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Isolation and Identification of Highly Effective Phosphate Solubilising Bacterial Strain from Coastal Soils to Promote Rice Growth

Simmanna Nakka[1], Uday Bhaskar Sajja[2]

[1, 2]Department of Biotechnology, Dr.B.R. Ambedkar University, Etcherla, Andhra Pradesh, India

Corresponding Author E-mail: *simmannan564[at]gmail.com*
Ph: +91 9603246067

Abstract: ***Phosphorous (P) is the second most vital macronutrient for plants that plays a significant role in the growth and development. Among the total soil phosphorous, plants can use only 0.1% of available phosphate, hence making the availability of soluble phosphate a limiting factor for plant growth. In order to overcome poor phosphorous availability, phosphate solubilising microorganisms can be used to ensure agriculture production. Hence, the study was aimed to isolate, identify, and characterise a potent bacterial species from coastal soils that can efficiently solubilise phosphate for plant absorption. From the five soil samples, a total of nine different discrete bacterial colonies were identified and they were named as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09. The screening of phosphate solubilisation demonstrates that the greatest phosphate solubilisation ability was found in the MGP-04 strain. Furthermore, the greatest phosphate solubilisations under increasing NaCl concentrations were found in the MGP-03 and MGP-04 strain. The highest plant growth promoting properties such as IAA and GA production were found with the MGP-03 and MGP-04 isolates. Due to better phosphate solubility and plant growth promoting activities, bacterial isolate MGP-04 was selected for molecular identification and bioinoculation studies. The molecular phylogeny revealed that the bacterial isolate MGP-04 is belongs to Bacillus toyonensisspecies. The bioinoculation studies revealed that the greatest germination percentage (98.2%), root length (5.8 cm), shoot length (6.5 cm), and dry weight (159.1 mg/gm) of rice seedlings was found with the treatment of $1x10^7$ bacterial concentrations. The present study concluded that the bacterial isolate MGP-04 can be used as a potential phosphate solubiliser and plant growth promoter to increase crop productivity in reclaimed soils.***

Keywords: Phosphate solubilising bacteria, 16s rRNA, Indole acetic acid, Gibberellic acid, Molecular phylogeny

1. Introduction

Phosphorous (P) is the second most vital macronutrient for plant development and it is involved in the synthesis of biomolecules such as DNA, RNA, ATP, proteins, and phospholipids. The plant's dry mass approximately contains 0.2% of phosphorous (Maharajan et al., 2018). Since phosphorus plays a key role in almost every aspect of metabolism and the regulation of enzymes, including kinases, it is considered a very important element in the ecosystem (Nesme et al., 2018). Additionally, phosphorous also plays a significant role in the growth of roots and stems, crop maturity, and fixation of nitrogen in pulses (Khan et al., 2009). In soils, phosphorous can be found in both organic (Po) and inorganic (Pi) forms, and the total percentage of phosphorous in soils is approximately 0.05%. Among the total soil phosphorous content, inorganic phosphorous accounts for 35 to 70% (Harrison, 1987). Plants can use only 0.1% of available phosphate, hence making the availability of soluble phosphate a limiting factor for plant growth (Lambers and Plaxton, 2018). Since certain phosphorous containing compounds such as apatites and strengites exhibit relatively slow dissolving kinetics, others, such as phosphates of calcium, aluminium, and iron, possess rapid solubilisation which depends upon the particle size and pH of the surrounding soil (Pierzynski et al., 2005). Because soil contains less accessible phosphorous than healthy plant tissues, mineral P fertilisers in the form of easily available monocalcium phosphate or monopotassium phosphate are commonly used in agriculture (Schachtman et al., 1998).

Soluble phosphate anions in synthetic fertilisers are highly reactive and interact with ions in soil such as Ca, Fe, and Al to form respective insoluble phosphate salts. Therefore, the plant utilisation ability of phosphorous in synthetic fertilisers is limited to 5-25% only, which results in phosphorous augmentation in the soils and thereby leads to loss of soil fertility (Schnug and Haneklaus, 2016). As a result, phosphorous fertilisers are becoming the world's biggest fertiliser market and the good phosphorous synthetic fertiliser production throughout the world will reach its maximum level by 2040 due to the gradual increase in agriculture's needs for global phosphorous reserves (Schroder et al., 2010). Therefore, it is better to utilise soil phosphorous by solubilising inorganic phosphates because the phosphate stocks are not simply refilled as like nitrogen (Cordell et al., 2009). Hence, there is an urgent necessity to develop strategies to improve soil phosphorous consumption by plants. In order to overcome poor phosphorous availability, plant growth promoting rhizobacteria (PGPR) can be used to ensure agriculture production.

PGPR are beneficial soil microorganisms that can be used as bioinoculants, biofertilizers, biopesticides, and biostimulants to enhance plant growth and development (Mishra et al., 2019). The utilisation of bioresources like PGPR to improve plant growth and biocontrol appears to be a novel agriculture-engineering strategy that attracts many scientists (Noumavo et al., 2016). The phosphate solubilising PGPR are involved in the soil phosphorous cycle by the release of chelating or mineral-dissolving substances, such as organic acids, lipopolysaccharides, protons, and hydroxyl ions, liberation of extracellular enzymes, thereby induces

phosphate solubilisation from inorganic insoluble phosphates (Vyas and Gulati, 2009). The administration of phosphate-solubilising PGPR lowers the soil pH and forms a P-available region around the plant rhizosphere, thereby increasing phosphorous absorption by the plant and also raising the plant growth promoting activities of other beneficial microorganisms. Additionally, PGPR benefits comprise their potential to improve nitrogen uptake in the rhizosphere, root growth, plant metabolic activity, IAA synthesis, and microbial photo protection. Consequently, knowing the dispersion and variety of indigenous phosphate solubilising microorganisms in the rhizosphere of certain crops necessitates information about the local microbial community, their characterisation, and identity (Chahboune et al., 2011). Hence, the aim of the study is to isolate, identify, and characterise a potent bacterial species from coastal soils that can efficiently solubilise phosphate for plant absorption.

2. Materials and Methods

Sample collection

The soil samples were collected from five distinct locations that covers three districts such as East Godavari, Visakhapatnam, and Srikakulam which are located in the Andhra Pradesh, India, along the coast line of Bay of Bengal. The samples were collected in order to identify the phosphate solubilising bacteria and to screen their phosphate solubilising capabilities as well as the plant growth promoting activity on rice plants. The samples were randomly collected from different coastal areas which are nearly situated 50 Km away from each other (Table. 1). Approximately, 500 gm of soil sample was collected from each site and the samples were stored in ice boxes and transported to the laboratory, where they were kept in refrigerator at 4°C for further analysis.

Table 1: Sampling sites and the characteristic parameters of respective soil samples

Soil Samples	Coordinates	Soil Characteristics			
		pH	Temp.(°C)	DO (mg/l)	Colour
Antervedi	16°19' 4.36"N; 83°43' 47.47" E	6.4	25.6	6.4	Brownish black
Kakinada	17°1' 20.10"N; 82°17' 4.39" E	5.8	27.9	5.9	Brownish black
Addaripeta	17°15' 2.18"N; 82°32' 32.23" E	6.2	23.8	5.9	Dark brown
Rushikonda	17°47' 33.04"N; 83°22' 25.17" E	6.4	24.6	6.5	Brownish black
Kalingapatnam	5.18°20' 46.56"N; 84°7'31.10" E	5.8	27.8	6.7	Brownish black

Isolation of phosphate solubilising bacteria

All the soil samples were mixed in equal quantities to make a single sample, then 1 gm of mixed soil sample was dissolved in 9 ml of sterile deionised water. The soil suspension was filtered and serial dilutions were made as follows: 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6}.Then, bacterial species in the soil samples were isolated and enriched by growing cultures in BMM mineral salt medium (Chen et al., 2003).Then, the bacteria capable of solubilising phosphate were isolated and enriched by growing on Pikovskaya's agar medium (PVK) containing insoluble tricalcium phosphate (Goenadi et al., 2000). Then, 10 gm of glucose was added to the media as a carbon source to enrich the culture. The pH of the medium was adjusted to 7.2 by using 0.1 M NaOH. Then, the Pikovskaya's agar plates were inoculated with 1 ml of soil suspension from respective dilutions and the plates were incubated at 27- 30°C in an incubator for 7 days. For the isolation of pure cultures, 7 days olds prominent colonies which exhibit clear zones around them were picked and subcultured repeatedly for 5 times on Pikovskaya's agar medium.

Table 2: Composition of Pikovskaya's agar medium supplemented with insoluble tricalcium phosphate.

S. No	Material	Weight (gm/L)
1	Yeast extract	0.5
2	$(NH_4)_2SO_4$	0.5
3	$MgSO_4.7H_2O$	0.1
4	NaCl	0.2
5	KCl	0.2
6	$MnSO_4.2H_2O$	0.002
7	$FeSO_4.7H_2O$	0.002
8	Glucose	10
9	$Ca_3(PO_4)_2$	5
10	Agar	1.5

Morphological and physiological characterisation of bacterial isolates

The morphological and phenotypic characteristics of pure bacterial isolates including colony shape, colour, Gram's reactivity, sporulation, Motility were done according to Holt et al., (1994) methodology. Furthermore, the physiological and biochemical characteristics including IMViC (Indole, Methyl-red, Voges-Proskauer, and Citrate), catalase, urease, oxidase, nitrate reductase, starch hydrolysis, lipid hydrolysis, casein hydrolysis, and gelatin hydrolysis were done by the methodologies of Harley and Prescott (2002), and Hammes and Hertel (2009).

Screening of phosphate solubilising ability of active bacterial isolates

The phosphate solubilisation ability of pureisolates was screened by inoculating of cultures in the 1mg/ml concentrated insoluble tricalcium phosphate supplemented PVK broth and these cultures were incubated at 35±2ºC for 15 days in an orbital shaking incubator at 120 rpm. Following incubation, at every three days interval up to 15 days of incubation period, the required quantity of broth was withdrawn from each conical flask and centrifuged at 14000 rpm for 5 minutes. The collected supernatant was used for the quantitative determination of phosphate. The PVK broth of containing soluble phosphate was measured by the methodology of Strickland and Parsons, (1968).Phosphate solubility in the broth were expressed as µg per ml of broth.

Effect of salinity on phosphate solubilisation by bacterial isolates

The phosphate solubilising ability of isolated pure strains under salt stress was screened by plating of cultures obtained from the PVK agar plates cultured on the PVK broth

supplemented with 1mg/ml tricalcium phosphate and increasing NaCl concentrations. The isolated colonies of bacteria were grown in insoluble tricalcium phosphate containing PVK broth which is supplemented with five different concentrations of salt such as 1, 2, 3, 4, and 5% NaCl. These cultures were incubated at 28±2ºC for 15 days in an orbital shaking incubator for adequate development. After incubation, the required quantity of broth was withdrawn from each conical flask and centrifuged at 14000 rpm for 5 minutes. Then the quantity of soluble phosphate in supernatant was measured by the methodology of Strickland and Parsons (1968).

Screening of plant growth promoting ability of isolated bacterial colonies

Plant growth promoting activities of the nine isolated bacterial strains were evaluated by the estimation of IAA (Indole acetic acid) production, and GA (Gibberellic acid) production.

i) Estimation of IAA production

The ability of the bacterial isolates to produce IAA was determined qualitatively according to the methodology of Dawwam et al., (2013) by using Yeast Extract Mannitol (YEM) broth. All the isolated pure bacterial cultures were inoculated onto 250 ml conical flasks containing 50 ml of YEM broth media along with 0.1% L-tryptophan. The flasks were incubated in orbital shaker at 28±2°C at 120 rpm for 15 days. After incubation, the culture broths were centrifuged at 12000 rpm for 5 min and the collected supernatant was used to estimate the IAA production. To estimate IAA quantity, 1 ml of salkowaski reagent was added to the 500 µl of supernatant. Salkowski reagent was prepared by dissolving 2% of 0.5M $FeCl_3$ in 35% perchloric acid. Then, the tubes were incubated for 30 min in dark at room temperature and the pink colour development indicates the presence of IAA. The developed colour intensity was measured at 530nm by using a spectrophotometer against blank. The concentration of IAA in the test samples were determined by using calibration graph of known concentrations of IAA ranged between 1.25-100µg/ml.

ii) Estimation of GA production

The gibberellins were quantitatively estimated by the methodology of Holbrook et al., (1961) with slight modifications. To estimate gibberellins, 48-hour old broth cultures of bacterial isolates were centrifuged at 10000rpm for 15 minutes and the supernatant collected. The pH of the supernatant was adjusted to 2.5 by using 3.75N HCl. Then, 2 ml of zinc acetate reagent (1.2 M zinc acetate and 1% of glacial acetic acid) added to the 15 ml of supernatant and allowed to stand for 2 minutes. Then, 2 ml of 10.6% potassium ferrocyanide was added to the reaction mixture, followed by centrifuge at 2000 rpm for 15 minutes and supernatant was collected. Then, 5ml of 30% HCl was added to the supernatant and the reaction mixture was incubated for 75 minutes at 20°C. Finally, the concertation of gibberellins was determined by measuring the absorbance at 254 nm using a UV spectrophotometer against blank. The blank was prepared by adding 5% HCl. The standard calibration curve was prepared by using known concentrations of gibberellins ranged between 100-1000µg/ml.

Effect of MGP-04 on the rice seed germination

The seed germination of rice was examined to find out the possible effects of the bacterial isolate MGP-04. Field collected healthy young seeds of rice were washed with tap water and then distilled water. Washed seeds were surface sterilized with 70% ethanol and 2% sodium hypochlorite. The experiment was conducted in four groups. In the first group, the rice seeds were grown in normal conditions, and they are referred as 'control'. As well as in the second, third, and fourth groups, the rice seeds were treated with bacterial culture MGP-04 with increasing cell concentration, such as $1x10^5$, $1x10^6$, and$1x10^7$ cells/ml. The seeds were then soaked overnight in their respective concentrated bacterial cultures and kept for 5 days for germination on moistened filter paper in the sterile Petridish. After the initiation of germination, seedlings were transferred to plastic pots filled with autoclaved vermiculture and the pots were grown at 25ºC under a 16-hour photoperiod in a green house. The pots were irrigated every day with a half-strength Hoagland solution. After 10 days of growth, seedling growth parameters were established by assessing the percentage of seed germination, root length, shoot length, and dry weight.

Molecular Identification

Among all nine bacterial isolates, the potent phosphate solubilising bacterial isolate with plant growth promoting activities was characterized molecularly to identify the species by using 16s rRNA gene sequencing. The molecular characterization was carried out by the isolation of genomic DNA from the bacteria isolate, Amplification and sequencing of 16s rRNA gene, and phylogenetic tree construction

Isolation of Genomic DNA

The CTAB lysozyme method was used to isolate the genomic DNA of the bacterial isolate. To isolate genomic DNA, 1% of 10% glycine (W/V) was added to the 24 hours old late log phase cultures before harvesting and the culture was incubated another 24 hours at 37°C. To isolate genomic DNA, 10 ml of 48 hours old culture of MGP-04 was transferred to a 15 ml centrifuge tube and centrifuged at 2000 rpm for 10 minutes at room temperature. Then, the supernatant was removed, and the cell pellet was resuspended in 450 µl of Glucose Tris EDTA solution. The resultant cell suspension was taken in a 2 ml eppendorf tube containing 50 µl of 10 mg/ml lysozyme and incubated at 37°C for overnight. After incubation, 150 µl of 2:1 ratio mixture of 10% SDS and 10 mg/ml proteinase K solution was added to the cell suspension and incubated at 55°C for 40 minutes. Then, 200 µl of 5 M NaCl and 160 µl of CTAB solutions were added, mixed gently, and again incubated at 65°C for 10 minutes at room temperature. After incubation, an equal volume of 24:1 ratio mixture of chloroform and isoamyl alcohol solution was added and centrifuged at 8000 rpm for 5 minutes at 4°C.After centrifugation, 900 µl of upper layer was transferred to fresh 2 ml eppendorf tube and extraction was repeated again with 24:1 ratio mixture of chloroform: isoamyl alcohol solution. After extraction,560 560 µl of isopropanol was added to the 800 µl of upper aqueous layer and the mixture was gently mixed by inversion until the DNA was precipitated out. Then, the supernatant was discarded and 1 ml of 70% ice cold ethanol was added to the precipitate and stirred to wash the DNA.

After washing, the ethanol was removed by centrifugation at 4°C for 10 minutes. The DNA pellet was maintained by resuspended in 50 µl of Tris EDTA buffer. Finally, the isolated genomic DNA quantity and quality is assessed by a UV spectrophotometer and 1% Agarose gel electrophoresis.

Amplification and sequencing of 16S rRNA gene

PCR amplification and 16S rRNA gene sequencing were carried out as described Li *et al.,* (2010).The 16S rRNA gene of bacterial isolate MGP-04 was amplified by using PCR. Two universal primers 27F (5'AGAGTTTGATCMTGGCTCAG 3') and 907R (5'CCGTCAATTCMTTTRAGTTT3') were used to amplify 16S rRNA genes. PCR reaction mixture of 25 µl total volume, containing 1/10 volume 10× *Taq* buffer, 2 mm $MgCl_2$, 1-unit *Taq* DNA polymerase, 0.2 mMdNTP, 20 pmol forward primer, 20 pmol reverse primer and 100 ng DNA. DNA amplification was carried out in a Biorad Mini thermocycler with the following procedure: an initial denaturing step at 94°C for 5 min; 40 cycles for 1 min at 94°C (denature), 1 min at 48°C (annealing), 2 min at 72°C (extension) and a final elongation step at 72°C for 5 min and then cooled to 4˚C. Amplified PCR products were separated by electrophoresis on 1.5 % agarose gel. The purified PCR products was subjected to Sanger's di-deoxy sequencing, in both forward and reverse direction ns, using Big Dye terminator v3.1 cycle sequencing kit on ABI Prism3700 DNA Analyzer (Applied Biosystems Inc., USA).

Sequence analysis and phylogenetic tree construction

Bacterial isolate MGP-04 16s rRNA gene nucleotide composition was computed by Seqstate V.1.21 server (Muller, 2005).The molecular identification of bacterial isolate was conducted by constructing a phylogenetic tree with some major bacterial species. Bacterial isolate 16S rRNA gene sequence was subjected to the BLASTn in NCBI server to identify the homologous sequence or species and from the NCBI database, homologous species were selected based on their degree of homology with the target rbcL gene sequence. The selected homologous species 16S rRNA gene sequences were derived from the nucleotide NCBI database. Multiple sequence alignment was conducted with UPGMA to search homology of 16S rRNA gene sequences between the bacterial isolate and the selected homologous species. Phylogenetic tree was constructed using MEGAX. The accuracy test of the tree was performed by the bootstrap method 1000 times.

3. Results and Discussion

Isolation of phosphate solubilising bacteria from soil sample

The isolation of phosphate-soluble bacterial strains was done from five different soil samples of Andhra Pradesh. From these five soil samples, a total of nine different discrete bacterial colonies were identified, which exhibit clear zones around the colonies in the PVK agar plates. These discrete bacterial colonies were sub-cultured onto PVK agar plates by streaking to obtain pure cultures. Thus, colonies that were single and had no crosses were picked and again streaked onto PVK agar plates, incubated at 37°C for 24 hrs, and stored at 4°C until further use. The purified strains were maintained on PVK agar slants and stored at 4°C with successive subcultures every two weeks. The nine bacterial isolates were named as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09. In figure 1&2, the isolation and purification plates of phosphate solubilising bacteria have been shown.

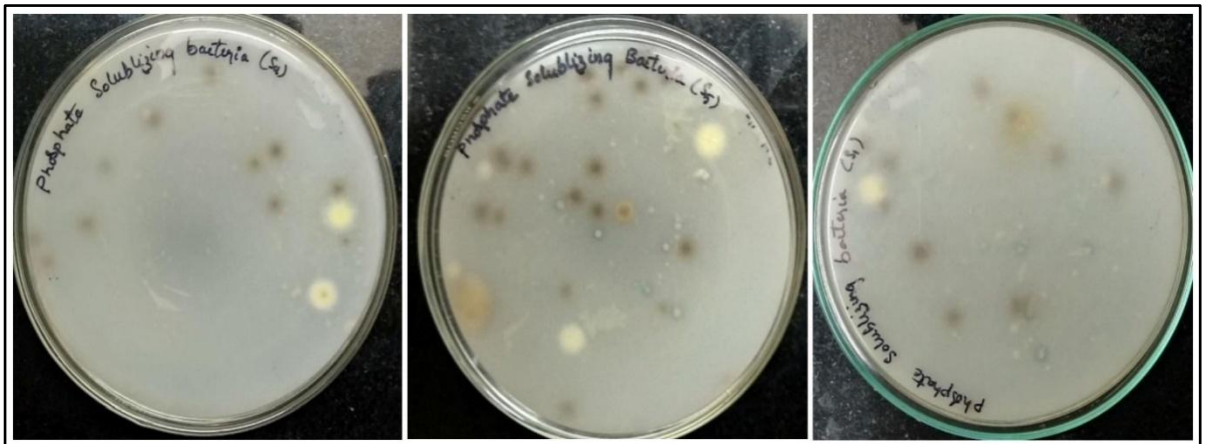

Figure 1: Isolation of phosphate solubilising bacteria from soil samples of coastal areas by culturing on the 5 gm of tricalcium phosphate amended fresh PVK agar plates

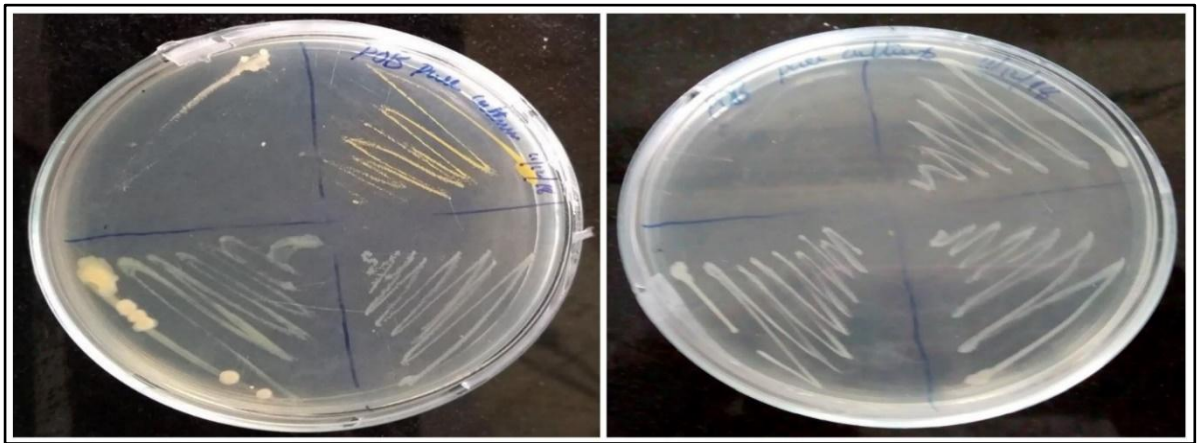

Figure 2: Pure cultures of isolated nine phosphate solubilising bacteria on the tricalcium phosphate amended fresh PVK agar plates

Due to the fixation of phosphorus in soil as insoluble forms such as phosphates of iron, aluminium, and calcium, the availability of free phosphates is limited for plants (Walpola and Yoon, 2012).The rhizosphere and soils are the great natural resources for several microorganisms that can liberate phosphorous from soil phosphates, essentially through mineralisation and this class of microbes is known as Phosphorous Solubilising Microorganisms (PSM) (Battacharyya and Jha, 2012). Furthermore, isolation is an essential way to get novel microorganisms, and evaluation of their physiological characteristics are important for the understanding of their environmental functions and potential applications in various fields (Vandamme et al., 1996). The present results are evident from the studies of Zhu et al., (2011) who reported that the halophilic soil microorganisms that are capable of solubilising insoluble phosphates are useful for saline alkaline agriculture. Previous studies such as Tajini et al., (2012), Postma et al., (2010), and David et al., (2014) reported that many bacterial species such as *Bradyrhizobium*, *Ralstonia*, *Rhizobium*, *Rhodococcus*, *Salmonella*, *Serratia*, *Sinomonas*, and *Thiobacillus* have effective inorganic phosphate solubilising properties.Andhra Pradesh is one of the states having the largest coastal areas in India, so there is a tremendous possibility for bacterial diversity to solubilise phosphates. The present study demonstrated that the soils from the coastal region of Andhra Pradesh are a potent source for phosphate-solubilising bacteria due to their large microbial diversity.

Morphological characterisation of bacterial isolates

The morphological characterization demonstrated that among the 9 isolates, 6 isolates, such as MGP-01, MGP-02, MGP-04, MGP-05, MGP-08, and MGP-09 exhibited a circular configuration, whereas the remaining 3 isolates (MGP-03, MGP-06, and MGP-07) exhibited an undulating configuration. As well as, among the 9 bacterial isolates, 5 colonies, including MGP-01, MGP-02, MGP-03, MGP-04, and MGP-07 appear in white. MGP-05, and MGP-08 colonies were in cream colour, while MGP-06, and MGP-09 colonies were yellow in colour.

Furthermore, among 9 isolates, 6 isolates includingMGP-01, MGP-02, MGP-04, MGP-06, MGP-07, and MGP-08 exhibit rod-shaped morphology whereas, 3 isolates (MGP-03, MGP-05, and MGP-09) were cocci. Eight isolates out of nine, such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-08, and MGP-09 were gram positive while the remaining 1 (MGP-07) was gram negative. Motility was observed in 5 bacterial isolates (MGP-01, MGP-03, MGP-06, MGP-07, and MGP-08), and the remaining 4 (MGP-02, MGP-04, MGP-05, and MGP-09) were nonmotile. The endospores were observed in 3 out of the 9 bacterial isolates, such as MGP-02, MGP-07, and MGP-09. The results of morphological and phenotypic characterisation of nine bacterial isolates are shown in table 1.

Table 1: Morphological and phenotypic characterization of the nine bacterial isolates

Characteristics	Bacterial isolates								
	MGP-01	MGP-02	MGP-03	MGP-04	MGP-05	MGP-06	MGP-07	MGP-08	MGP-09
Configuration	Circular	Circular	Undulate	Circular	Circular	Undulate	Undulate	Circular	Circular
Colony colour	White	White	White	White	Cream	Yellow	White	Cream	Yellow
Surface	Chalky	Smooth	Smooth	Smooth	Smooth	Smooth	Chalky	Smooth	Smooth
Grams Reaction	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Positive
Shape	Rods	Rods	Cocci	Rods	Cocci	Rods	Rods	Rods	Cocci
Spore	-	+	-	-	-	-	+	-	+
Motility	+	-	+	-	-	+	+	+	-

Physiological and biochemical characterisation

Several physiological and biochemical assays were performed to characterise isolated bacterial species, including various enzymes such as oxidases, catalase, nitrate reductase, and urease, which might be involved in mineralisation. Table 2 shows the physiological and biochemical properties of nine isolated bacterial strains from soils of coastal areas. From these results, among the nine isolates, three bacterial isolates MGP-03, MGP-04, and MGP-05 were indole positive while, the remaining isolates, MGP-01, MGP-02, MGP-06, MGP-07, MGP-08, and MGP-09 were indole negative. The methyl red test demonstrates

that the bacterial isolates MGP-01, MGP-02, MGP-03, MGP-06, MGP-07, and MGP-09 were positive whereas, MGP-04, MGP-05, and MGP-08 isolates were negative. Among the nine isolates, five isolates such as MGP-01, MGP-03, MGP-05, MGP-06, and MGP-09 were positive for VP test whereas, four isolates including MGP-02, MGP-04, MGP-07, and MGP-08 negative for VP test. In the current study, six isolates such as MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, and MGP-08 tested as positive for citrate utilisation, whereas the remaining three isolates, such as MGP-01, MGP-02, and MGP-09tested as negative for citrate utilisation.

Catalase test demonstrated that the MGP-01, MGP-02, MGP-05, MGP-06, MGP-07, and MGP-08 bacterial isolates possess catalase among nine isolates (i.e., are catalase-positive) and the remaining isolates such as MGP-03, MGP-04, and MGP-09 showed negative result for catalase presence. The urease test indicates that only two bacterial isolates (MGP-03 and MGP-06) exhibit the presence of urease while the remaining bacterial isolates, such as MGP-01, MGP-02, MGP-04, MGP-05, MGP-07, MGP-08, and MGP-09 negative for urease presence. The oxidase test determines that the eight isolates were (MGP-01, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09) tested as positive for oxidase and one isolate, such as MGP-02 tested as negative for oxidase. The present study demonstrated that four bacterial isolates among the nine isolates, such as MGP-04, MGP-05, MGP-06, and MGP-09 possess an enzyme nitrate reductase, whereas the remaining five isolates, such as MGP-01, MGP-02, MGP-03, MGP-07, and MGP-08 negative for the nitrate reductase.

Starch hydrolysis test revealed that six bacterial isolates including MGP-02, MGP-04, MGP-05, MGP-07, MGP-08, and MGP-09 out of nine were positive for starch hydrolysis, while the remaining three isolates, MGP-01, MGP-03, and MGP-06 were negative for starch hydrolysis. The lipid hydrolysis test demonstrates that, Among the nine isolates, five bacterial isolates MGP-02, MGP-03, MGP-04, MGP-06, and MGP-09 exhibit lipid hydrolysis whereas, four isolates, MGP-01, MGP-05, MGP-07, and MGP-08 didn't exhibit lipid hydrolysis. The casein hydrolysis test determines that five bacterial isolates such as MGP-01, MGP-02, MGP-04, MGP-05, and MGP-09 possesses casein hydrolysis, while the remaining four isolates, MGP-03, MGP-06, MGP-07, and MGP-08 didn't possesses casein hydrolysis. The gelatin hydrolysis test determines that, among the nine isolates, only three bacterial isolates including MGP-02, MGP-05, and MGP-08 were tested positive for gelatin hydrolysis and remaining six isolates, MGP-01, MGP-03, MGP-04, MGP-06, MGP-07, and MGP-09 were tested as negative for gelatin hydrolysis.

Table 2: Physiological and biochemical characterization of the nine bacterial isolates

Test Name	Bacterial isolate names								
	MGP-01	MGP-02	MGP-03	MGP-04	MGP-05	MGP-06	MGP-07	MGP-08	MGP-09
Indole	-	-	+	+	+	-	-	-	-
Methyl Red	+	+	+	-	-	+	+	-	+
Voges-Proskauer	+	-	+	-	+	+	-	-	+
Citrate	-	-	+	+	+	+	+	+	-
Catalase	+	+	-	-	+	+	+	+	-
Urease	-	-	+	-	-	+	-	-	-
Oxidase	+	-	+	+	+	+	+	+	+
Nitrate reductase	-	-	-	+	+	+	-	-	+
Starch hydrolysis	-	+	-	+	+	-	+	+	+
Lipid hydrolysis	-	+	+	+	-	+	-	-	+
Casein hydrolysis	+	+	-	+	+	-	-	-	+
Gelatin hydrolysis	-	+	-	-	+	-	-	+	-

Microorganisms have important geoactive functions in the biosphere, especially in biotransformation, biogeochemical cycling, metal, mineral, and soil formation. Furthermore, internal factors, including enzymes and metabolites, have an important role in the biochemical characterisation of microbes. Therefore, microorganisms isolated from soil samples have great significance in various fields such as agriculture, biochemical, pharmaceutical, and environmental biotechnology (Alexander, 1961). Even though the microbial species with plant growth promoting properties are not yet saturated, several microorganisms have been established to enhance agriculture productivity by applying as biofertilizers. Therefore, there is a need to establish novel bacterial species that are associated with plant development and growth processes.

Screening of phosphate solubilising ability

The results of phosphate solubilising abilities for nine isolated bacterial strains are shown in table 3. From these results, the greatest phosphate solubilisation ability was found in the MGP-04 strains, whereas MGP-01 had the lowest phosphate solubilisation ability. On the third day of incubation, the phosphate solubilisation of nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was observed to be 89, 197, 325, 381, 274, 88, 260, 164, and 185 µg/ml respectively. On the sixth day of incubation, the phosphate solubilisation of nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was observed to be 121, 248, 584, 593, 338, 119, 319, 220, and 231 µg/ml respectively. On the ninth day of incubation, the phosphate solubilisation of nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was observed to be 139, 494, 840, 870, 396, 185, 386, 269, and 294 µg/ml respectively. On the 12^{th} day of incubation, the phosphate solubilisation of nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was observed to be 249, 530, 980, 982, 451, 228, 440,

318, and 406 µg/ml respectively. On the 15th day of incubation, the phosphate solubilisation of nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was observed to be 131, 429, 418, 510, 389, 270, 419, 186, and 304 µg/ml respectively. The present study demonstrates that, all the isolated bacterial strains showed significant phosphate solubilisation with increased incubation times.

Table 3: Screening of phosphate solubilisation abilities from the nine bacterial isolates with increasing incubation times

S. No	Isolate	Phosphate solubility (µg/ml)				
		3rd day	6th day	9th day	12th day	15th day
1	MGP-01	89	121	139	249	131
2	MGP-02	197	248	494	530	429
3	MGP-03	325	584	840	980	418
4	MGP-04	381	593	870	982	510
5	MGP-05	274	338	396	451	389
6	MGP-06	88	119	185	228	270
7	MGP-07	260	319	386	440	419
8	MGP-08	164	220	269	318	186
9	MGP-09	185	231	294	406	304

Effect of salinity on phosphate solubilisation by bacterial isolates

The results of phosphate solubilisation abilities under increasing salt concentrations for nine isolated bacterial strains are shown in table 4.From these results, the greatest phosphate solubilisation under all NaCl concentrations were found in the MGP-03 and MGP-04 strains, whereas MGP-01 had the lowest phosphate solubilisation ability under all NaCl concentrations. At 1% NaCl concentration, the phosphate solubilisation ability of nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was observed to be 174, 296, 778, 739, 317, 559, 493, 401, and 285 µg/ml respectively. At 2% NaCl concentration, the phosphate solubilisation ability of nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was observed to be 153, 248, 721, 701, 300, 517, 428, 372, and 238 µg/ml respectively. At 3% NaCl concentration, the phosphate solubilisation ability of nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was observed to be 144, 231, 704, 647, 286, 338, 389, 330, and 210 µg/ml respectively. At 4% NaCl concentration, the phosphate solubilisation ability of nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was observed to be 89, 196, 653, 621, 255, 310, 317, 281, and 165 µg/ml respectively. At 5% NaCl concentration, the phosphate solubilisation ability of nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was observed to be 73, 145, 610, 584, 210, 287, 278, 187, and 126 µg/ml respectively. The present study demonstrates that, all the isolated bacterial strains showed significant reductions in phosphate solubilisation with increasing NaCl concentrations.

Table 4: Effect of salt on the phosphate solubilisation abilities of the nine bacterial isolates with increasing NaCl concentrations

S. No	Isolate	Phosphate solubilization at 15 days of incubation (µg/ml)				
		1% NaCl	2% NaCl	3% NaCl	4% NaCl	5% NaCl
1	MGP-01	174	153	144	89	73
2	MGP-02	296	248	231	196	145
3	MGP-03	778	721	704	653	610
4	MGP-04	739	701	647	621	584
5	MGP-05	317	300	286	255	210
6	MGP-06	559	517	338	310	287
7	MGP-07	493	428	389	317	278
8	MGP-08	401	372	330	281	187
9	MGP-09	285	238	210	165	126

Estimation of IAA production

The quantities of IAA from all the nine bacterial isolates with increasing incubation times were shown in table 5.From these results, the greatest IAA production was found with the MGP-03 and MGP-04 strains, whereas MGP-01 had the lowest IAA production. On the third day of incubation, the production of IAA from nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was quantified to be 82, 186, 684, 549, 240, 417, 352, 228, and 217 µg/ml respectively. On the sixth day of incubation, the production of IAA from nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was quantified to be 119, 227, 743, 712, 259, 487, 381, 278, and 236 µg/ml respectively. On the ninth day of incubation, the production of IAA from nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was quantified to be 148, 248, 786, 740, 271, 528, 421, 315, and 240 µg/ml respectively. On the 12th day of incubation, the production of IAA from nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was quantified to be 190, 274, 838, 764, 310, 569, 486, 389, and 278 µg/ml respectively. On the 15th day of incubation, the production of IAA from nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was quantified to be 198, 318, 847, 795, 338, 595, 513, 418, and 295 µg/ml respectively. These results revealed that all the isolated bacterial strains significantly increase IAA production with increased incubation times.

Table 5: Quantitative estimation of IAA production from nine bacterial isolates

S. No	Isolate	IAA (µg/ml)				
		3rd day	6th day	9th day	12th day	15th day
1	MGP-01	82	119	148	190	198
2	MGP-02	186	227	248	274	318
3	MGP-03	684	743	786	838	847
4	MGP-04	549	712	740	764	795
5	MGP-05	240	259	271	310	338
6	MGP-06	417	487	528	569	595
7	MGP-07	352	381	421	486	513
8	MGP-08	228	278	315	389	418
9	MGP-09	217	236	240	278	295

Indole acetic acid (IAA) is an auxin that plays a crucial role in root growth by inducing cell division. Some microorganisms produce IAA, which promotes root development and root length, resulting in a larger root surface area that allows the plant to absorb more nutrients from the soil (Boiero et al., 2007). The current study is evident from the earlier reports of several researchers who identified several soil bacterial species that produce IAA. For example, Kaydan et al., (2013) reported that bacterial species such as *J. bacillus*, *T. saccharophilus*, *T. goriensis*, *B. megaterium*, *B. simplex*, and *B. aryabhattai*. It has been reported by Rajput et al., (2013) that the soil bacteria *Planococcusrifietoensis* promotes the plant growth of Triticum aestivum by producing IAA. Similarly, Siddikee et al., (2010) reported that several bacterial species, such as *Oceanobacillus* sp., *Halomonas* sp., *Exiguobacterium* sp., *Zhihengliuella* sp., and *Bacillus* sp., exhibit plant growth promoting activities by producing IAA.

Estimation of gibberellic acid

The quantities of GA from all the nine bacterial isolates with increasing incubation times were shown in table 6. The present results demonstrated that, among the nine bacterial isolates, six isolates such as MGP-01, MGP-03, MGP-04, MGP-05, MGP-07, and MGP-09 exhibit GA production in all the tested incubation periods, whereas MGP-08 didn't exhibit GA production. The isolates MGP-2 and MGP-06 exhibit GA production on the 15th and 9th day of incubation respectively. From these results, the greatest GA production was found with the MGP-03 strains. Whereas MGP-02 had the lowest GA production. On the third day of incubation, the production of GA from nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was quantified to be 17.6, 0, 35.9, 32.6, 10.5, 0, 15.5, 0, and 26.9 µg/ml respectively. On the sixth day of incubation, the production of GA from nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was quantified to be 23.5, 0, 49.3, 36.8, 18.0, 0, 28.8, 0, and 33.8 µg/ml respectively. On the ninth day of incubation, the production of GA from nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was quantified to be 37.4, 0, 58.3, 51.5, 27.4, 13.7, 37.1, 0, and 39.2 µg/ml respectively. On the 12th day of incubation, the production of GA from nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was quantified to be 44.8, 0, 89.5, 76, 33.2, 15.9, 44, 0, and 46 µg/ml respectively. On the 15th day of incubation, the production of IAA from nine bacterial isolates such as MGP-01, MGP-02, MGP-03, MGP-04, MGP-05, MGP-06, MGP-07, MGP-08, and MGP-09 was quantified to be 47.3, 10.5, 93.2, 88.3, 38.4, 22.5, 47.3, 0, and 53.8 µg/ml respectively. These results revealed that, all the isolated bacterial strains significantly increase GA production with increased incubation times.

Table 6: Quantitative estimation of GA production from nine bacterial isolates

S. No	Isolate	GA (µg/ml)				
		3rd day	6th day	9th day	12th day	15th day
1	MGP-01	17.6	23.5	37.4	44.8	47.3
2	MGP-02	0	0	0	0	10.5
3	MGP-03	35.9	49.3	58.3	89.5	93.2
4	MGP-04	32.6	36.8	51.5	76.0	88.3
5	MGP-05	10.5	18.0	27.4	33.2	38.4
6	MGP-06	0	0	13.7	15.9	22.5
7	MGP-07	15.5	28.8	37.1	44.0	47.3
8	MGP-08	0	0	0	0	0
9	MGP-09	26.9	33.8	39.2	46.0	53.8

Gibberellins (GA) are secondary metabolites that can work as plant growth hormones. They are biotechnologically and economically important because of their use in agriculture, horticulture, gardening, and other plant-allied fields (Shukla et al., 2005). In plants, gibberellins are involved in a wide range of developmental processes such as breaking of flower dormancy, fruit senescence, seed dormancy, stem elongation, and sex expression (Kang et al., 2014). In addition to plants, many bacterial and fungal species also produce gibberellins (Gutierrez et al., 2001). Mansour and Aldesuquy, (2014) has been reported that several bacterial species, including *Acetobacter*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholedria*, and *Pseudomonas*, produce gibberellins in significant quantities. Piccoli et al., (1997) observed that the plant growth promoting rhizobacteria (PGPR) enhances the growth and yield of many crop plants by producing gibberellins. These results indicated that the bacterial isolate MGP-04 exhibit better phosphate solubility and plant growth promoting activities hence, this bacterial isolate was identified molecularly and applied as bioinoculant to study the beneficial effect on the growth and yield of Rice.

Effect of MGP-04 on the rice seed germination

The results of rice seed growth parameters under the treatment of different MGP-04 cell concentrations are shown in table 7. From these results, the greatest germination percentage of rice seedlings was found with the treatment of 1x10^7 bacterial concentrations, and the lowest germination percentage was found in the control group. The germination percentages of rice seedlings with the different treatment groups including control, 1x10^5, 1x10^6, and1x10^7 cells/ml MGP-04 bacterial isolate, were measured as 95.8, 96.6, 96.9, and 98.2% respectively. As well as, the highest root growth was observed in the seedlings that were treated with 1x10^7 bacterial concentration, and the lowest root growth was observed in the control group. The root length of rice seedlings with the different treatment groups including control, 1x10^5, 1x10^6, and1x10^7 cells/ml MGP-04 bacterial isolate, were measured as 3.1, 3.5, 4.5, and 5.8 cm respectively. It was observed that the shoot lenght of rice seedlings was highest with the 1x10^7 cells/ml concentration of MGP-04 isolate, whereas the lowest shoot length was found in the control group. The corresponding shoot lengths of different treatment groups of rice seedlings such as control, 1x10^5, 1x10^6, and1x10^7 cells/ml MGP-04 bacterial isolate was measured as 4.1, 4.9, 5.5, and 6.5 cm. The dry weight of the rice seedlings was significantly increased with the treatment groups containing increasing concentrations of bacterial cells. The highest dry weight was obtained from the

seedlings that were treated with $1x10^7$ cells/ml of MGP-04 bacterial isolate, while the lowest dry weight was obtained from the control group. The respective dry weights of rice seedlings with the different treatment groups including control, $1x10^5$, $1x10^6$, and$1x10^7$ cells/ml MGP-04 bacterial isolate was measured as 116.8, 121.3, 148.9, and 159.1 mg/gm.

Table 7: Effect of MGE-04 on growth parameters of rice seedlings

S. No	Bacterial concentration/ml	Percent germination	Root Length (cm)	Shoot length (cm)	Dry Weight (mg)
1	Control	95.8	3.1	4.1	116.8
2	1×10^5	96.6	3.5	4.9	121.3
3	1×10^6	96.9	4.5	5.5	148.9
4	1×10^7	98.2	5.8	6.5	159.1

In the present study, it was observed that the increasing bacterial cell concentration (MGP-04) increases the seed germination percentage, root growth, shoot growth, and dry weight in rice cultivar. The current results imply that phosphate solubilising bacteria treatment is advantageous for rice cultivar, since a significant rise in germination of seeds, and also root and shoot length, has been observed when compared to the control. It has been stated by Kannaiyan et al., (2004) that the PGPRs play a significant role in the production of biofertilizers to increase agriculture productivity. The present results are evident from the results of Chaykovskaya et al., (2001) who reported that phosphate solubilising bacteria increase the yield of pea and barely by releasing plant growth regulators along with phosphate solubilisation. Linu et al., (2009) observed that phosphorous and nitrogen concentrations, as well as nodulation, biomass, and grain yield in cowpea are significantly increased with the treatment of phosphate solubilising bacteria, *Burkholderia* sp. Similarly, Korir et al., (2017) found that the treatment of rhizobacteria along with the PGPR greatly enhances the root nodulation and biomass of common bean. It has been reported by Walpola and Yoon, (2013) that tomato plants exhibit higher plant growth and biomass when they are inoculated with PGPRs such as *Pantoeaagglomerans* and *Burkhoderiaanthina*.

Molecular and Phylogenetic Characterization of bacterial Isolate MGP-04

Total DNA quantification

Genomic DNA was isolated from the bacterial strain MGP-04 and the total DNA content was found as 482 ± 12 µg/g wet weight. Absorbance ratio of isolated DNA at A260/280 was in the range of 1.87–1.96 respectively. The results were given as Mean ± Standard Deviation (SD) obtained from three independent experiments. The mol% G + C content of DNA of bacterial strain MGP-04 was calculated by using the following equation of Marmur and Doty (1962). On 1% agarose gel the isolated genomic DNA was appeared as prominent band.

PCR amplification and sequencing of 16s rRNA gene

The 16S rRNA regions of genomic DNA of bacterial isolate MGP-04 yielded clear single and good quality bands. In agarose gel electrophoresis the 1st well shows marker DNA and the second wells shows amplified gene product with approximately 1500bp length. Figure 3 showed the PCR amplified DNA band in 1.5% agarose gel. Due to the high primer universality and discriminatory power, the 16S rRNA gene has been routinely used for phylogenetic studies (Schneider et al., 2004).

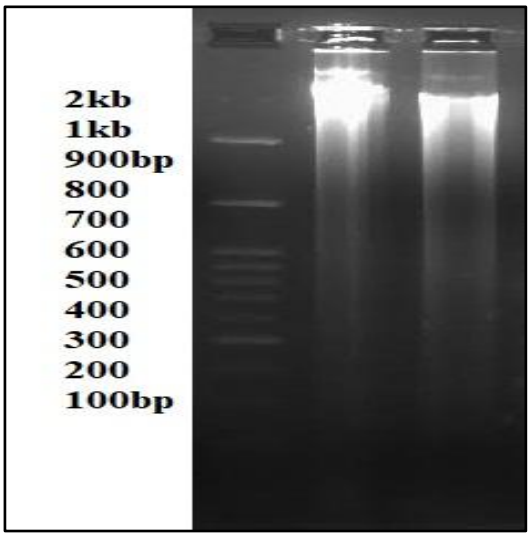

Figure 3: Photograph showing the PCR amplified 16S rRNA gene band in 1.5% agarose gel.

Microbiology strives to develop rapidly by identifying and characterizing microbial species to establish a correct phylogenetic relationship. Clegg and Zurawski, (1991) stated that phylogenetic and evolutionary studies of microorganisms are rapidly increasing due to sequencing of PCR amplified gene products. The 16S rRNA gene is known as the best characterized but not the most variable region in bacterial genomes and it is easily retrievable with common primers for PCR. Sabat et al., (2017) reported that the 16S rRNA is a highly conserved 1.5 kilobase pair length gene, which is considered as a prime target of phylogenetic studies because it is found throughout all bacteria, as either a single copy or numerous copies. It has been stated by Woo et al., (2008) that identification and phylogenetic relationships of uncultivable bacteria have been made possible by 16S rRNA sequencing. The present study acquired the advantage of the 16S rRNA gene, taking into consideration that the coding 16S rRNAgene is easily amplified and sequenced in prokaryotes and has an impact in phylogenetic investigations by providing a reliable placement of a taxon into a group and genus.

Sequence analysis and multiple sequence alignment

A sequence characteristic of 16S rRNA gene was calculated by seqstate v.1.21 (Muller, 2005). The sequence of PCR amplified bacterial isolate MGP-04 16S rRNA gene have 1428 nucleotides showed in figure 3. The sequence of 16S rRNA gene have 368 bp of Adenine(A), 301 bp of Thymine (T), 434 bp of Guanine (G) and 325 bp of Cytosine (C). The % of GC was calculated as 53.2.The 16S rRNA gene (1-1428 bp) sequence of bacterial isolate MGP-04was used as a query against the nucleotide sequence database. The BLASTn search of the Gen Bank database using the 1428 bp 16S rRNA gene sequence of MGP-04showed its similarity to many species of the *Bacillus* genus. From the BLASTn results, it was observed that the 16S rRNA gene sequence of

bacterial isolate MGP-04 showed the highest similarity to strain *Bacillus toyonensis* strain Se8, with an identity of 99.93% and an E-value of 0.0. From the hits, a total of 9 sequences which belongs to the *Bacillusgenus* were selected from the NCBI data base, and the selected sequences were used for multiple sequence alignment. Multiple sequence alignments of the 16S rRNA regions were obtained using the CLUSTAL program (Higgins *et al.*, 1992).

From the present results also concluded that the 16S rRNA from bacterial isolate MGP-04 have comparatively high GC content, which is evident with the previous studies. Hildebrand et al., (2010) reported that the GC content varies highly in bacterial species and also stated that the variation of GC content in independent bacterial species ranging from 13 to more than 75%. Even though the precise reasons for these differences in GC content within and across species are unknown, according to Mann and Chen, (2010) it is expected that a variety of variables, including both evolutionary development and environmental influences, are involved. Hence, high G/C content leaves microbial genomes less frequent than A/T nucleotides, and this suggests bacterial genes contain high GC content. This is also evident with the results of Zhang and Gao, (2017) who reported that linear regression studies demonstrate that the proportion of amino acids translated by GC rich codons makes positive contributions to increased evolutionary rates, whereas the amino acids preferentially acquired to be in GC rich species. Foerstner et al., (2005) has been observed that the microbial groups coinhabiting same environments tend to have similar %GC irrespective of taxa. Multiple sequence alignment can be used to analyse DNS, RNA, and protein sequences to find out closely related sequences. Multiple sequence alignment procedures are a combination of computational solutions for aligning evolutionary related sequences by accounting for all evolutionary events including mutations, insertions, deletions, and rearrangements under particular conditions (Wallace et al., 2005), Notredame, (2007) has stated that multiple sequence alignments give more information than pairwise alignments because they reveal structural and functionally important areas within a family.

Phylogenetic tree analysis

The selected homology sequences for the target 16S rRNA gene were extracted from the NCBI database for constructing a phylogenetic tree. The evolutionary divergence of phosphate solubilising bacterial isolate MGP-04with its relative members was determined. A satisfactory result was established by the use of 16S rRNA gene as a marker to evaluate the phylogenetic relationship. In the phylogenetic tree, there were 2 main clades. The first main clade consists of three subclades. The first subclade of main clade 1 was composed of *Bacillus cereus* strain 1BS20 (KM015450.1), *Bacillus megaterium* (KY007586.1). The second subclade of the main clade 1 was composed with the *Bacillus cereus* strain AHR3 (KT456542.1). The third subclade of the main clade 1 was composed with the *Bacillus cereus* strain CP133 (KU312197.1), *Bacillus cereus* strain B88 (LN890264.1), *Bacillus cereus* strain B77 (LN890253.1). The second main clade consists of three subclades. The first and second subclade of main clade 2 was composed of *Bacillus cereus* strain Se1 (KX959974.1) and *Bacillus thoringiensis* (EU153549.1). The third subclade of main clade 2 was composed of MGP-04and *Bacillus toyonensis* strain 1BS2 (KX959981.1). The phylogenetic tree indicated that the phosphate solubilising bacterial isolate MGP-04 has a close branch with *Bacillus toyonensis* strain 1BS2 (GenBank Accession No. KX959981.1). On the basis of morphological studies, the molecular phylogenetics, it is revealed that the bacterial isolate MGP-04 is belongs to *Bacillus toyonensis* species. The maximum-parsimony phylogenetic tree was showed in figure 4.

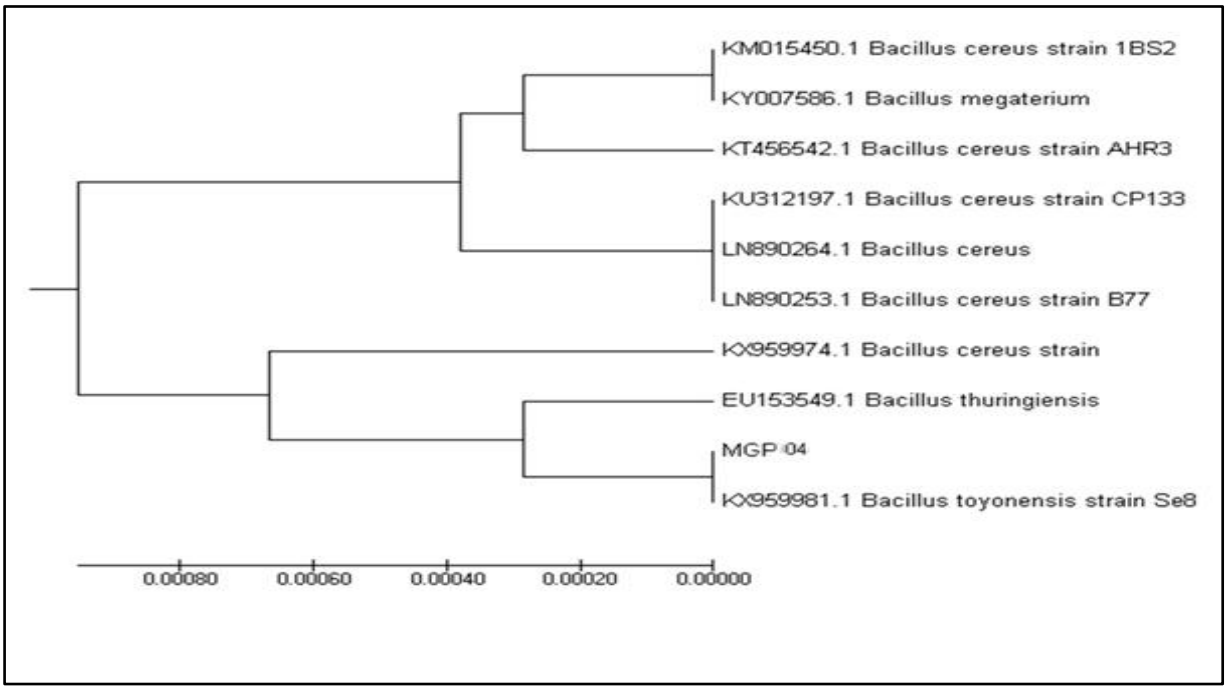

Figure 4: Maximum Parsimony tree of phosphate solubilising bacterial isolate MGP-04 and other relative species based on the 16S rRNA gene

DNA sequence analysis in several species provides valuable information about their taxonomy, gene makeup, and utilizations. DeGroot et al., (2011) reported that the generic level identification was deemed effective when a single genus was involved in all hits with maximum percent identification scores are greater than 95%. While species identification was deemed effective only when a single species was included with the highest percent identity score of greater than 95%. In the phylogenetic tree, the clades are organised mostly with the combination of several species and strains. Therefore, generating a local barcode database is necessary for a wide range of ecological applications, including the construction of community phylogenetics (Kress et al., 2009).

4. Conclusion

The present study demonstrates that the bacterial strain MGP-04, isolated from the coastal soils of Andhra Pradesh state, possesses high dissolving abilities of insoluble phosphate under normal as well as saline conditions. Furthermore, the bacterial isolate can also promote plant growth by producing plant growth hormones such as IAA and GA. The molecular identification by the 16S rRNA gene revealed that the bacterial isolate MGP-04 belongs to the *Bacillus toyonensis* species. Furthermore, increasing bacterial cell concentration (MGP-04) increases the seed germination percentages, root growth, shoot growth, and dry weight in rice cultivar. The current results imply that phosphate solubilising bacteria treatment is advantageous for rice cultivar, since a significant rise in germination of seeds, and also root and shoot length, has been observed when compared to the control. Hence, the present study concluded that the bacterial isolate MGP-04 can be used as a potential phosphate solubiliser and plant growth promoter to increase crop productivity in reclaimed soils.

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Conflicts of interest
The authors declare no conflicts of interest.

References

[1] Alexander, M. (1961). Introduction to soil microbiology. Wiley Estern Ltd. New Delhi.
[2] Walpola, B.C. and Yoon, M. (2012): "Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils: a review," African Journal of Microbiology Research., 6: 6600–6605.
[3] Bhattacharyya, P.N. and Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J. Microbiol. Biotechnology., 28: 1327–1350. doi: 10.1007/s11274-011-0979-9.
[4] Boiero, L., Perrig, D., Masciarelli, O. (2007). Phytohormone production by three strains of *Bradyrhizobiumjaponicum* and possible physiological and technological implications. ApplMicrobiol Biotechnology., 74: 874–880.
[5] Chahboune, R., Said, B., Silvia, M., Eulogio, J.B. (2011). Characterization of Bradyrhizobium species isolated from root nodules of *Cytisusvillosus* grown in Morocco, Systematic and Applied Microbiology., 34(6): 440-445. doi.org/10.1016/j.syapm.2011.04.008.
[6] Chaykovskaya, L.A., Patyka, V.P., Melnychuk, T.M. (2001). Phosphorus mobilizing microorganisms and their influence on the productivity of plants. In: Plant Nutrition-Food Security and Sustainability of Agroecosystem. Horst WJ (Ed) pp: 668-669.
[7] Chen, K., Wu, J., Liou, D., Hwang, S. (2003). Decolorization of the textile dyes by newly isolated bacterial strains, J. Biotechnology., 101: 57-68.
[8] Clegg, M.T. and Zurawski, G. (1991). Chloroplast DNA and the study of plant phylogeny. Pp. 1- 13 in P. S. Soltis, D. E. Soltis, and J. J. Doyle, eds. Molecular systematics of plants. Chapman & Hall, New York.
[9] Cordell, D., Drangert, J.O., White, S. (2009). The story of phosphorus: global food security and food for thought. Global Environ. Chang., 19: 292-305.
[10] David, P., Raj, R.S., Linda, R., Rhema, S.B. (2014). Molecular characterization of phosphate solubilizing bacteria (PSB) and plant growth promoting rhizobacteria (PGPR) from pristine soils. Int. J. Innov. Sci. Eng. Technol., 1: 317–324.
[11] Dawwam, G.E., Elbeltagy, A., Emara, H.M., Abbas, I.H., Hassan, M.M. (2013). Beneficial effect of plant growth promoting bacterianisolated from the roots of potato plant. Annals of Agricultural Science., 58(2):195–201.
[12] De Groot, G.A., During, H.J., Maas, J.W., Schneider, H., Vogel, J.C., Erkens, R.H.J. (2011). Use of rbcL and trnL-F as a two-locus DNA barcode for identification of NW-European ferns: an ecological perspective. PLoS ONE., 6: e16371.
[13] Foerstner, K.U., Von Mering, C., Hooper, S.D., Bork, P. (2005). Environments shape the nucleotide composition of genomes. EMBO Rep., 6: 1208–1213.
[14] Goenadi, D.H., Sisweto, I., Sugiarto, Y. (2000). Bioactivation of poorly soluble phosphate rocks with a phosphorus-solubilizing fungus. Soil Sci Soc Am J., 64: 927–32.
[15] Gutiérrez-Mañero, F., Ramos-Solano, B., Probanza, A., Mehouachi, J., Tadeo, F.R., Talon, M. (2001). The plant-growth-promoting rhizobacteria Bacillus pumilus and Bacillus licheniformis produce high amounts of physiologically active gibberellins. Physiol Plant., 111: 206–211.
[16] Hammes, W.P. and Hertel, C. (2009). Genus I. Lactobacillus. Bergey's Manual of Systematic Bacteriology., 3: 465-511.
[17] Harley, J.P. and Prescott, L.M. (2002). Laboratory Exercises in Microbiology. 5th Edition, The McGraw-Hill Companies.
[18] Harrison, A.F. (1987): Soil organic phosphorus: a review of world literature. Cab International Wallingford, Wallingford, UK.
[19] Hildebrand, F., Meyer, A., Eyre-Walker, A. (2010). Evidence of selection upon genomic GCcontent in bacteria. PLoS Genet., 6: e1001107. doi: 10.1371/journal.pgen.1001107.

[20] Holbrook, A.A., Edge, W.L.W., Bailey, F. (1961). Spectrophotometric method for determination of gibberellic acid in gibberellins. ACS Washington, D.C., 159-167.
[21] Holt, J.G., Krieg, N.R., Sneath, P.H.A., Stanley, J.T., William, S.T. (1994). Bergey's Manual of Determinative Bacteriology. Williams and Wilikins, Baltimore, 786-788.
[22] Kadyan, S., Panghal, M., Kumar, S. (2013). Assessment of functional and genetic diversity of aerobic endospore forming Bacilli from rhizospheric soil of *Phyllanthusamarus* L. World J MicrobiolBiotechnol., 29: 1597–1610. doi.org/10.1007/s11274-013-1323-3.
[23] Kang, S.M., Radhakrishnan, R., Khan, A.L., Kim, M.J., Park, J.M., Kim, B.R., Shin, D.H., Lee, I.J. (2014). Gibberellin secreting rhizobacterium, Pseudomonas putida H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. Plant Physiol. Biochem., 84: 115–124.
[24] Kannaiyan, S., Kumar, K., Govindarajan, K. (2004). Biojertilizer Technology jor Rice-based Cropping System. Scientific Pub. (India)., Jodhpur.
[25] Khan, A.A., Jilani, G., Akhtar, M.S., Naqvi, S.M.S., Rasheed, M. (2009). Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J. Agric. Biol. Sci., 1: 48-58.
[26] Korir, H., Mungai, N.W., Thuita, M., Hamba, Y., Masso, C., (2017). Co-inoculation effect of rhizobia and plant growth promoting rhizobacteria on common bean growth in a low phosphorus soil. Front. Plant Sci., 8: 141.
[27] Kress, W.J., Erickson, D.L., Jones, F.A., Swenson, N.G., Perez, R., Sanjur, O., Bermingham, E. (2009). Plant DNA barcodes and a community phylogeny of a tropical forest dynamics plot in Panama. Proc. Natl. Acad. Sci., USA. 106: 18621-18626.
[28] Lambers, H. and Plaxton, W.C. (2018). P: back to the roots. Annu. Plant Rev., 48: 3–22.
[29] Li, M., Hong, Y., Klotz, M.G. (2010). A comparison of primer sets for detecting 16S rRNA and hydrazine oxidoreductase genes of anaerobic ammonium-oxidizing bacteria in marine sediments. ApplMicrobiol Biotechnology., 86: 781–790. doi.org/10.1007/s00253-009-2361-5.
[30] Linu, M.S., Stephen, J., Jisha., M.S. (2009). Phosphate solubilizing Gluconacetobacter sp. Burkholderia sp. and their potential interaction with Cowpea (Vigna unguiculata (L.) Walp). Intern. J. Agri. Res., 4: 79 - 87.
[31] Maharajan, T., Ceasar, S. A., Ajeesh Krishna, T. P., Ramakrishnan, M., Duraipandiyan, V., Naif Abdulla, A. D. (2018). Utilization of molecular markers for improving the P efficiency in crop plants. Plant Breed., 137: 10–26. doi: 10.1111/pbr.12537.
[32] Mann, S. and Chen, Y.P.P. (2010). Bacterial genomic GþC composition-eliciting environmental adaptation. Genomics., 95: 7–15.
[33] Mansour, F.A. and Aldesuquy, H.S.H. (2014). Studies on plant growth regulators and enzymes production by some bacteria. Qatar QatarUnivSci J., 159: 121–128.
[34] Marmur, J. and Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J. mol. Biol., 5: 109.
[35] Mishra, B. K., Lal, G., Sharma, Y. K., Kant, K., Saxena, S. N., and Dubey, P. N. (2019). Effect of microbial inoculants on cumin (Cuminum cyminum Linn.) growth and yield. Int. J. Seed Spices., 53: 53–56.
[36] Muller, K. (2005): SeqState: primer design and sequence statistics for phylogenetic DNA datasets. Appl. Bioinforma., 4: 65-69. doi.org/10.2165/00822942-200504010-00008.
[37] Nesme, T., Metson, G.S., Bennett, E.M. (2018). Global P flows through agricultural trade. Glob. Environ. Change., 50: 133–141. doi: 10.1016/j.gloenvcha.
[38] Notredame, C. (2007): Recent evolutions of multiple sequence alignment algorithms. PLoSComput Biol., 3(8): e123. doi:10.1371/journal.pcbi.0030123.
[39] Noumavo, P.A., Agbodjato, N.A., Baba-Moussa, F., Adjanohoun, A., Baba-Moussa, L. (2016). Plant growth promoting rhizobacteria: Beneficial effects for healthy and sustainable agriculture. African Journal of Biotechnology., 15: 1452–1463.
[40] Piccoli, P., Lucangeli, D., Schneider, G., Bottini, R. (1997). Hydrolysis of [17,17-2 H2] Gibberellin A20-Glucoside and [17,17-2 H2] Gibberellin A20-glucosyl ester by *Azospirillumlipoferum* cultured in a nitrogen-free biotin-based chemically-defined medium. Plant Growth Regul., 23: 179–182.
[41] Pierzynski, G.M., McDowell, R.W., Sims, J.T., Sharpley, A.N. (2005). Chemistry, cycling, and potential movement of inorganic phosphorus in soils, in: Sims, J.T., Sharpley, A.N. (Eds.), Phosphorus: Agriculture and the environment. American Society of Agronomy, Madison, WI, 53-86.
[42] Postma, J., Nijhuis, E.H., Someus, E. (2010). Selection of phosphorus solubilizing bacteria with biocontrol potential for growth in phosphorus rich animal bone charcoal. Appl. Soil Ecol., 46: 464–469. doi: 10.1016/j.apsoil.2010. 08.016.
[43] Rajput, L., Imran, A., Mubeen, F., Hafeez, F.Y. (2013). Salt-tolerant PGPR strain *Planococcusrifietoensis* promotes the growth and yield of wheat (Triticum aestivum) cultivated in saline soil. Pak J Bot., 45: 1955–1962.
[44] Sabat, A.J., Van Zanten, E., Akkerboom, V., Wisselink, G., Van Slochteren, K., De Boer, R.F. (2017). Targeted next-generation sequencing of the 16S23S rRNA region for culture-independent bacterial identification - increased discrimination of closely related species. Sci. Rep., 7: 3434. doi: 10.1038/s41598- 017-03458-6.
[45] Schachtman, D.P., Reid, R.J., Ayling, S.M. (1998). Phosphorus Uptake by Plants: From Soil to Cell. Plant Physiol., 116: 447-453.
[46] Schneider, W., Sherman, D., Stone, A., Damsteegt, V., Frederick, R. (2004). Specific detection and quantification of Plum pox virus by real-time fluorescent reverse transcription-PCR. Journal of virological methods., 120: 97-105. 10.1016/j.jviromet.2004.04.010.
[47] Schnug, E. and Haneklaus, S.H. (2016). The enigma of fertilizer P utilization, in P in Agriculture: 100% Zero,

eds E. Schnug, and L. De Kok, (Dordrecht: Springer)., 7–26. doi: 10.1007/978-94-017-7612-7_2.

[48] Schroder, J.J., Cordell, D., Smit, A.L., Rosemarin, A. (2010). Sustainable use of phosphorus: EU tender ENV. B1/ETU/2009/0025, Wageningen, The Netherlands.

[49] Shukla, R., Chand, S., Srivastava, A. K. (2005). Batch kinetics and modeling of gibberellic acid production by Gibberellafujikuroi. Enzyme and Microbial Technology., 36: 492-7.

[50] Siddikee, M.A., Chauhan, P.S., Anandham, R., Han, G.H., Sa, T. (2010). Isolation, Characterization, and Use for Plant Growth Promotion Under Salt Stress, of ACC Deaminase-Producing Halotolerant Bacteria Derived from Coastal Soil. J. Microbiol. Biotechnology., 20: 1577-1584. doi.org/10.4014/jmb.1007.07011.

[51] Strickland, J.D.H. and Parsons, T.R. (1968). A Practical Handbook of Seawater Analysis. Bulletin of Fisheries Research Board of Canada, 167: 1-311.

[52] Tajini, F., Trabelsi, M., Drevon, J.J. (2012). Combined inoculation with Glomusintraradices and Rhizobium tropici CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.). Saudi J. Biol. Sci., 19, 157–163. doi: 10.1016/j.sjbs.2011.11.003.

[53] Vandamme, P., Pot, B., Gillis, M., DeVos, P., Kersters, K., Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematic. Microbiol Rev., 60: 407–438.

[54] Vyas, P. and Gulati, A. (2009). Organic acid production in vitro and plant growth promotion in maize under controlled environment by phosphate-solubilizing fluorescent Pseudomonas. BMC Microbiology., 9: 174.

[55] Wallace, I., Blackshields, G., Higgins, D. (2005). Multiple sequence alignments. Current opinion in structural biology., 15: 261-6. 10.1016/j.sbi.2005.04.002.

[56] Walpola, B. and Yoon, M.H. (2013). Isolation and characterization of phosphate solubilizing bacteria and their co-inoculation efficiency on tomato plant growth and phosphorous uptake. Afr J Microbiol Res., 7: 266-275. 10.5897/AJMR12.2282.

[57] Woo, P.C.Y., Lau, S.K.P., Teng, J.L.L., Tse, H., Yuen, K.Y. (2008). Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories, Clinical Microbiology and Infection., 14(10): 908-934.

[58] Zhang, G. and Gao, F. (2017). Quantitative analysis of correlation between AT and GC biases among bacterial genomes. PLoS One., 12: e0171408–11.

[59] Zhu, F., Qu, L., Hong, X., Sun, X. (2011). Isolation and characterization of a phosphate solubilizing halophilic bacterium Kushneria sp. YCWA18 from DaqiaoSaltern on the coast of yellow sea of China. Evid. Based Complement. Alternat. Med., 2011: 615032. doi: 10.1155/2011/615032.