

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

2) Phosphate
0.5g of phosphate dissolved in 100 ml of tap water.

3) Phosphate Solubilizing Bacteria
20 ml of bacterial culture filtrate containing enzyme dissolved in 100ml of faucet water.

Procedure:

A pot experiment for phosphate utilization in Black gram (*Vigna mungo*) was made in surface sterilized pots (Plastic pots of about 6 X 10 inches). 8 kg soil was prepared. The soil was divided into ten equal parts and sterilized. After sterilization the 1kgof soil was filled in Ten pots, labeled as control, phosphate(Chemical Fertilizer), Vermicompost, Phosphate solubilizing bacteria,phosphate solubiling bacteria + Vermicompost. 10g seeds were soaked overnight and sowed in each pot 12 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.

Preparation of Source:

S. NO	EXPERIMENTAL GROUP	COMPOSITION	TREATMENT VOLUME
1	Test 1	Control	9 ml of tap water
2	Test 2	PSB	9 ml of PSB
3	Test 3	Vermicompost	9 ml of dissolved Vermicompost
4	Test 4	Phosphate	9 ml of dissolved Phosphate
5	Test 5	PSB + Vermicompost	4.5ml PSB + 4.5ml Vermicompost

Data collection and analysis:

The plant had been taken out from the pot and used for observation. Then by slow movement of the roots in water, the roots had been separated from the soil. Roots had been similarly wiped clean in gently running faucet water and peat loads had been eliminated with forceps. The root became separated from shoot. The length of the shoot became measured from the bottom of the shoot to the developing factor of the youngest leaf. The length of root became measured from the developing factor of root to the longest to be had lateral root apex. Root and Shoot became blotted dry and the burden became recorded earlier than the substances

should get desiccated. The number of roots and leaves became additionally counted through eye estimation.

Chlorophyll study:

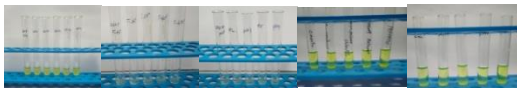
Estimation of Chlorophyll Content by Arnon Method:

An identified amount of black gram leaf tissue a hundred mg became suspended in 10 mL of eighty percent acetone, blended well and stored at 4°C in a single day in dark. Supernatant became withdrawn after centrifugation (5000 rpm) and absorbance became recorded at 663 and 645nm in Spectrophotometer. The quantity of chlorophyll became calculated by Arnon method (1949).

III. RESULTS AND DISCUSSION

Isolated Bacterial Strain: *Pseudomonas sp.*

Chemical Assay:



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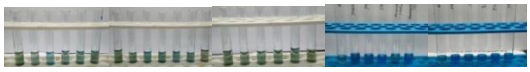
Protein Assay:



a b c d e

a) Time
b) Temperature
c) pH
d) Carbon Source
e) Nitrogen Source

Plate Method Resulst:

a b c d e

a) Control
b) PSB
c) Vermicompost
d) Phosphate
e) PSB + Vermicompost

Pot Method Result:

a b c

d e

a) Control
b) PSB
c) Vermicompost
d) Phosphate
e) PSB + Vermicompost

Phosphate Solubilizing Bacteria is regarded to enhance solubilization of fixed soil phosphorus and carried out phosphates, ensuing in better crop yield. It automatically isolated from rhizosphere soil of numerous flora including wheat, soybean, mustard and chili. Some bacteria are also capable of solbulize the phosphate.

Hence, the PSB are widely distributed and man are isolated from pond water (Milan and Ken 1987), rock phosphate (Takeda and Knight, 2006), poultry waste (Pai et al., 2003), leguminous plant soil, rice rhizosphere soil (Khan et al., 2003) manure (Rajput et al., 2007).

In this present investigation about the PSB species were isolated from rhizospheric soil (Khan et al.,) of Chennai district, Tamil Nadu India.

From the samples, the bacteria consortium had been developed in Pikovskaya liquid medium. On screening the consortium numerous colonies had been discovered at the plates, which gave region of clearing. Four exceptional bacterial colonies had been picked up from the plates of Pikovskaya agar displaying the maximum region of the

clearing round those colonies. From the four bacterial species, based on solubilization performance test higher halo region formed bacterial strain became used for in addition study. The solibilization by the examined bacterial traces on Pikovskaya medium. The Pseudomonas species had been capable of dissolve phosphate effectively, and recorded better dissolving performance as much as 325 (Nguyen et al,. 1992).

The growth examine of the organism is vital for the manufacturing of enzyme because maximum of the extracellular enzymes are produced throughout log segment of the organisms. Since it is an enzyme manufacturing, the changed growth study became carried out.

Effect Of Various Parameters On Phosphatase Production:

Optimization carried out by physical and chemical method:

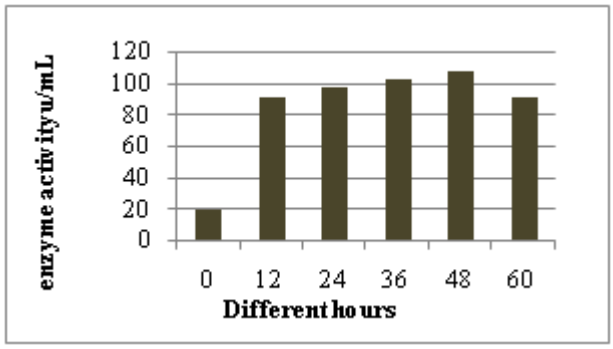
Physical method:

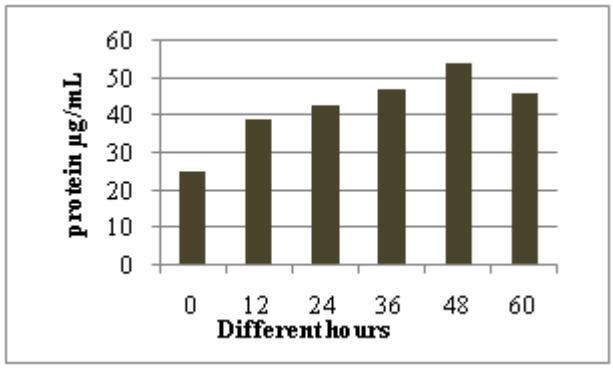
Effect of time:

The bacterial culture was withdrawn and checked for enzyme activity at every 12 hrs and the enzyme activity was investigated. The result revealed that least amount has been produced during 0th hrs (19 U/ml) and high amount was found at 48th hrs (107 U/ml). In the case of total protein of production time experimental group, the high amount recorded at 48th hrs (54µg/ml) and least at 0th hrs (25µg/ml).

Phosphatase production on different incubation times:

S.No	Time of culture withdrawal (hr)	Total Protein (µg/ml)	Total Enzyme Activity (U/ml)
1	0	25	19
2	12	39	91
3	24	43	97
4	36	47	102
5	**48**	**54**	**107**
6	60	46	90



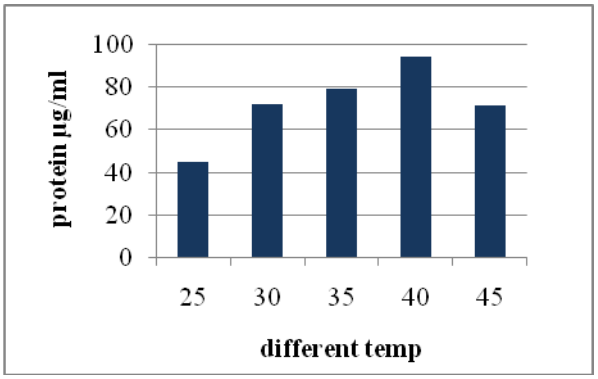


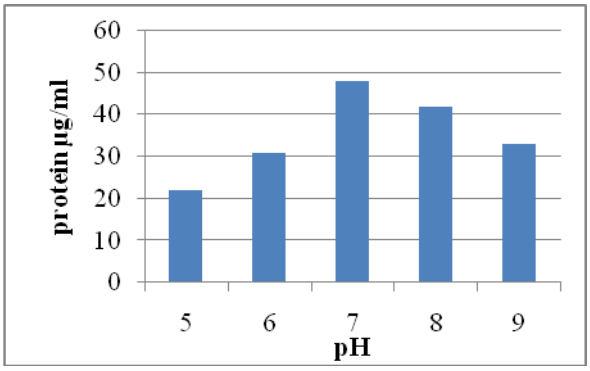
Effect of temperature:

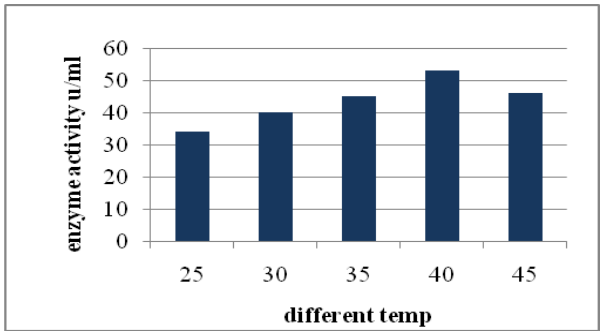
The environmental parameters are displaying tremendous impact in the growth of the organism and the manufacturing of enzyme. The essential parameters like temperature, pH, carbon supply and nitrogen supply are very vital parameters of the manufacturing. To optimize the foremost temperature for the higher yielding, productions have been made in several temperatures. It became determined that the isolate has proven better phosphatase activity (53U/ml) at 40ºC. The excessive quantity of protein recorded at 40ºC (94µg/ml) and least at 25ºC (45µg/ml).

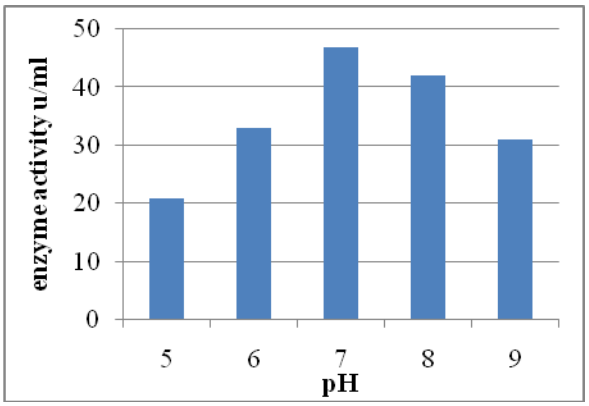
Effect of temperature on phosphatase production:

S.No	Temperature (°C)	Total Protein (µg/ml)	Total Enzyme Activity (U/ml)
1.	25	45	34
2.	30	72	40
3.	35	79	45
4.	**40**	**94**	**53**
5.	45	71	46









Effect of pH:

Next to the temperature, the pH is the essential parameter which determines the growth of the organism and phosphatase manufacturing. Usually maximum of the micro organism require medium pH for its development. The requirement of the pH for its higher growth is also based on the environmental pH wherein they grow. The Pseudomonas species isolated from the soil sample has proven better manufacturing at pH 7. (Ben Mesaoud et al., 2004).

In the existing study consequences showed that the better manufacturing had been recorded at best pH variety from 7 (47 U/ml), so this Pseudomonas species need an alkaline pH for their maximum enzyme manufacturing. In the case of protein of pH experimental group, the excessive quantity recorded at 7 (48µg/ml) and least at (22µg/ml).

Influence of pH on phosphatase production:

S.No	pH	Total Protein (µg/ml)	Total Enzyme Activity(U/ml)
1.	5	22	21
2.	6	31	33
3.	**7**	**48**	**47**
4.	8	42	42
5.	9	33	31

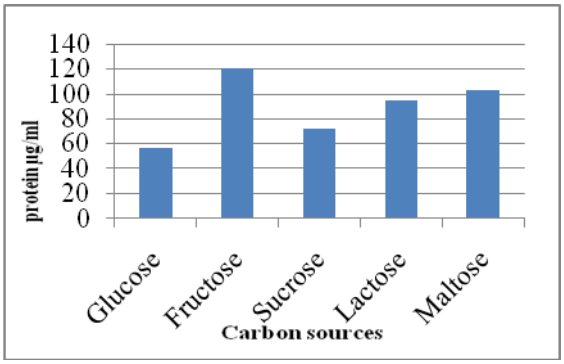
Chemical Method:

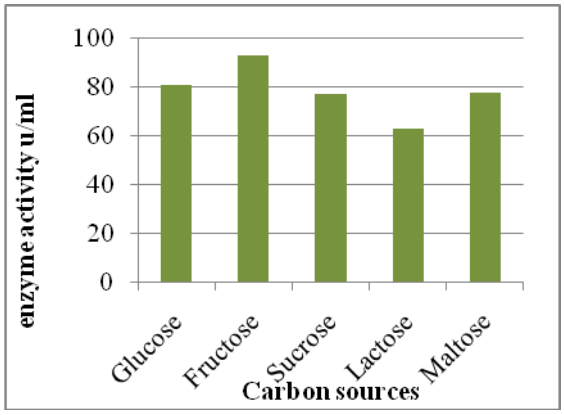
Effect of carbon source:

The carbohydrates are essence strength supply for maximum of heterotrophic organisms. The isolate has produced higher quantity of phosphatase from Fructose (93 U/ml). In the case of experimental group, the excessive quantity of protein recorded at fructose (120µg/ml) and least at glucose (57µg/ml). (Ben mesaoud et al., 2004).

Carbon source optimization for the production of phosphatase:

S.No	Carbon sources	Total Protein (µg/ml)	Total Enzyme Activity (U/ml)
1.	Glucose	57	81
2.	**Fructose**	**120**	**93**
3.	Lactose	72	77
4.	Sucrose	85	63
5.	Maltose	103	78



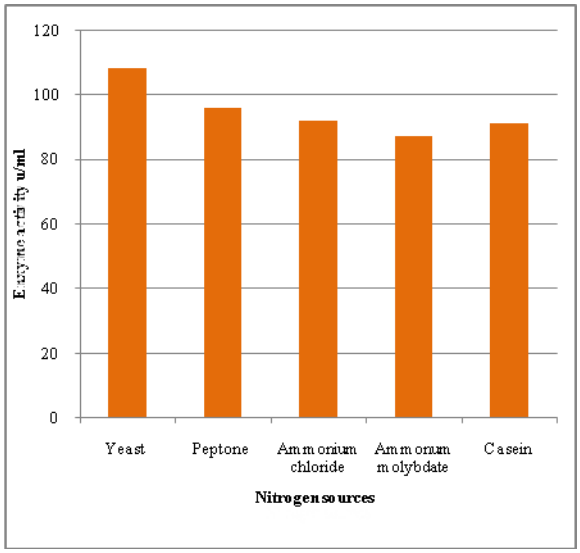


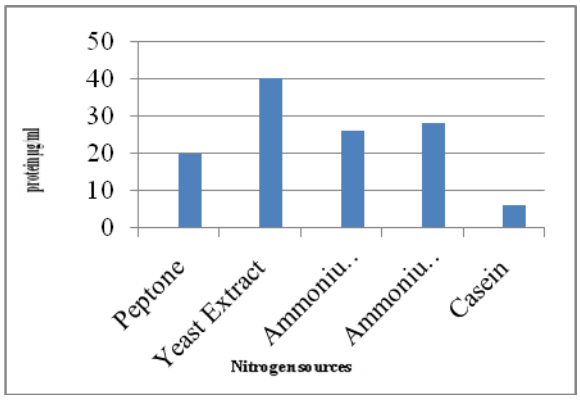
Effect of nitrogen source:

The nitrogen supply is of secondary strength supply for the organisms, which play an vital function withinside the development of the organism and the manufacturing. The nature of the compound and the concentration that we're using can also additionally stimulate or down modulate the manufacturing of enzymes. The inorganic nitrogen supply Yeast extract (108 U/ml) became discovered to be a higher nitrogen supply for this isolated bacterial culture and withinside the case of overall protein of pH experimental group, the excessive quantity recorded at Yeast extract (40µg/ml) and least at Casein (6µg/ml). (Qureshi et al.,2010).

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.	Peptone	22	96
2.	**Yeast extract**	**40**	**108**
3.	Ammonium chloride	26	92
4.	Ammonium molybdate	28	87
5	Casein	6	91





Accessment Of Phosphate Solubilizing Bacteria And Its Growth Of Shoot And Root In Black Gram *Vigna mungo*:

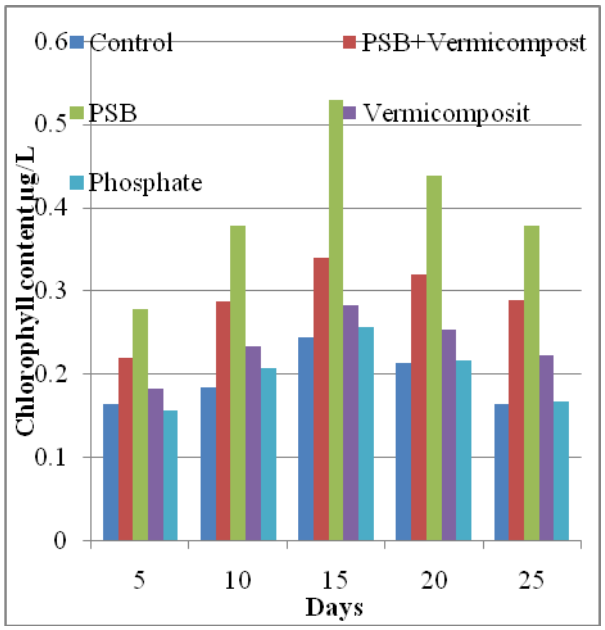
The fifteenth day old plants were taken for the shoot and root length, number of leaves, number of roots. The recorded consequences in application part of in this present research provided following. All together, the test 2 showed best development than the all different group. In the case of total moist mass maximum found in Test 2, and followed by 5, 4, 3 and 1. Best dry weight found in Second Test, and followed by 5, 3, 4 and 1. Highest stem length became found in Test two and followed by 5, 3, 4 and 1. Best root length became found in T 2 and followed by 3, 5, 4 and 1. The overall length became found maximum in Test 2 and followed by 5, 3, 4 and 1. Number of leaves became found maximum in Test 2 and followed by 3, 5, 4 and 1. Many roots became found in Test 2 and followed by 5, 3, 4 and 1.

Test 1: Control **Test; 2:** Phosphate Solubilizing Bacteria; **Test 3:** Vermicompost; **Test 4:** Phosphate; **Test 5:** PSB + vermicompost.

	Shoot length			Root length			Total length			No. of leaves			No. of roots			Wet	Dry wt.
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	wt.	
Control	8.5	9.2	7.4	3.5	5.5	2	17.3	19.2	14	16	15	15	11	10	8	0.95	0.19
PSB	**9.8**	**10**	**8.9**	**4.5**	**3.8**	**3.5**	**22**	**17.5**	**19.1**	**17**	**15**	**16**	**11**	**12**	**14**	**2.40**	**0.80**
Vermicompost	10	8.5	8.6	3.7	4	1.5	16.2	17.8	15.9	10	15	13	9	11	10	1.09	0.32
Phosphate	9	7.6	6.5	3.7	1.5	1.6	14	12.8	12.6	7	10	12	8	7	5	1.17	0.25
PSB + Vermicompost	8.8	8.5	10	3.8	3.3	5	16	17.2	17.3	12	14	13	7	12	8	1.84	0.56

Chlorophyll Study Result:

DAYS	5	10	15	20	25	AVERAGE
Control	0.163012	0.183012	0.243012	0.213012	0.163012	0.061759
PSB+Vermicompost	0.218671	0.286707	0.338671	0.318671	0.288671	0.041536
PSB	**0.278379**	**0.378379**	**0.528379**	**0.438379**	**0.378379**	**0.042304**
Vermicomposit	0.182287	0.232287	0.282287	0.252287	0.222287	0.062813
Phosphate	0.15606	0.20606	0.25606	0.21606	0.16606	0.048212



IV. CONCLUSION

- In this present study *P. species* become isolated and used for manufacturing phosphatase in LB Broth medium.
- The parameters like Time, Temperature, pH, Carbon and Nitrogen supply have been optimized for higher manufacturing of phosphatase.
- In consideration of time at forty eight hours showed most enzyme activity manufactured.
- Among unique temperatures the most phosphatase became recorded at 40°C temperature.
- In pH parameter, pH 7 showed most manufacturing of phosphatase activity Pseudomonas species. In the case of carbon fructose and in nitrogen yeast extract have been showed the better manufacturing of phosphatase.
- Pot trials become done with *Vigna mungo* for phosphate utilization and most measurement become best in Test 2.
- The above highlighted outcomes are preliminary records for confirming the performance through *V. mungo*. Further study will need for the nutrients value, yield, flowering etc. for identify the ability.

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