Comparative efficiency of five potash and phosphate solubilizing bacteria and their key enzymes useful for enhancing and improvement of soil fertility

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Abstract- Series of experiments were carried out to investigate K mobilization and their abilities of native KMB & P solubilization and their abilities of native PMB. Total five isolates showed their prominent efficiency of K mobilization & P mobilization. Among five isolates two best cultures were PBA16 *Bacillus coagulans* & M29 *Bacillus megaterium* for PSB and almost all five in same range for KMB. P solubilizing index was carried out in Sperber & Aleksandrov agar media supplemented with ROCK phosphate and Tri- calcium phosphate (TCP). ,PBA16 gave 4.60 solubilizing index and maximum P release 36.69 at µg/ml in 6 DAI supplemented with TCP and 68.80 µg/ml in 6 DAI supplemented with Rock phosphate. All KMB isolates exhibited acid production in media with tolerate wide range of pH concentration In mass production, all five cultures showed well growth in Sperber, Aleksandrov & Phytate media after 5 to 6 day after incubation. Their colonies were round or irregular, white and their shape was rod and motile. They were capable of dissolving both phosphate and potassium and PBA16 & M29 strains had high phosphate and potassium dissolution capacity effectively. All five cultures for KMB & PSB strains were characterized through morphological, physiological characteristics and their enzymatic activity.

I. INTRODUCTION

In the last century, when the chemical fertilizers were first introduced into the agriculture, most of the problems faced by farmers to increase crop yield were solved. However, chemical fertilizer slowly started to show their side effect in agro ecosystem and environment ultimately harming human and other animals. In view of that use of biofertilizers and organic manners were recommended to apply in integrated manner for improvement of plant nutrient supply and there by sustainable crop production (Han *et al*., 2006). Basically, biofertilizers are living microorganisms that colonizes the rhizosphere or the zone that surrounds the roots of plants (Shen, 1997). These microorganisms have ability to convert nutritionally important macro elements such as nitrogen, phosphorus and potassium (NPK) from unavailable to available form through biological processes (*Ahmed*, 2009). This group of bacteria are also broadly termed as 'plant growth promoting rhizobacteria' (PGPR) [6] and Amongs them key genera are *Pseudomonas, Azospirillum, Burkholderia, Bacillus, Enterobacter, Rhizobium, Erwinia, Serratia, Alcaligenes, Arthrobacter, Acinetobacter* and *Flavobacterium.* etc. (*Rodriguez, Fraga, 1999*).

Most of the essential plant nutrients, including phosphorus and potash, are naturally found as insoluble forms in soil. A large portion of the inorganic phosphate applied to soil as fertilizer is rapidly immobilized after application and becomes unavailable to plants. Thus, the release of insoluble and fixed forms of phosphorus and natural potash of soil are important aspect of increasing soil macro nutrient availability. Microorganisms such as phosphate solubilizers and potash mobilizer can play a vital role in release of available forms of phosphorous and potash from insoluble forms present in natural pool.

II. PHOSPHATE SOLUBILIZING MICROORGANISMS

A large portion of inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized and becomes unavailable to plants. Thus, the release of insoluble and fixed forms of phosphorus is an important aspect of increasing soil phosphorus availability. Microorganisms have an important role in the phosphorus conversion processes in the soil. Phosphorus found in the chemical structure of microorganisms as phosphates and plays a significant part in the energy metabolism like in the phosphorylation reaction of high energy compounds formation etc. in respiratory and fermentative processes in addition; also it has a plastic function, as involved in the synthesis of nucleoproteins and lipids. The prime mechanism of mineral phosphate solubilization by microorganism is the production of organic acids and acid phosphatases which mineralize and mobilize phosphorus in soil. It is generally accepted that the major mechanism of mineral phosphate solubilization is the action of organic acids synthesized by soil microorganisms resulting in acidification of its surroundings. Both gram + ve and - ve organisms like *Pseudomonas, Bacillus, Burkholderia* etc. are among the most powerful phosphate solubilizers (Mehta and Nautiyal, 2001; Singh *et al*., 2011). Since 1956, based on their phosphorus nutrition, the microorganisms have been classified in five groups based on cherecteristics. Bacteria assimilating to the same extent of mineral and organic phosphorus; bacteria assimilating particularly mineral phosphorus; bacteria assimilating particularly organic phosphorus; bacteria solubilizing tricalcium phosphorus in the form of glycerophosphate; bacteria solubilizing triclcium phosphorus.

Phosphorus is used differently by the various microorganisms due to their participation in the processes of the solubilisation and fixation of phosphates in the soil mineralization. There is evidence that the chemical nature of

most of the organic phosphorus in the soil is of microbial origin. Most of the microorganisms possess an enzymatic system which enables them to mineralize phosphorus containing organic compounds, and the phosphatase activity of the microorganisms has been known for a long time. Up to 50% of the microorganism isolated from the soil and rhizosphere exhibit phytase activity. Atlas (1998) considers that bacteria have both phytase and nuclease and a result of it able to mineralize not only glycerophosphate but also lecithin. In 1964, Mazkin and Kuznetova divided soil microorganisms according to their enzymatic mechanism in five groups as follows: microorganisms with low phosphatase activity (e.g. mycobancteria, micrococci, bacillus mycoides); microorganisms with high phosphatase activity (e.g. pseudomonadaceae family representatives); microorganisms with high phosphatase and ribonuclease activities (e.g. *Bacillus megaterium*, which possesses a glucose induced adaptive ribonuclease) and microorganisms with high phosphatase, ribonuclease and deoxyribonuclease activities (e.g. *B. subtilis*, *B. cereus*).

III. POTASH MOBILIZING MICROORGANISMS

Plants absorb potassium from the soil and its availability in soil is dependent upon the K dynamics as well as on total K content. Out of the three forms of potassium found in the soil, soil minerals make up more than 90 to 98 per cent of soil potassium [2]. The second non-exchangeable form of potassium up to 10 per cent of soil potassium is predominantly the interlayer K of non-expanded form as elite and lattice in K-feldspars [3, 4]. Release of non-exchangeable K to the third exchangeable form occurs when level of exchangeable and solution K is decreased by crop removal, runoff, erosion and/or leaching [2, 5] (*Priyanka Parmar et al.*, 2013).

Many microorganisms in the soil are able to solubilize 'unavailable' forms of K-bearing minerals, such as micas, illite and orthoclases by excreting organic acids which either directly dissolves rock K or chelate silicon ions to bring the K into solution (Groudev, 1987; Friedrich *et al*., 1991; Ullman *et al*., 1996; Bennett *et al*., 1998). Therefore, the application of K solubilizing microorganisms (KSM) (Zahra *et al*., 1984; Vandevivere *et al*., 1994; Barker *et al.*, 1998) is a promising approach for increasing K availability in soil. Production of carboxylic acids like citric, tartaric and oxalic acids is also associated with feldspar solubilization by microrganisms (Malinovskaya *et al.*, 1990; Sheng and Huang, 2002b Sheng, 2005).

Plant growth-promoting bacteria (PGPR) have been reported to be the key elements for plant establishment under nutrient-imbalance conditions. Their use in agriculture can favours the reduction of agro-chemical use and support eco-friendly crop production (Herrera *et al*., 1993; Glick, 1995; Requena *et al*., 1997). PGPR can also improves of plant growth, plant nutrition uptake root growth pattern, plant competitiveness and responses to external stresses. Different PGPR including associative bacteria such as *Azospirillum, Bacillus, Pseudomonas, Enterobacter* have been used for their beneficial effects on plant growth (Kloepper and Beauchamp, 1992; Hoflich *et al*., 1994 Sheng, 2005).

IV. MATERIALS AND METHODS

The present investigation was undertaken with the aim of comparative efficacy of five potash and phosphate solubilizing bacteria and their key enzymes useful for enhancing and improvement of soil fertility to exploit their.

The detailed information about materials used and methods adopted for the present research work has been elaborated in this chapter. The study was conducted at Department of Agricultural Microbiology, B.A. College of Agriculture, Anand Agricultural University, Anand, during Feb 2014 to April 2014.

Qualitative estimation of phosphate and potash solubilizing efficiency of test cultures on agar plates.

All the isolates were spot inoculated on Sperber's medium supplemented with tri calcium phosphate and Aleksandrove's media containing mica for testing mineral phosphate and potash substrate solubilisation respectively. Plates were incubated at 30±2˚C for five days with observation on colony diameter every 24 h. Clear zone formation around the growing colony indicated phosphate solubilization activity. Solubilization Index (SI) was calculated according to method described by Collavino *et al.* (2010) using following formula. Experiment was also carried out for checking culture's efficiency by replacing Calcium phytate in place of TCP.

Qualitative estimation of phosphate solubilization efficiency in liquid medium.

Erlenmeyer flasks (250 ml) containing 100 ml of the sterilized liquid Sperber's medium were inoculated with 100 µl of bacterial suspension (approx. 10^8 cfu/ml). For each isolate three flasks were inoculated. The flasks were incubated on rotary shaker (150 rpm) at 30+ 2˚C. After 3, 5, 7 and 10 days, measurement of pH using pH meter and liberated P in broth following Vanado-molybdate method was carried out (Jha *et al*., 2009). The graph of OD versus concentration of phosphate in µg was plotted for the standard and samples were compared to calculate P concentration.

Qualitative estimation of potash solubilizing efficiency in liquid medium

Hundred (100) ml of sterilized Aleksandrov media containing mica as mineral potash source. 250 ml Erlenmeyer flask were inoculated with 100 µl of bacterial suspension (approx. 10^8 cfu/ml) and incubated at 30±2˚C for 10 days. For each isolate three flasks were inoculated. The flasks were incubated on rotary shaker (150 rpm) at 30±2˚C. After 3, 5, 7 and 10 days, each flask was checked for potassium release by flame photometry. The suspension was centrifuged at 10,000 rpm for 10 min and supernatant was retained. 1ml of supernatant was taken in 50 ml volumetric flask and volume was made to 50 ml with distilled water and mixed thoroughly. After that the solution was fed to flame photometer for estimating K (Hu *et al*., 2006).released from the mineral mica individually by test bacteria.

Qualitative estimation of phosphatase enzyme activity
Estimation of acid phosphatase activity

Incubated 3 ml of substrate (pera nitrophenol) at 37°C for 5 min. Added 0.5 ml of enzyme extract and mix well. Remove

immediately 0.5 ml from this mixture and mix it with 9.5 ml 0.085N NaOH. This corresponds to zero time assays (Blank). Incubated the remaining solution with Substrate enzyme for 15 min at 37°C. Drawn 0.5 ml of sample and mixed it with 9.5 ml NaOH solution. Measured absorbance of blank and incubated tubes at 405 nm on spectrophotometer. Taken 0.2 to 1.0 ml (4 to 20 mH) of standard, dilute to 10.0 ml with NaOH solution. Read the colour following standard curve was prepared to find out unit activity.

Estimation of alkaline phosphatase activity

Incubated 3 ml of substrate (pera nitrophenol) at 37°C for 5 min. added 0.5 ml of enzyme extract and mixed well. Removed immediately 0.5 ml from this mixture and mixed it with 9.5 ml 0.085N NaOH. This corresponds to zero time assay (Blank). Incubated the remaining solution with substrate enzyme for 15 min at 37°C. Drawn 0.5 ml of sample and mix it with 9.5 ml NaOH solution. Measured absorbance of blank and incubated tubes at 405 nm on spectrophotometer. Take 0.2 to 1.0 ml (4 to 20 mH) of standard, diluted to 10.0 ml with NaOH solution. Read the colour following standard curve was prepared to find out unit activity.

Determination of phytase enzyme activity

For primary screening, 100µl suspension of these flasks was plated onto a phytase screening turbid agar media plates (PSM) containing 1.5% glucose, 0.1% Na- phytate (Hi media, India), 0.2% NH4NO3, 0.05% KCl, 0.05% MgSO4.7H2O, 0.03% MnSO4, 0.03% FeSO4.7H2O and 2.0% agar (pH 7.5) and incubated at 50°C for 2-5 days. Bacterial isolates, capable of hydrolyzing Na-phytate which were recognized by their surrounding clear halo, were further selected and repeatedly streaked on nutrient agar plates. Hydrolytic zone was calculated by subtracting the diameter of zone of growth from diameter of total halo area.

Quantitative analysis of phytase producing microorganisms by spectrometry

Each of the bacterial isolates were grown in 50ml of liquid medium containing 0.1 % sodium phytate, 1% peptone, 0.2% (NH4)2SO4, 0.05% KCl, 0.05% MgSO4.7H2O, 0.03% MnSO4, 0.03% FeSO4.7H2O, pH 7.5 in a 250ml flask and incubated at 50°C for three days on a rotary shaker at 200rpm. Crude enzyme was harvested by centrifugation at 10,000g for 10mins at 4°C and the clear supernatant was used as the source of extracellular phytases.

Sodium phytate was used as substrate for assaying the activity of phytase. Phytase activity was determined by measuring the amount of liberated inorganic phosphate. The reaction mixture consisted of sodium phytate (Sigma; 0.5% w/v) prepared in sodium acetate buffer (0.2 M, pH 5.5) and 0.2ml of supernatant. After incubation at 50°C for 30mins, the reaction was stopped by adding an equal volume of 15% Trichloroacetic acid. The liberated phosphate ions were quantified by mixing 100µl of assay mixture with 900µl of 1.0 M H2SO4 10% ascorbic acid- 2.5% ammonium molybdate (3:1:0.1) (v/v). After 20mins of incubation at 50°C, absorbance was measured at 700 nm.

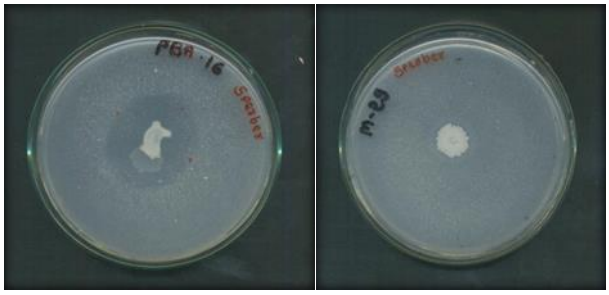
V. Result and Discussion

Screening and selection of efficient microorganisms having beneficial characters to optimize higher crop yield and to conserve the sustainability of the agricultural ecosystem is an important approach in Agricultural Microbiology. The present investigation was aimed to have comparative efficacy of five phosphate and potash solubilizing native bacteria and their key enzymes useful for enhancing soil fertility *in vitro*.

Qualitative estimation of phosphate solubilization efficiency of test cultures

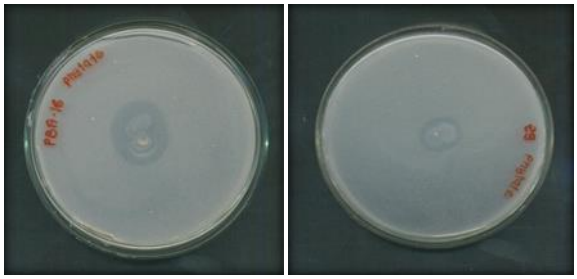
Phosphate solubilization efficiency of test cultures was tested on Sperber's media supplemented with 'P' substrates like Tri Calcium phosphate (TCP) and Calcium phytate.

Tri calcium Phosphate solubilization by selected strains



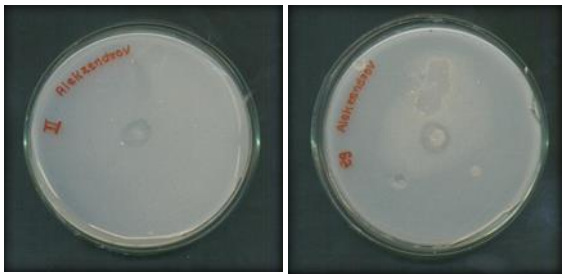
Total 5 native microbial strains were screened for P solubilization activity on Sperber's agar media supplemented with TCP as source of phosphorous. The solubilization zone formation was observed at 5 days. The SI for different isolates was ranging from 1.0 to 4.60 and 1.0 to 3.65 in Sperber's medium. The highest SI was recorded for *B. coagulans* II (4.60) followed by *E. asburiae*, *B. coagulans* I, *Bu. metallica* and *B. megaterium* as 1.0, 4.41, 1.00 and 2.13 respectively. While the lowest SI was recorded to be 1.0 for isolate *Bu. metallica* and *E. asburiae.*

Solubilization of Ca-phytate by native strains



The ability of the isolates to solubilize insoluble organic phosphate in form of Ca-Phytate was also tested in media containing Ca- Phytate as sole source of phosphate. After 5 days of incubation the zone diameter were recorded and SI was worked out. The highest SI 3.13 was recorded for isolate B. coagulans II followed by isolate *E. asburiae*, *B. coagulans* I, *Bu. metallica* and *B. megaterium*. Standard check *B. coagulans* I was recorded with SI 3.13. SI for all the isolates was found statistically different from each other.

Qualitative estimation of potash (K) solubilization efficiency of test cultures



Potash solubilization efficiency of five cultures was tested on Alekzendrov's media containing mica as source of mineral potash. The highest SI in Alekzendrov's medium was recorded for isolate *B. coagulans* I (3.55) followed by *B. megaterium*, *E. asburiae*, *B. coagulans* II and *Bu. metallica*.

Quantitative estimation of mineral phosphate and potash solubilisation by selected test cultures

Estimation of phosphate by VM method

Phosphate solubilization assay in liquid medium was performed with two insoluble P substrates, TCP and rock phosphate in MS broth. Observations were recorded at 3, 5, 7 and 10 days after inoculation and release of P was estimated by vanedo-molybdate method. Prior to media preparation the substrates were thoroughly washed with sterile double distilled water to remove free P residues.

Total P release from TCP in Sperber's broth

All of 5 strains showed significantly higher solubilization of phosphate than control and in Sperber broth with tri calcium phosphate, strain *E. asburiae* and *B. megaterium* had P-solubilizing ability up to 51.78 µg/ml and 53.41 µg/ml after 6 day of incubation respectively and *B. coagulans* I had 77.47 µg/ml after 8 days of incubation respectively.

All the five strains showed decrease in pH of the medium as then soluble P content increases this shows that the organisms release phosphorous as a result of organic acid production in the medium.

Total P release from rock phosphate in Sperber's broth

P solubilizing bacteria plays an important role in plant nutrition through the increase in P uptake by the plants. Applications of phosphate solubilizing microorganisms have been used as P-biofertilizer for crop cultivation and reported increased soil fertility. In present study the strain effective in P-solubilization are *B. coagulans* II and *B. megaterium* which are found is have good potential to be applied biofertilizer to increase 'P' in soil and assimilation of phosphate which will be useful to increase crop yield. In Sperber broth with rock phosphate, *B. coagulans* II & *B. megaterium* were found as most efficient strains and P-solubilizing ability up to 68.80 µg/ml and 103.57 µg/ml after 6 days of incubation respectively. pH values were measured at 2 to 8 days of incubation and the results showed that pH value were always higher than 6.5.

Quantitative estimation of mineral potash solubilisation by test cultures

Testing of potash mobilizing capacity of bacteria in liquid assay by flame photometry

After observing the positive solubilization index of potential KMB isolates on Aleksandrov agar plates supplemented with mica, the isolates were further analyzed in Aleksandrov liquid media for K release with broth from minerals feldspar and mica from 2 DAI to 8 DAI. In Aleksandrov media supplemented with feldspar isolate *E. asburiae* gave highest K release 4.85 µg ml^{-1} at 6 DAI, which was declined to 4.50 µg ml^{-1} at 8 DAI and considered as best K solubilizer, next was *Bu. metallica* which showed 4.78 µg ml^{-1} of K release at 8 DAI. Least K release was exhibited by *B. coagulans* I which gave 3.07 µg ml^{-1} at 8 DAI and *B. coagulans* II exhibited 3.51 µg ml^{-1} at 8 DAI, K release in broth.

Qualitative and quantitative analysis of organic acids production in liquid media by HPLC

HPLC analysis of culture filtrate was carried out to identify and quantify the organic acids produced during solubilization of TCP by selected two cultures *Bu. metallica* and *B. megaterium* at 5 DAI. During TCP solubilization, it was found that the major organic acids produced by both the bacteria are lactic and gluconic acids.

Total organic acid production was recorded as 35,708.78 µg/ml and 5122.3 µg/ml of *Bu. metallica and B. megaterium* respectively, which was lowest among all the isolates which can be correlated with its P solubilization efficiency of this culture. Overall results showed that lactic acid is the major organic acid produced by both the isolates followed by gluconic acid. Among the five native cultures, it was observed that two cultures *Bu. metallica* and *B. megaterium* were found overall best for 'P' solubilizers and their organic acid profiling was carried out using HPLC.

Sr. No.	Organic acid	Concentration (µg/ml) after 5 DAI	
		Bu. metallica	***B. megaterium***
1	**Oxalic acid**	-	0.0
2	**Gluconic acid**	35205.03	1772.2
3	**Tartaric acid**	-	0.0
4	**Pyruvic acid**	-	0.0
5	**Malic acid**	-	0.0
6	**Malonic acid**	-	0.0
7	**Lactic acid**	80.80	3185.8
8	**Acetic acid**	106.66	0.0
9	**Citric acid**	43.87	0.0
10	**2-Keto Gluconic acid**	153.94	38.6
11	**Propionic acid**	118.47	125.7
12	**Butyric acid**	-	0.0
13	**Succinic acid**	-	0.0
Total Organic acids		**35708.78**	**5122.3**

Quantitative estimation of mineral phosphatase and phytase enzymes of selected cultures

Determination of acid phosphatase activity by phosphorous solubilizing bacteria

Extracellular acid phosphatase was eluted on rock phosphate and TCP, after elution the unit activity of five strains of bacteria was increased in 8 days. While, in contrast to our results, extracellular acid phosphatase was obtained after 6 days in *Bu. metallica* 0.143 µg ml^{-1} and *E. asburiae* 0.061 µg ml^{-1} in rock phosphate and *B. coagulans* II 0.061 µg ml^{-1} as higher concentration and after 8 days in *B. coagulans* I 0.097 µg ml^{-1} in rock phosphate and *E. asburiae* 0.112 µg $ml^{-1}min^{-1}$, *B. megaterium* 0.164 µg ml^{-1} as higher concentration than other strains respectively.

Determination of alkaline phosphatase activity by phosphorous solubilizing bacteria

Extracellular alkaline phosphatase was obtained after 6 days in *B. megaterium* (0.159 µg/ml/min), *Bu. metallica* (0.143µg/ml/min) in rock phosphate broth and *B. coagulans* II (0.148 µg/ml/min), *Bu. metallica* (0.143 µg/ml/min) as higher concentration and after 8 days in *B. coagulans* I (0.097 µg/ml/min) in rock phosphate and *E. asburiae* (0.112 µg/ml/min), *B. megaterium* (0.164 µg/ml/min) as higher concentration than other strains respectively.

One unit of phosphatase is the amount which hydrolyses 1µmol of substrate per minute at acidic pH and temperature 37°C

***In vitro* testing of phytase producing capacity of bacteria in liquid assay and plate assay**

The data from the quantitative determination of phytase activity in phytate medium showed that total phytase enzyme produced by *B. coagulans strain PBA 16* strains showed activity above 0.008 U/ml after 6 days. Whereas, after 8 days other isolates activity reduced expect *B. megaterium*, which is increased above 0.003 U/ml.

One unit of phytase is defined as the amount of enzyme that liberates one µ mol inorganic phosphate mL^{-1} min^{-1} under the assay condition.

VI. Summery and Conclusion

Five native cultures were screened for P solubilization and their P solubilizing index was worked out in Sperber agar media supplemented with TCP as source of phosphorus and observed that three isolates viz. *Bu. metallica*, *B. coagulans* and *B. megaterium* gave good halo than others. Whereas solubilization index was carried out in phytate medium was also showedgood halo zone of two isolates.

P solubilization efficiency in liquid medium indicated that isolate *B. megaterium* and *E. asburiae* are found best and solubilize maximum amount of TCP releasing 53.41 µg/ml P and 51.78 µg/ml at 6 DAI respectively. For rock phosphate maximum P release 103.58 µg/ml and 68.80 µg/ml recorded on 6 DAI for isolates *B. coagulans* II and *B. megaterium* respectively.

The overall results indicated that two native PSB isolates have been established as more potent cultures and have significantly importance for soil fertility and P fertilizer. Entire scenario of different experimentations signifies that, PSB isolate *B. megaterium* and *B. coagulans* II used as P-biofertilizer to increase 'P' in soil and also used to increase crop yield with best potency for enhancement of soil fertility.

Selected two native PSB isolates one gram negative and one are gram positive viz. *B. megaterium* and *Bu. metallica* respectively, were further screened for organic acid production on media supplemented with pH indicator dye bromo phenol blue (BPB) primarily exhibited positive results of acid secretion. HPLC profiles for qualitative and quantitative analysis of organic acids showed presence of different organic acids *viz*. oxalic, gluconic, malonic, lactic, acetic, citric, 2-KG, butaric, propionic, malic and tartaric acid in culture filtrates of isolates among which gluconic acid was predominant in all the isolates tested. Isolate *Bu. metallica* found to produce highest amount of organic acid i.e. 35,708.78 µg/ml on 5 DAI. Isolate *B. megaterium* also found to produce total 5122.3µg/ml of organic acid.

Among the all enzymatic activity indicate that extracellular phosphatase was produced by the five native isolates was noticed to be low compared to 'P' solubilizers sited in literature. All five cultures produce low acid and alkaline phosphatase and thus there was lesser hydrolysis of the substrate hence show lower activity.

Five native cultures of K and P solubilizers showed their K solubilizing index was carried out in Aleksandrov agar media supplemented with mica as source of mineral potash and observed that two isolates gave good zone of solubilization than others.

Potash mobilizing efficiency in Aleksandrov liquid media supplemented with mica as a source of mineral potash indicated that isolate *E. asburiae* was best potash mobilizer showing 4.85µg/ml of K release from feldspar at 6 DAI and 4.50 µg/ml of K release from mica at 8 DAI.

Overall results indicated that five native cultures can significantly improves plant growth if used in field due to their PGPR activity like P and K solubilization. Entire scenario of different experimentation signifies that native isolates have best potency for 'P' & 'K' mobilizing and their by for the improvement of soil fertility ultimately for better crop productivity.

References

[1] Aleksandrov, V. G., Blagodyr, R. N. and Iiiev, I. P. (1967). Liberation of phosphoric acid from apatite by silicate bacteria. Mikrobiyol Zh. (Kiev), 29: 111-114.

[2] Ahmed, Hala F. S. and El-Araby, Magda M. I. (2012). Evaluation of the influence of nitrogen fixing, phosphate solubilizing and potash mobilizing biofertilizers on growth, yield, and fatty acid constituents of oil in peanut and sunflower.African Journal of Biotechnology, 11(43): 10079-10088.

[3] Archana (2007). Studies on potassium solubilizing bacteria. M.Sc. Thesis submitted to the University of Agricultural Sciences, Dharwad.

[4] Bae HD, Yanke LJ, Cheng, KJ , Selinger LB (1999). A novel staining method for detecting phytase activity. J. Microbiol. Meth. 39: 17-22.

[5] B. C. Antanaitis & P. Aisen (1983). "Uteroferrin and the purple acid phosphatases". Advances in Inorganic Biochemistry 5: 111–136. PMID 6382957.

[6] Beacham IR. Periplasmic enzymes in gram-negative bacteria. Int J Biochem. 1979;10:877–883.

[7] Baoudene-Assali, F., J. Baratti, and G. P. F. Michel. 1993. Purification and properties of a phosphate-irrepressible membrane-bound alkaline phosphatase from Zymomonas mobilis. J. Gen. Microbiol. 139:229–235.

[8] Barre, P., Velde, B., Abbadie, L. (2007). Dynamic role of "illite-like" clay minerals in temperate soils: facts and hypothesis. Biogeochemistry, 82: 77-88.

[9] Craven PA, Hayasaka SS. (1982) Inorganic phosphate solubilization by rhizosphere bacteria in a Zostera marina community. Can J Microbiol;28:605–10.

[10] Carmany DO, Hollingsworth K, Mc-Cleary WR. (2003) Genetic and biochemical studies of phosphatase activity of PhoR., J Bacteriol.;185:1112–1115.

[11] Cho JS, Lee W Ch, Kang SH, Lee J Ch, Bok JD, Moon YS, Lee H G, Kim S Ch, Chio YJ (2003). Purification and characterization of a phytase from Pseudomonas syringae MOK1. Curr. Microbiol. 47: 290-294.

[12] Chung, H., Park, C. H., M., Madhaiyan, M., Seshadri, S., Song, J., Cho, H. and Sa, T. (2005). Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biology & Biochemistry, 37: 1970 1974.

[13] FAI (2007). Fertiliser Statistics 2006–2007. The Fertiliser Association of India, New Delhi.

[14] Fiske, C.H. and subbarow, Y. (1925). The colorimetric determination of phosphorous. J. Biol. Chem. 65: 375-380.

[15] Gerhardt, P., Murray, R. G. E., Costelow, R. N., Nexter, E. W., Wood, W. A., Kreig, N. P. and Phillips, G. B.,(1981). Manual of Methods of General Bacteriology. American Society of Microbiology, Washington, D.C. pp. 400-450.

[16] Girgis, M. G. Z., Khalil, H. M. A., Sharaf, M. S., (2008). Invitro evaluation of rock phosphate and potassium solubilizing potential of some Bacillus strains. Australian Journal of Basic and Applied Sciences, 2(1):68-81.

[17] Glew, R. H., and E. C. Heath. 1971 a. Studies on the extracellular alkaline phosphatase of Micrococcus sodonensis. II. Factors affecting secretion. J. Biol. Chem. 246:1566–1574.

[18] Han HS, Lee KD (2005) Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability and growth of eggplant. Res J Agric Biol Sci 1:176–180

[19] Han HS, Supanjani, Lee KD (2006). Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ 52:130–136

[20] Halder AK, Chakrabartty PK. Solubilization of inorganic phosphate by Rhizobium. Folia Microbiol 1993;38:325–30.

[21] Hulett, F. M., E. E. Kim, C. Bookstein, N. V. Kapp, C. W. Edwards, and VOL. 62, (1996). Phosphatases from a psychrophilic arthrobacter strain 3737

[22] H. W. Wyckoff. (1991). Bacillus subtilis alkaline phosphatase III and IV. J. Biol. Chem. 266:1077–1084.

[23] Howson, S. J. and Davis, R.P. (1983), production of phytase- hydrolysing enzyme by some fungi, Enzyme Microb, Technol. 5: 377-382.

[24] Jha, B.K., Pragash, M.G., Cletus, J., Raman, G. and Sakthivel. (2009). Simultaneous phosphate solubilization potential and antifungal activity of new fluorescent pseudomonas strains, P. aeruginosa, P. plecoglossicida and P. mosselii. World J Microbiol Biotechnol. 25:573-581.

[25] Kobori, H., and N. Taga. (1980). Extracellular alkaline phosphatase from marine bacteria: purification and properties of extracellular phosphatase from a marine Pseudomonas sp. Can. J. Microbiol. 26:833–838.

[26] Lian, B., Fu, Mo, D. M. and Liu, C. Q. (2002). A comprehensive review of the mechanism of potassium release by silicate bacteria. Acta Mineral Sinica, 22: 179-183.

[27] Liu, D., Lian, B and Dong, H., (2012). Isolation of Paenibacillus sp. and assesment of its potential for enhancing mineral weathering. Geomicrobiology Journal, 29: 413-421.

[28] Memon, Y. M., Fergus, I. F., Hughes, J. D., Page, D. W. (1988). Utilization of non-exchangeable soil potassium in relation to soil types, plant species and stage of growth. Australian Journal of Soil Research, (26): 489–496.

[29] Mikhailouskaya, N. and Tcherhysh, A. (2005). K-mobilizing bacteria and their effect on wheat yield. Latvian Journal Agronom, 8:154-157

[30] Mehta, S. and Nautiyal, C.S. (2001). An efficient method for qualitative screening of Phosphate-solubilizing bacteria. Curr Microbiol. 43: 51–56.

[31] Nomoto, M., M. Ohsawa, H.-L. Wang, C.-C. Chen, and K.-W. Yeh (1988). Purification and characterization of extracellular alkaline phosphatase from an alkalophilic bacterium. Agric. Biol. Chem. 52:1643–1647.

[32] Olsen, S.R. (1954). In: Estimation of available phosphorous in soils by extraction with sodium carbonate. U.S.D.A., Washington, D.C., Circ. pp: 939.

[33] Panday, A. Szakacs, A. Soccol, C.R. Rodriguez-leon, J.A. Soccol, V.T., 2001. Production, purification and properties of microbial phytase. Biores. Technol. 77: 203-214

[34] Powar VK, Jagannathan V (1982). Purification and properties of phytate-specific phosphate from Bacillus Subtilis. J. Bacteriol. 151: 1102-1108.

[35] Priyanka Parmar, S. S. Sindhu, (2013)Potassium Solubilization by Rhizosphere Bacteria: Influence of Nutritional and Environmental Conditions. Journal of Microbiology Research 2013, 3(1): 25-31

[36] Richardson A, Pankhurst C, Doube B, Gupta V and Grace P (1994). Soil microorganisms and phosphorus availability. Soil biota: management in sustainable farming systems 50-62.

[37] Rodriguez, H. and Fraga, R. (1999), Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech Adva. 17:319-339.

[38] Rossolini GM, Schippa S, Riccio ML, Berlutti F, Macaskie LE, Thaller MC (1998). Bacterial nonspecific acid phosphohydrolases: physiology, evolution and use as tools in microbial biotechnology. Cell Mol Life Sci.;54:833–850.

[39] Rodriguez H, Fraga R (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol. Adv. 17: 319-339.

[40] Singh M.K., Singh, D.P., Mesapogu, S., Babu, B.K. and Bontemps, C. (2011). Concomitant colonization of nifH positive endophytic Burkholderia sp. In rice (Oryza sativa L.) promotes plant growth. World J Microbiol Biotechnol. 27:2023–2031.

[41] Sugumaran P and Janartham B (2007). Solubilization of potassium minerals by bacteria and their effect on plant growth. World Journal of Agricultural Sciences 3(3) 350-355.

[42] Sheng XF, Huang WY and Yin YX (2003). Effects of application of silicate bacteria fertilizer and its potassium release. Journal of Nanjing Agricultural University 23 43-46.

[43] Sheng, X. F., (2005). Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of Bacillus edaphicus. Soil Biology & Biochemistry,37: 1918–1922.

[44] Sparks, D.L., Huang, P.M. (1985). Release of soil potassium by weathering reactions. In: Munson RD (ed) Potassium in agriculture. American Society of Agronomy, Madison, WI, pp: 202–276.

[45]

[46] Thaller MC, Berlutti F, Schippa S, Lombardi G, Rossolini GM. b (1994) Characterization and sequence of PhoC, the principal phosphate-irrepressible acid phosphatase of Morganella morganii. Microbiology.;140:1341–1350

[47] Thaller MC, Berlutti F, Schippa S, Iori P, Passariello C, Rossolini GM. a (1995). Heterogeneous patterns of acid phosphatases containing low molecular mass polypeptides in members of the family Enterobacteriaceae. In1 J Syst Bacteriol.;45:255–261.

[48] Yamane, K., and B. Maruo, (1978). Alkaline phosphatase possessing alkaline phosphodiesterase activity and other phosphodiesterases in Bacillus subtilis. J. Bacteriol. 134:108–114.

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