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Optimisation of Growth Factors for Effective Use of Phosphate Solubilizing Bacterial Strains and Its Use as Bioinoculants for the Growth of Groundnut (*Arachis hypogaea*) Plant

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Authors' contributions

This work was carried out in collaboration between both authors. Author PA managed the literature searches, performed the experimental analysis, wrote the protocol and wrote the first draft of the manuscript. Author VGK designed the study, managed the analyses of the study and edited the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Phosphorus (P) is one of the essential macronutrients for plant growth. Phosphate solubilizing bacteria (PSB) are organisms which are used as bioinoculants to enhance the plant growth. Plants take phosphate in the form of soluble orthophosphate ions but due to the presence of calcium, magnesium, potassium, sodium, aluminium and ferrous ions in soil, the soluble orthophosphate is converted in to insoluble form. Because of this process plants utilize very little amount of phosphate, even though phosphorus containing fertilizers are added to the plants. Phosphate-solubilizing bacteria (PSB) has been added as fertilizer to increase phosphorus uptake and plant growth. It is usually observed that the solubilization of phosphate by the phosphate solubilizing bacteria would drop the pH of the medium. Acidification of the medium may be due to production of organic acids by the bacterial strains. Such plant growth-promoting bacteria has the ability to produce enzymes such as Phosphatase and 1-aminocyclopropane-1-carboxylate (ACC) deaminase to lower plant ethylene levels, that often results in various stresses, which shows the

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efficacious functioning of these bacteria. This study aims in the optimization of growth factors of the isolated PSB strains for use as a Biofertilizers, specifically to study the effects of temperature, pH and different carbon, nitrogen sources and NaCl concentrations on phosphate solubilization ability. The results showed phosphate solubilization was expressed maximum at pH 6, temperature 25 °C, dextrose as carbon source, ammonium sulphate as Nitrogen source and (1.2%) NaCl concentration by the PSB strains. Further the PSB was applied as bioinoculants to enhance the root, shoot length and phosphorus accumulation content of ground nut (*Arachis hypogaea*) seedlings. Present study highlights the importance of these plants growth promoting bacterial strains and their uses for agriculture purposes as a Biofertilizer.

Keywords: Phosphate solubilizing bacteria (PSB); optimization; phosphatase; ACC deaminase; bioinoculants; groundnut; biofertilizer.

1. INTRODUCTION

For growth of any microorganism and its synthesis of cellular compounds, the culture conditions plays an important role. The environmental factors are very essential to increase the growth and it influences the production of secondary metabolites. The soil microbes are depended upon physico- chemical properties of soil such as pH, temperature and other factors like carbon, nitrogen, phosphorus, sulphur for their growth. Generally, for expression of secondary metabolism of microorganisms usually they need different types of media. Selection of appropriate media is complex since the possible variations are so large. Simple media works very well as broth and agar and this has been validated many times with novel bioactive compounds being produced [1]. The development of media, which leads to increase the production of bioactive compounds. By using this approach, chances of finding novel compounds increased and could be worth investigating for microorganism's secondary metabolism. Although a good growth may occur in many media but secondary metabolites may only be produced in a specific medium. Microorganism may produce one metabolite on one medium and a totally different one on another medium. To promote the growth of plants many growth promoting bacteria are employed which use different mechanisms. For the plant growth they need many enzymes and hormones, there are some growth-promoting bacteria use a number of different mechanisms to promote the growth of plants [2], arguably, the bacterial trait that is key in facilitating plant growth is the possession of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and phosphatase. This enzyme is responsible for the cleavage of the plant ethylene precursor, ACC, into ammonia and ketobutyrate [3]. ACC deaminase- producing organisms decrease plant ethylene levels, by decreasing ACC levels in plants, [4], it leads to the plant growth inhibition and even death in case of present in high concentrations of ACC level.

Phosphorus in soils is immobilized or becomes less soluble either by absorption, chemical precipitation or by both processes [5]. The soil as insoluble form of phosphate to enhance the plant growth using phosphate solubilizing bacteria under the poor phosphorus conditions [6]. *Pseudomonas* genera is one of most efficient phosphate solubilizers based on the reported research. Phosphate solubilizing bacteria completely depend on the culture conditions for the activity of phosphate solubilization.

Each species or a strain has a characteristic minimum, optimum and maximum temperature. The optimal temperature for growth may not be that best suited to product formation especially where the product is predominantly non growth associated as in the case of many secondary metabolite [7].

This present objectives of the study focused on the optimization of suitable growth parameters like pH, temperature, different carbon, nitrogen sources and different NaCl concentrations, for the isolated PSB strains, that could induce higher levels of plant growth by solubilizing phosphate and producing phosphatase and ACC deaminase enzymes. Further the PSB strains were used as bioinoculants in greenhouse experiments with Groundnut (*Arachis hypogaea*) plants.

2. MATERIALS AND METHODS

2.1 Enrichment and Isolation of the Bacterial Strains

Soil samples were collected from Rhizosphere of paddy and groundnut cultivated area, mappedu

village, Thiruvallur district. The bacterial strains were enriched in Pikovskaya's medium (g/l) yeast extract 0.500, dextrose 10.000, calcium phosphate 5.000, ammonium sulphate 0.500, potassium chloride 0.200, magnesium sulphate 0.100, manganese sulphate 0.0001, ferrous sulphate 0.0001, adjusted to pH -7 and distilled water 1 L [8]. The medium was autoclaved, cooled to room temperature and was amended with TCP as a phosphorus source (5g/L). The bacterial strains was enriched on pikovskaya;s medium. The culture was then transferred in to medium. Increase in cell count of the bacterial strains from 10^5 to10^6 cfu/mL with phosphate, the bacterial strains was taken as a confirmation for phosphate solubilization.

2.2 Bacterial Strains Used as a Bioinoculants

The bacterial strains used in the present study were APKVG02- *Maricaulis virginensis,* APKVG07-*Kosakonia oryzae,* APKVG10 – *Klebsiella pneumoniae*.

2.3 Optimization of Growth Parameters Effecting Phosphate Solubilization

2.3.1 Effect of pH and temperature

Optimal media and temperature was used, but the pH of the media was set at different pH (3, 4, 5, 6, 7) using NaOH or HCl and the culture was maintained at different temperature (25°C, 35°C and 45°C) in media supplemented with 0.5% tri-calcium phosphate as sole phosphorus source. The isolates were checked for solubilization activity in PVK broth amended with Tricalcium phosphate. Inoculation was carried out by using pure colony of individual bacterial culture from PVK Agar plate. Flasks were incubated 37°C for 5 days respectively [9]. Turbidity was monitored every 24 hours interval. After incubation, Phosphate solubilization by the isolates was quantified spectrophotometrically, and based on solubilization using the protocol, pH and temperature was determined.

2.3.2 Effect of various carbon and nitrogen sources

Effect of various carbon sources like Glucose, Fructose, Sucrose, Maltose, Starch, Lactose and Nitrogen sources like Ammonium sulphate, Urea, Casein, and sodium nitrate, peptone were studied in PVK Broth. The isolates were checked for solubilization activity in PVK broth amended with Tricalcium phosphate. Inoculation was carried out by using pure colony of individual bacterial cultures from PVK Agar plate. Flasks were incubated at 37°C for 5 days respectively [9]. Turbidity was monitored every 24 hours interval. After incubation, Phosphate solubilization by the isolates was quantified spectrophotometrically, and based on solubilization using the protocol best carbon and Nitrogen source was determined.

2.3.3 Effect of different NaCl concentrations

Effect of different NaCl concentrations (0.4%, 0.8%, 1.2%, 1.6%, and 2%) was studied in PVK broth. The isolates were checked for solubilization activity in PVK broth. Inoculation was carried out by using pure colony of individual bacterial culture from PVK Agar plate. Flasks were incubated 37°C for 5 days respectively [9]. Turbidity was monitored every 24 hours interval. After incubation, Phosphate solubilization by the isolates was quantified spectrophotometrically, and based on solubilization using the protocol same as above, the best NaCl concentration was determined.

2.4 Determination of Enzymes

2.4.1 Determination of phosphatase enzyme activity

Determination of Phosphatase enzyme was done by the protocol given by Ponmurugan et al. [10]. Bacterial colonies were inoculated in Pikovskaya's broth, poured in test tubes (10 ml/tube) and autoclaved at 15 psi for 15 min. The tubes were incubated in incubator shaker at 120 rpm, 37°C for overnight. 10 ml of above grown bacterial culture was taken and filtered through Whatman no. 1 filter paper. This was considered as enzyme or protein sample. The enzyme acid phosphatase was assayed using para nitrophenyl phosphate (PNP-P) as a substrate. The reaction mixture contained 2.5 ml (0.1 M) sodium acetate buffer (pH 5.8), 1 ml (1 mM) magnesium chloride, 0.5 ml 1 % PNP-P and 0.5 ml of a suitable dilution of enzyme preparation. One ml of the reaction mixture was transferred to 2 ml of 0.2 M sodium hydroxide before and after 15 min incubation at 37°C to stop the reaction. The sodium hydroxide solution added before incubation acts as a control sample for each analysis. The amount of para nitro phenol (PNP) liberated was measured by recording the absorbance at 420 nm using an appropriate

calibration curve. Activity is expressed as µmol PNP liberated min^{-1}. The blank was run in a similar manner using distilled water [11].

2.4.2 Determination 1-aminocyclopropane-1-carboxylate (Acc) deaminase activity

ACC deaminase activity was assayed according to a modified method proposed by Honma and Shimomura (1978). The bacterial extracts were prepared in 1 mL of 0.1 M Tris HCl (pH 7.6) and transferred to a 1.5 mL microcentrifuge tube. The contents of the microcentrifuge tube were centrifuged at 16,000 × *g* for 5 min and the supernatant was removed. The pellet was suspended in 600 mL 0.1 M Tris HCl (pH 8.5). Subsequently, 30µL toluene was added to the cell suspension and vortexed at the highest setting for 30 seconds. Then, 200 µL of the toluenised cells were transferred to a clean 1.5 mL microcentrifuge tube; 20 µL of 0.5 M ACC was added to the suspension, vortexed for 5 secs, then incubated at 30°C for 15 min. After the incubation, 1 mL 0.56 M HCl was added to the mixture, vortexed and centrifuged for 5 min at 16,000 × *g* at room temperature. To 1mL of this suspension, 800 µL of 0.56 M HCl was added and mixed in glass tubes. 300 µL of 2,4-dinitrophenylhydrazine reagent (0.2% 2,4-dinitrophenylhydrazine in 2 M HCl) was added to the glass tube, vortexed and then incubated at 30°C for 30 min. The absorbance of the mixture was measured at 540 nm after the addition of 2 mL of 2 N NaOH [12].

2.5 Phosphorus Accumulating Granules in Bacterial Strain Using Transmission Electron Microscopy

PSB were grown for 48 h on PVK medium, and cells were recovered from the plates with phosphate-buffered saline (PBS; pH 7.4). After being washed with PBS, the bacteria were fixed with 3% paraformaldehyde plus 0.5% glutaraldehyde and embedded in agarose. Samples in agarose were washed with PBS, treated with 2% osmium tetroxide and dehydrated with ethanol. The samples were included in LR White resin (Electron Microscopy Sciences) that was polymerized at 60°C. Ultrathin sections (60 nm) were obtained with a Leica UC6 ultramicrotome and stained with uranyl acetate and lead citrate. Samples were observed with a JEOL JEM-1010 transmission electron microscope, and images were digitally captured with a MegaView III camera (Olympus) in Cancer Institute, Chennai.

2.6 Application of Phosphate Solubilizing Bacteria in Plant Growth- Pot Experiment Preparation of Bacterial Inoculants

Effective Phosphate solubilizing bacterial cultures were inoculated into 100 ml of PVK broth medium and were incubated at 28±1°C for 5 days in a shaker. The culture was centrifuged at 12,000 rpm for 15 min. The pellets were suspended in phosphate buffer ($NaH_2Po_4.2H_2O$: 32.2g; Na_2HPO_4: 28.39g; sterile distilled water: 100 ml) and washed repeatly with the buffer and were resuspended in the same buffer solution [13].

2.7 Induction of Growth in Ground Nut (*Arachis hypogaea*) Plants Using PSB Strains

To study effect of phosphobacteria on the growth of ground nut and tomato Plant, an experiment was conducted. Certified seeds of ground nut (*Arachis hypogaea*) and tomato (*Solanum lycopersicum*) were produced from Horticulture and Agriculture soecity, cathedral road, Chennai. The seeds were surface sterilized with 0.1% $HgCl_2$ for 5 minutes and the seeds were soaked for 1 hour in liquid cell suspension (10^{-8} cells ml) separately and spreaded on to the soil moisture with sterile distilled water. Three replicates of 100 seeds of ground nut and tomato were maintained for each bacterial treatment served as control. After 20 days of treatment, the plant growth characteristics viz, average root length, average shoot length, root and shoot biomass were analyzed [14].

2.8 Dry Matter Content of Plants and Phosphorus Uptake

The root and shoot portions of tomato plants were separated and air dried for two days. They were then oven dried at 70°C to a constant weight. The shoot and root dry weights were recorded separately and the average dry weight of plants was expressed in g/plant. Plant samples were finely ground after drying and used to determine phosphorous content following vandomolybdate phosphoric yellow colour [15].

3. RESULTS AND DISCUSSION

3.1 Effect of pH and Temperature

The mechanism of PSB is dependent upon different parameters such as pH, temperature,

NaCl concentration and different carbon, nitrogen and phosphorus sources. Thus optimization studies on various physicochemical parameters are necessary for the maximum solubilization of phosphorous by the isolated bacterial strains. The Phosphate solubilizing bacteria are very sensitive to various environmental conditions like temperature and pH. The optimal temperature for growth may not be that best suited for the product formation especially where the product is predominantly non growth associated as in the case of many secondary metabolites. So, in the present study the effect of varying temperature i.e. 25°C, 35°C, and 45°C on the growth and production of phosphate solubilizing activity was studied for 5 days' interval. All the isolated PSB strains showed optimum incubation time for the growth and Phosphate solubilizing activity was found to be 3rd day which is at 72 hours of incubation time [16].

The pH of the medium forms major criteria for phosphate solubilizing bacteria to grow in the PVK medium. As they produce acids during their metabolism of phosphate solubilization pH of the medium affects the growth of the phosphate solubilizing bacteria. In the present study the isolated bacterial strains were able to grow in pH less than neutral. Especially at pH 5, it was noted that the PSB strains showed an OD value of APKVG02 (0.989), APKVG07 (0.846) and APKVG10 (0.526) given in Figs. 1, 2, 3 and Temperature plays a major role on the growth of the phosphate solubilizing bacteria. Hence, the isolated PSB strains were tested with different temperature like (25°C, 35°C and 45°C) for growth at 25°C the bacterial strains produced the maximum growth APKVG02 (0.943), APKVG07 (0.775) and APKVG10 (0.935) respectively given in Figs. 4, 5, 6. Jena (2013) reported on isolate *Pseudomonas* which showed best maximum phosphate solubilizing activity at incubation time of 72 h in their experimental study. Each microorganism has its own specific minimum, optimum and maximum temperature. Phosphate solubilizing activity has been found to be dependent on the temperature as well. In our study, the maximum Phosphate solubilization activity was produced at 25°C by all three isolates APKVG02- *Maricaulis virginensis,* APKVG07-*Kosakonia oryzae,* APKVG10–*Klebsiella pneumonia* respectively. However, there was a decline in yield of their activity above and below 28°C. This behavior of all the PSB isolates showed similarity to usual response of mesophilic organisms where the latter's metabolic activities usually slow down below and above the optimum temperature. This proves the isolated phosphate solubilizing bacteria are mesophilic in nature. Our results coincide with (Mishra et al., 2009) who has reported that Phosphate solubilizing bacteria grew at temperatures ranging from 4 to 30°C, with a growth optimum at 28°C. Also Behrendt et al. (2007) reported that for *Pseudomonas lurida,* theoptimal growth temperature is 21°C. The pH has a strong influence on the pathways of metabolism and product generation by microorganism. The optimum pH for growth rate may be different from that for growth yield and

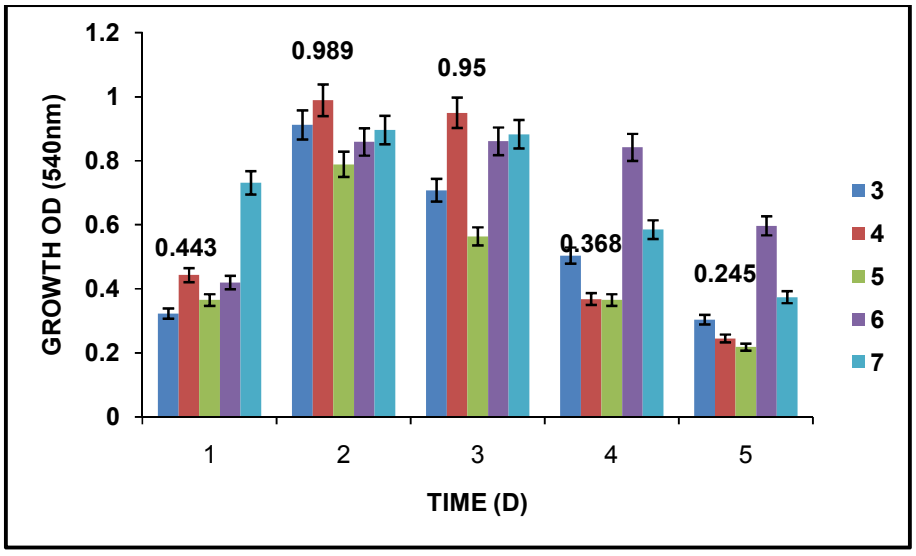

Fig. 1. Effect of pH on APKVG02

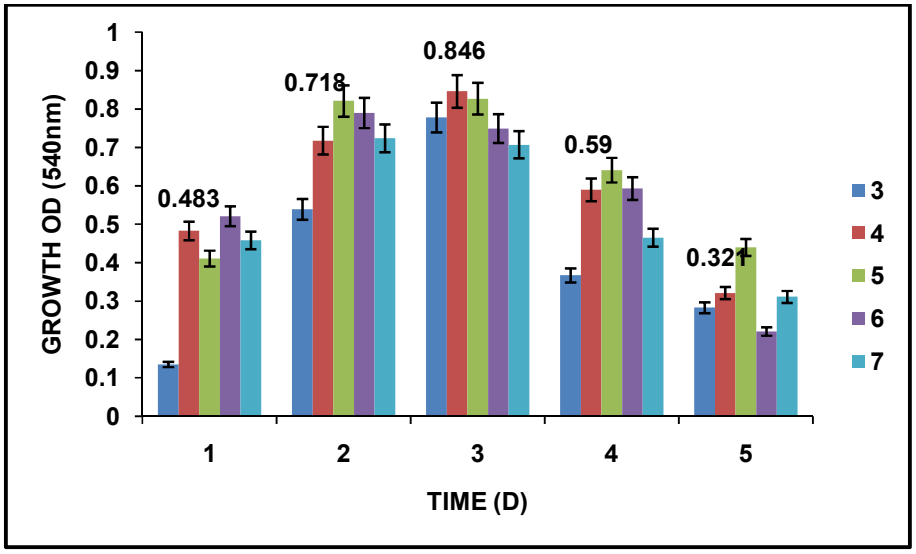

Fig. 2. Effect of pH on APKVG07

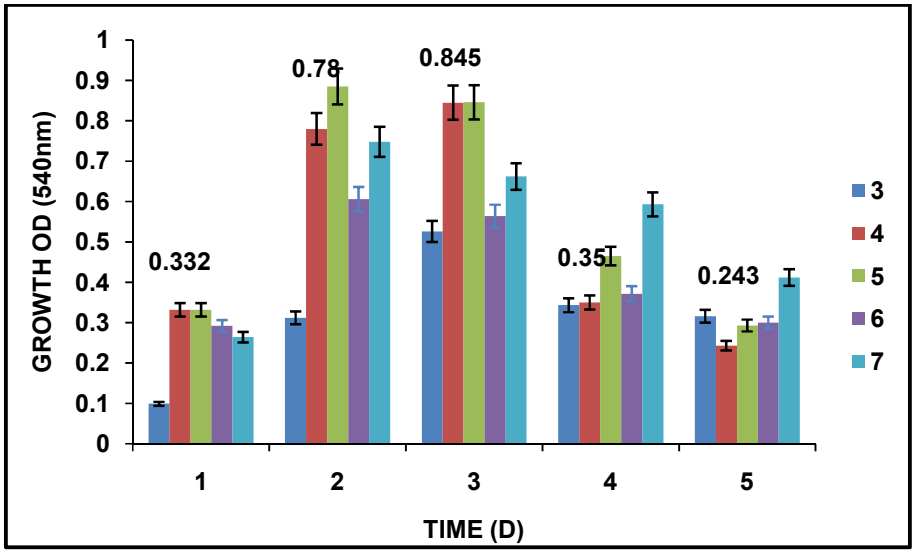

Fig. 3. Effect of pH on APKVG10

entirely different from the optimum for product formation. In our study, pH 5 is found to be best for the growth as well as Phosphate solubilizing activity which is in accordance with Yadav, (2013) who concluded that pH 7.5 and Jena, (2013) showed the efficiency at pH 5.0 as optimum for the Phosphate solubilizing activity of their isolated strains [17].

In the present study, lowering in pH in liquid culture accompanied phosphate solubilization is noted which may be due to the production of organic acids. But we were not able to correlate between acidic pH and the inorganic phosphorous produced [18]. Such observations on solibilization of phosphorous and their influence on the decrease in pH were not been correlated and are also being reported by Pallavi and Gupta, 2013 [13]; Goenadi *et al.,* 2000; Kundu and Gera, 2002.

Probably this may be due to the reason that solubilization depends not only on the pH and acid concentration but also on the structure and type of organic molecule in which inorganic phosphate exist. In another study by Fankem et al. (2006) there was a decrease in pH which was not strictly proportional to the amount of

Phosphate solubilized. In our study, the pH was found to decline in the medium from 7.00 (control) to minimum of 5. In another study by Pandey et al. (2006) [16] the pH of the broth was found to decline from 6.00 (control) to 4.11, 3.91, 3.73 and 3.81 at temperatures 4, 9, 21 and 28°C respectively.

3.2 Effect of Various Carbon and Nitrogen Sources

Carbon is one of the primary source of nutrition for the metabolic activity and growth of the microorganism. Effect of different carbon sources (Glucose, Fructose, Sucrose, Maltose, Starch) were tested on the growth of the PSB strains in the PVK medium for 5 days. It could be observed that in the presence of glucose a basic monosaccharide the phosphate solubilizing bacterial strains showed maximum growth APKVG02 (0.922), APKVG07 (0.819) and APKVG10 (0.712) respectively showed in Figs. 10, 11, 12 and The phosphate solubilizing bacterial strains were grown in PVK medium in the presence of different nitrogen sources like Ammonium sulphate, Urea, Casein and Sodium

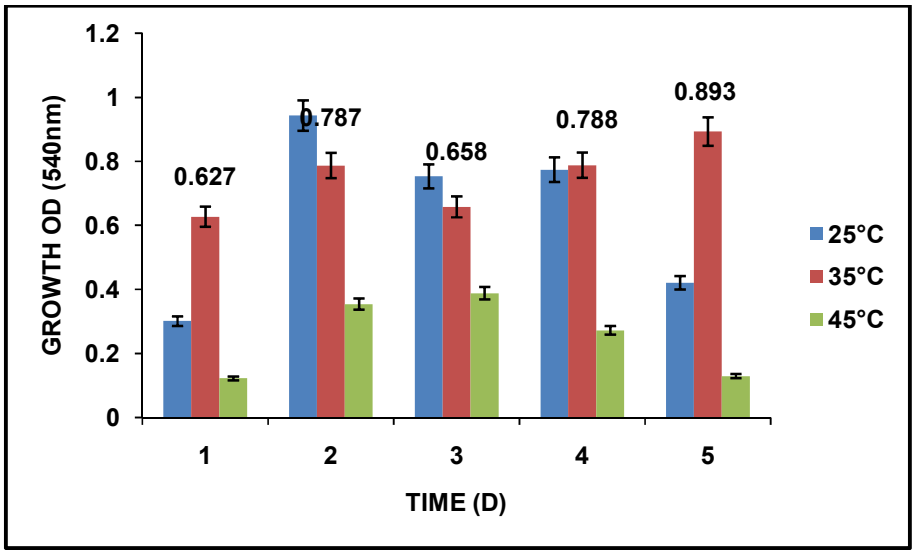

Fig. 4. Effect of temperature on APKVG02

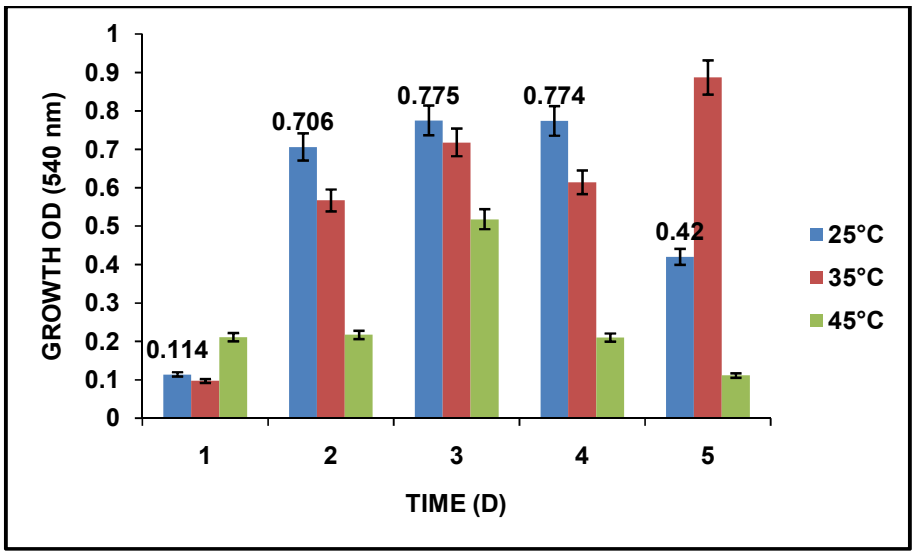

Fig. 5. Effect of temperature on APKVG07

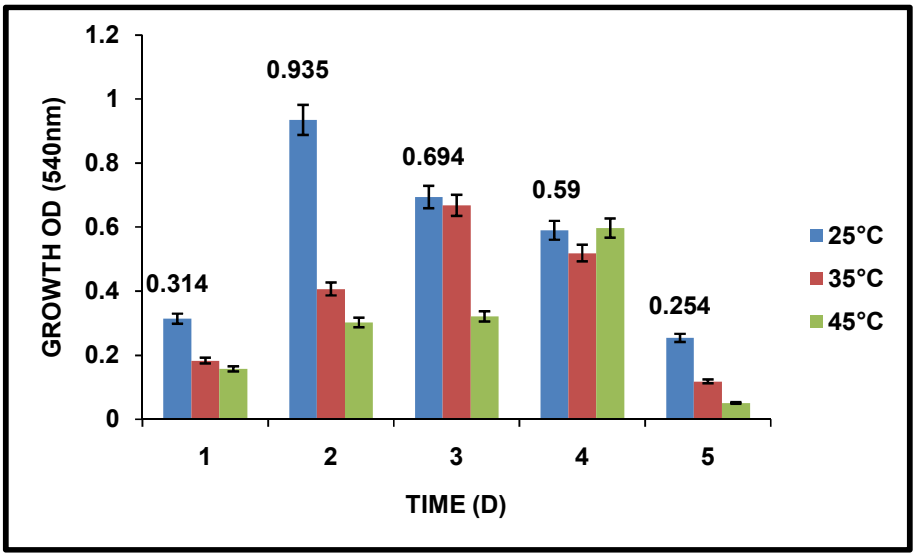

Fig. 6. Effect of temperature on APKVG10

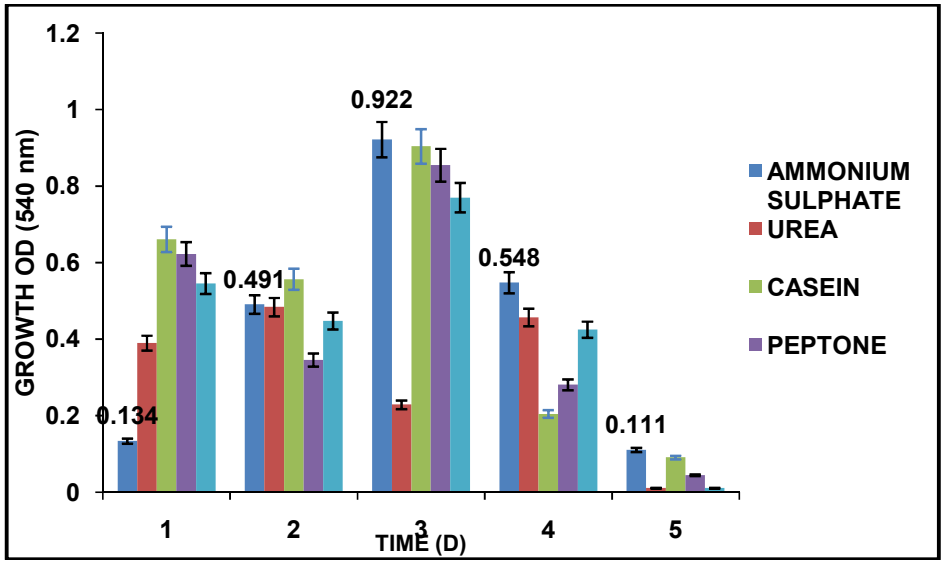

Fig. 7. Effect of various nitrogen sources on APKVG02

nitrate for a period of 5 days. Nitrogen sources play a key role in enhancing the growing of the bacterial strains in the medium. It could be observed that ammonium sulphate proved to be the best source of nitrogen. The bacterial strain showed APKVG02 (0.922) and APKVG07 (0.914), APKVG10 (0.804) respectively given in 7, 8, 9.

Thus, the present investigation deals with the analysis of different carbon sources suitable for the phosphate solubilizing bacteria as these bacteria play a major role as biofertilizers. The *Maricaulis virginensis,* APKVG07-*Kosakonia oryzae,* APKVG10 – *Klebsiella pneumonia* isolate showed maximum growth with glucose as a primary source of carbon in the media. Shahab and Ahmed (2008) studied the optimization of different carbon sources in their medium. The carbon sources used were Glucose, Fructose, Sucrose and Lactose in which the optimized source was Glucose. In the present investigation, *Maricaulis virginensis,* APKVG07-*Kosakonia oryzae,* APKVG10 – *Klebsiella pneumonia* the isolates were grown with different nitrogen sources to check their effect on the growth of the microorganisms and it was found that all the strains showed maximum growth with ammonium

sulphate followed by casein, peptone and urea. Amit Sagervanshi et al., (2012) [3] studied the effect of different nitrogen sources for the maximum solubilization. The different nitrogen sources used were ammonium sulphate, Casein, sodium nitrate and Urea which showed optimized source as Ammonium sulphate.

3.3 Effect of Different NaCl Concentrations

Saline – alkaline soil affects the growth of the plants there are microbes which can sustain in such environment and convert the phosphorous forms and solubilize them [19]. Some microbes are affected by such salinity stress. To study the salinity stress isolated Phosphate solubilizing bacterial strains were grown in PVK medium in the presence of different concentrations of NaCl (0.4%, 0.8%, 1.2%, 1.6%, 2%) for 5 days. NaCl plays an important role in the growth of bacteria in the enriched medium. In the present work it was observed that the maximum growth on NaCl was observed at 1.2% by APKVG 07 (0.991), APKVG 10 showed (0.968) and APKVG 02 showed (0.889) respectively given in Figs. 13, 14, 15. Srinivasan et al. (2012) optimized various NaCl concentrations for maximum

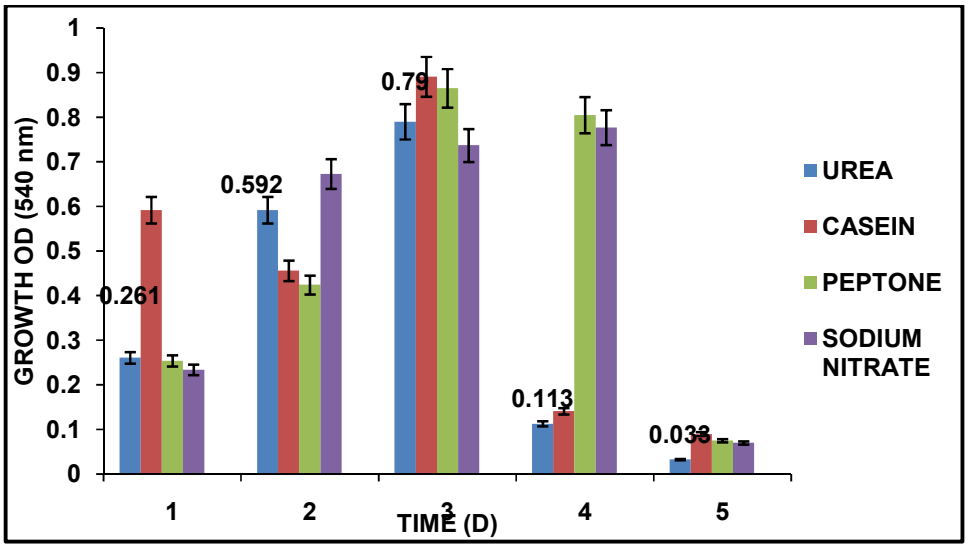

Fig. 8. Effect of various nitrogen sources on APKVG07

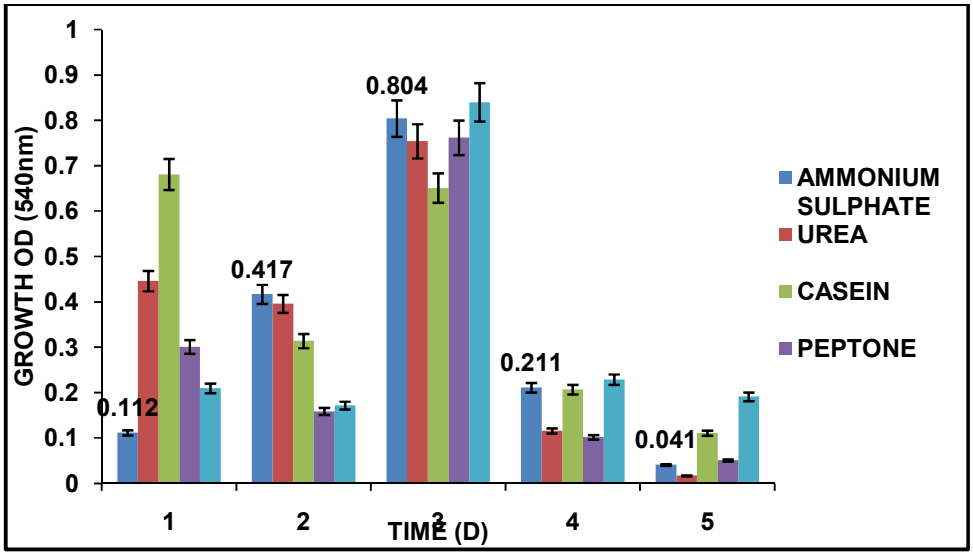

Fig. 9. Effect of various nitrogen sources on APKVG10

solubilization of phosphate in the medium. The different NaCl concentrations were 0.4%, 0.8%, 1.2%, 1.6% and 2.0% out of which the optimum concentration was seen at 0.4%. In this study, the different NaCl concentrations used were 0.4%, 0.8%, 1.2%, 1.6% and 2.0% in which all the strains showed the optimized concentration was 1.2 %.

3.4 Determination of Enzymes- Determination of Phosphatase Activity and 1-aminocyclopropane-1-carboxylate (ACC) Deaminase Activity

Glick (2012) reported that plant growth promoting bacteria requires enzymes and hormones, which requires many different mechanisms to increase plant growth and the enzymes enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase and phosphatase play an important role in phosphate soluibilization [20]. This enzyme is responsible for the cleavage of the plant ethylene precursor, ACC, into ammonia and ketobutyrate (Honma and Shimomura 1978). ACC deaminase- producing organisms decrease plant ethylene levels, by decreasing ACC levels in plants, (Glick et al. 1998, 2007), it leads to the plant growth inhibition and even death in case of present in high concentrations of ACC level. Phosphate solubilization by phosphobacteria is always oriented with phosphatase enzyme. Hence, in this study attempt was made, to study the phosphatase activity in response to the phosphorus enrichment, by using the isolated Phosphate solubilizing bacteria. All the isolated PSB strains were able to show phosphatase activity. The results showed that the activity proved to be the maximum with the strains APKVG 10 (0.23 µg/ml) followed by APKVG07 (0.075 µg/ml) and APKVG 02 (0.006 µg/ml) and the bacterial isolates showed APKVG02 (0.17 mg/ml), APKVG07 (0.189 mg/ml) and APKVG10 (0.214 mg/ml) ACC deaminase potential respectively given in Table 1. Ponmurugan and Gopi (2006) showed phosphatase activity in the strain GPO2 which was about 36.87 µg/ml followed by SP03 at 32.08 µg/ml and the highest ACC deaminase activity was reported to be 60%. The present study showed maximum phosphatase activity of 0.25 µg/ml and ACC deaminase activity was at 0.214 µg/ml. There is increasing evidence that phosphobacteria improve plant growth due to biosynthesis of plant growth substances rather than their action in releasing available phosphorous.

3.5 Phosphorus Accumulating Granules in Bacterial Strain Using TEM

Phosphorus granules present in the PSB which converted inorganic phosphate into soluble form were identified by Transmission Electron Microscope and it could be observed the Bacterial size was nearly 500 nm [21]. The phosphorus granules were seen as dark colour spots, which were accumulated inside Bacterial cell at 72 h given in Fig. 16.

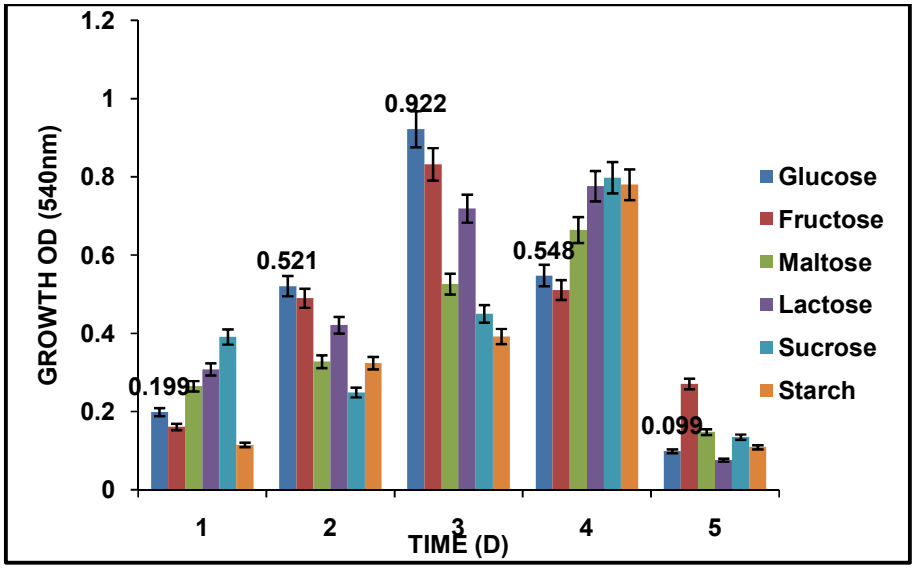

Fig. 10. Effect of various carbon sources on APKVG02

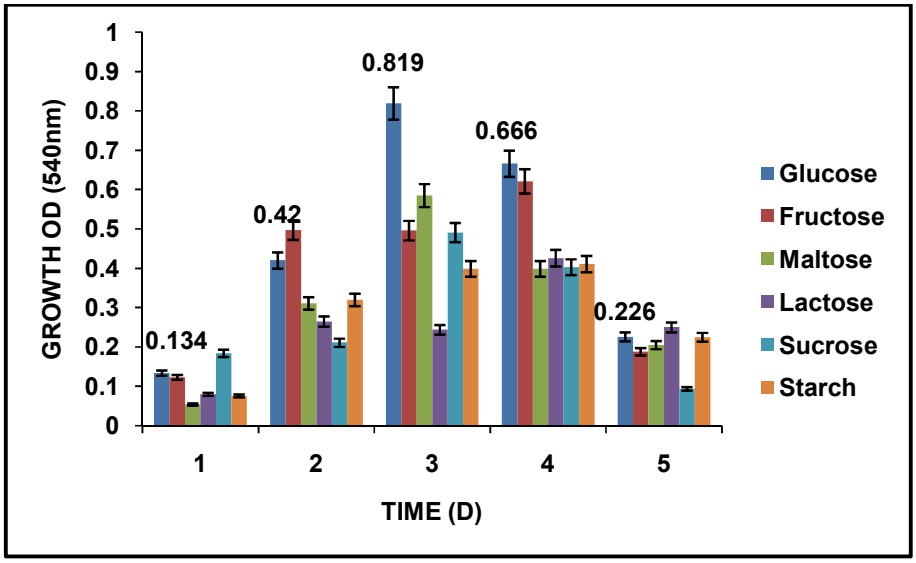

Fig. 11. Effect of various carbon sources on APKVG07

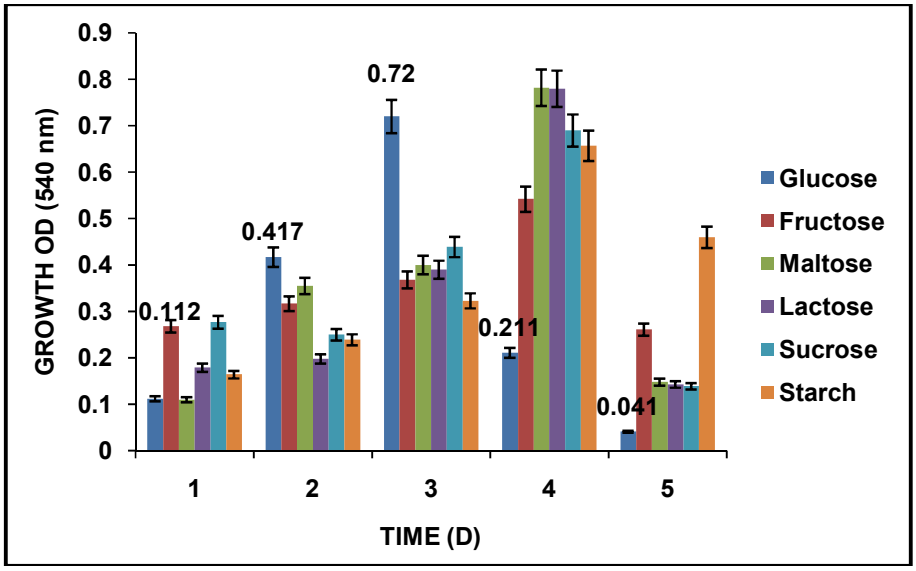

Fig. 12. Effect of various carbon sources on APKVG10

3.6 Application of Phosphate Solubilizing Bacteria in Plant Growth- Pot Experiment Induction of Growth in Groundnut Plant (*Arachis hypogaea*) by PSB

The use of phosphate solubilizing bacteria as inoculants increases Phosphorus uptake by the plant thereby increasing crop yield. For the mineral phosphate solubilization which helps to the production of organic acids and phosphatases which acts a major role in the mineralization of organic phosphorous present in the soil. In this pot experiment, inoculation of Ground nut plants was inoculated with PSB bacterial strains. The inoculated bacterial strains enhanced the shoot length, phosphorus content and dry weight of the plant and the results obtained were compared with uninoculated plant given in Fig. 17. Charana et al. [14] (2014) worked on the PSB strains to enhance growth of the tomato and ground nut the seed and showed 31.88% shoot and 45.53% root growth of the plants inoculated with PSB strains. Shahid et al., (2014) worked on the inoculation of sunflower plants with *Bacillus sp.* which enhanced the

sunflower root length to 6.53 cm, shoot length to 26.7 cm, fresh weight was 2.22 g, dry weight 0.29 g and phosphorus content was seen as 0.77 µg/ml. According to Walpola and Yoon (2013), inoculation of tomato plant with *P. agglomerans* and *B. anthina* showed an increase in plant phosphorus content which was seen at 144.17 mg/plant.

In the present study, PSB the strains used with Ground nut plant showed increase in shoot length from 14 cm to 30 cm, increase in root length from 8 cm to 10 cm, root dry weight was 0.211 µg/ml to 0.992 µg/ml, shoot dry weight was seen in the range of 0.169 µg/ml to 807 µg/ml and phosphorus content increased from 15.1 µg/ml to 70.9 µg/ml given in Table 2.

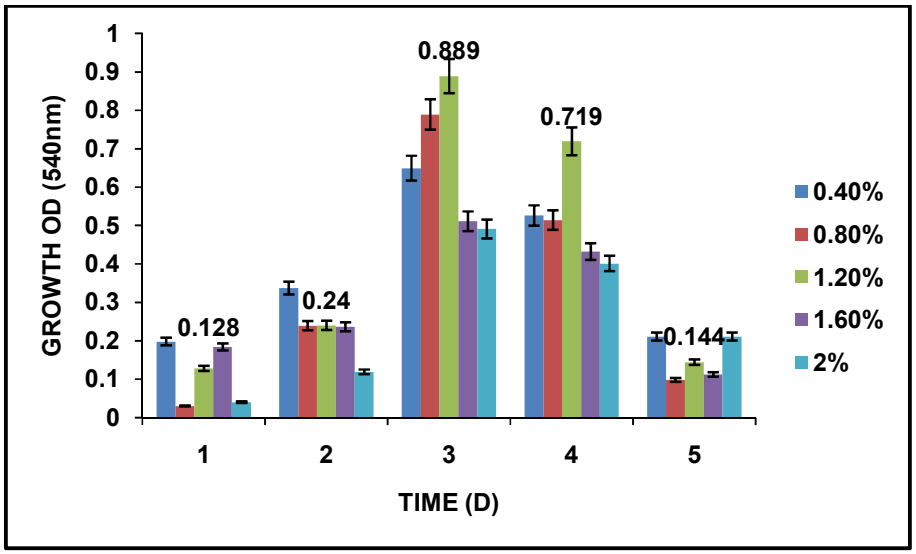

Fig. 13. Effect of different NaCl concentrations on APKVG02

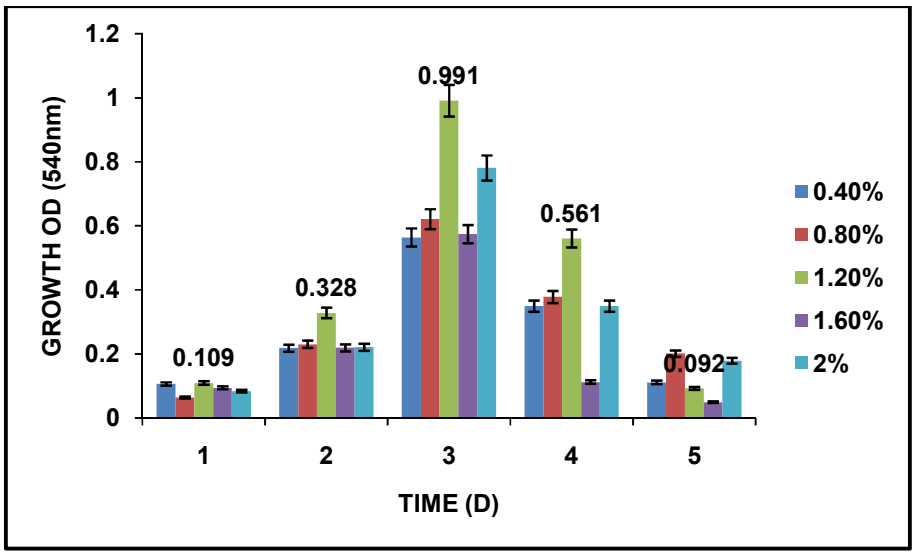

Fig 14. Effect of different NaCl concentrations on APKVG07

Table 1. Phosphatase activity and ACC deaminase activity

Isolates	Phosphatase activity (mg/ml)	ACC Deaminase activity (mg/ml)
APKVG02	0.006	0.170
APKVG07	0.075	0.189
APKVG10	0.230	0.214

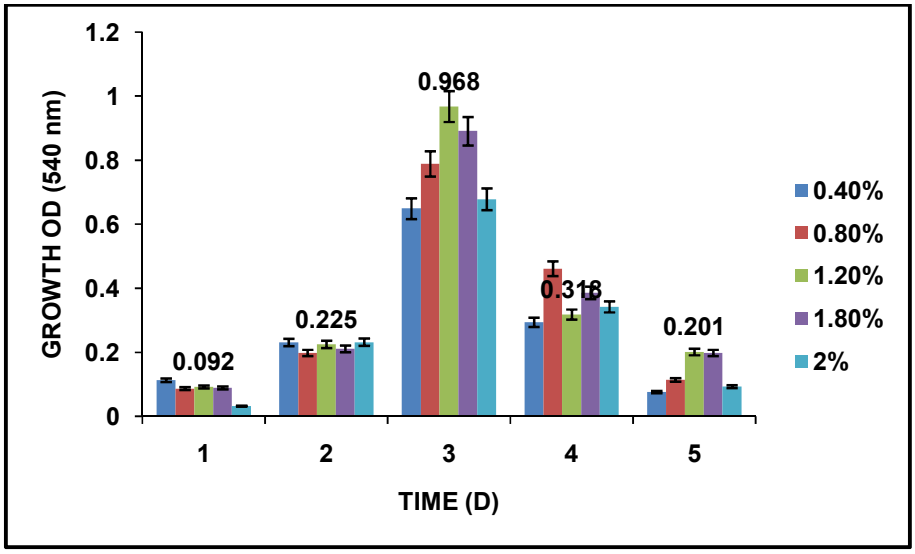

Fig. 15. Effect of different NaCl concentrations on APKVG10

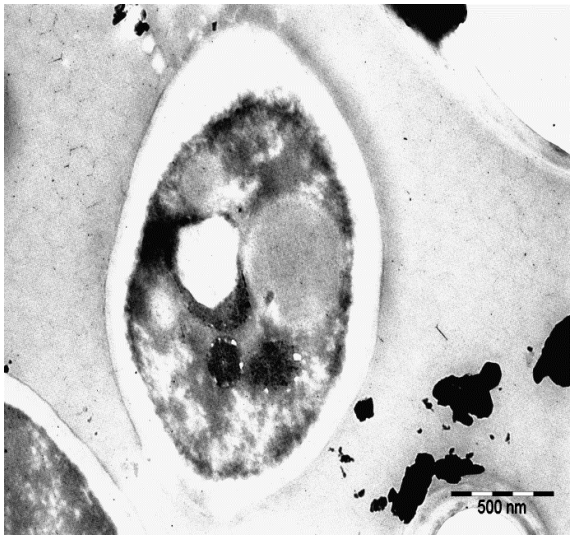

Fig. 16. Transmission electron microscope of PSB strains showing (1) exopolymeric substance (2) dark phosphate granules

Table 2. Growth, yield and physiological parameters of Ground nut plant *(Arachis hypogaea)*

Treatments	Shoot length (cm)	Root length (cm)	Root Dry weigth (g)	Shoot Dry weight (g)	P content (µg/ml)	
					Shoot	Root
Control	14.3	8.8	0.211	0.169	15.1	21.5
APKVG02	28.9	8.9	0.675	0.511	29.3	80.0
APKVG07	23.7	9.3	0.891	0.432	45.3	50.2
APKVG10	30.7	10.2	0.992	0.807	70.6	45.7

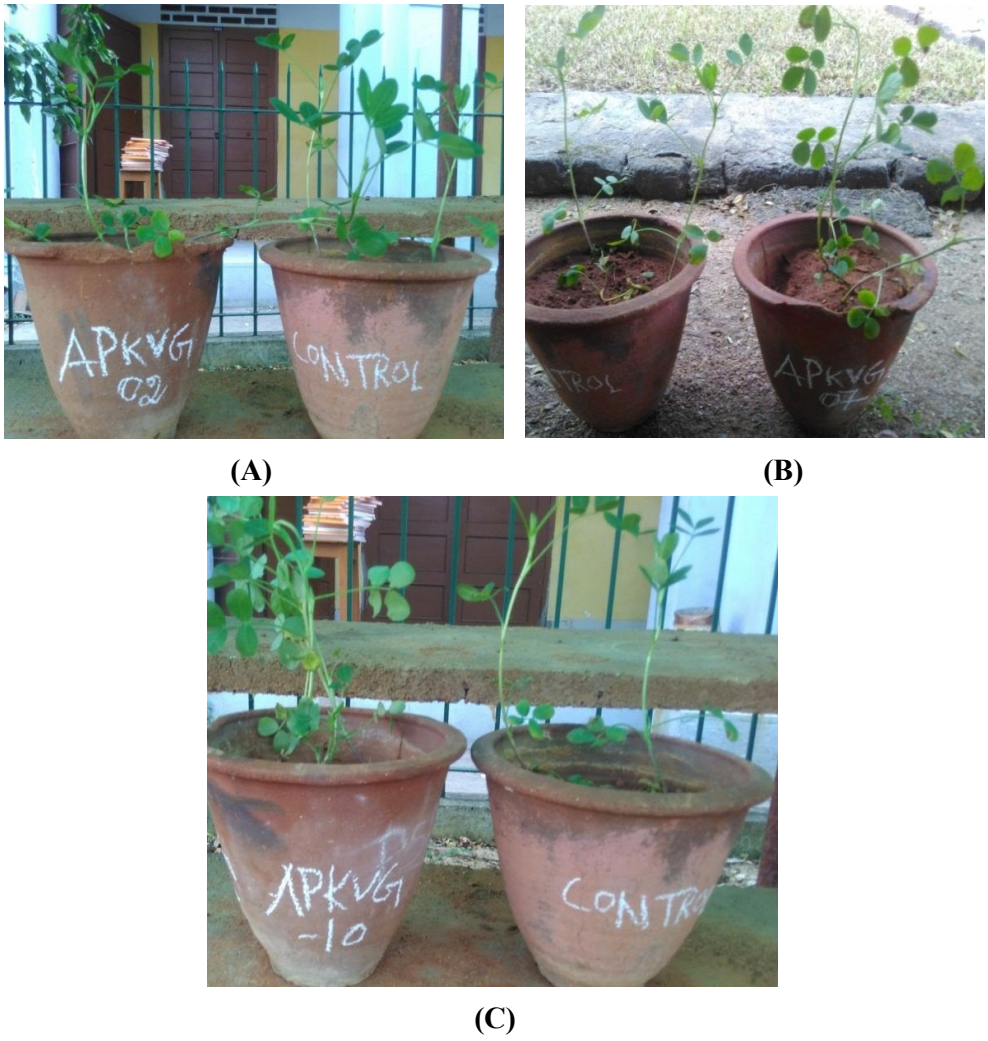

Fig. 17. Growth of the Ground nut plant *(Arachis hypogaea)* inoculated with (a) APKVG02, (b) APKVG07 and (c) APKVG10 PSB strains

4. CONCLUSION

Phosphorus is said to be the most important key element which affects the nutrient uptake of the plant from the soil and it also plays an important role in all major metabolic processes of plants including photosynthesis, energy transmission, signal transduction, macromolecule synthesis, respiration and nitrogen fixation in legumes. There is an abundance of the phosphorous content in the soil in the form of both organic and inorganic forms but it is not made available for the plants for the root to uptake it and hence it becomes a major limiting factor for plant growth. Several reactions are done to remove the available phosphate from the soil solution into the soil solid phase. But most of these methods have not proved to be efficient. It is because of this reason that phosphorus becomes fixed and available phosphorus and nitrogen is supplemented to the agriculture soil by adding chemical fertilizers. Addition of such chemical fertilizers accounts for an increase in the cost of agriculture production and also has a major environmental impact on soil health. This study highlights on the optimization parameters with the isolated phosphate solubilizing bacterial strains *Maricaulis virginensis, Kosakonia oryzae, Klebsiella pneumonia* isolated from rhizosphere of paddy and groundnut plants. The study also indicated they grow well with optimum temperature of 25°C and increases the acidity (decrease in pH 5) of the growth medium, glucose is the carbon source, Ammonium

sulphate is the nitrogen source best sources and 1.2% NaCl concentrations. The phosphate solubilization was further confirmed by their high phosphatase and 1-aminocyclopropane 3-carboxylate deaminase enzyme activities. Moreover, these isolates brought about significant increase in the growth of the groundnut plant under glasshouse trials, suggesting their applicability for crop improvement. Such isolates would better serve as bioincoculants by replacing the chemical fertilizers and show a more ecofriendly method.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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