

Exploration of a novel plant growth promoting bacteria *Stenotrophomonas maltophilia* AVP27 isolated from the chilli rhizosphere soil

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ABSTRACT: The present research is aimed for isolation and characterization multiple plant growth promoting activities of chilli rhizobacteria and enhancement of chilli plant growth. Bacterial strains were isolated from chilli rhizosphere by using Jensen's media and screened for plant growth promoting traits such as Indole acetic acid production, phosphate solubilization, Hydrogen cyanide production, Siderophore production and Ammonia production. Three bacterial strains (AVP 22, AVP 23 and AVP 27) were identified as potential PGPR and they were identified as genus *Klebsiella*, *Alcaligenes* and *Stenotrophomonas* by biochemical and physiological characterization. AVP 27 was identified as *Stenotrophomonas maltophilia* by 16s rRNA partial sequence and their multiple plant growth promoting traits were characterized. The bacterial isolate AVP 27 (KM14433) showed of inorganic phosphate solubilization (818ppm), Acid phosphatase activity (1.62 IU/ml), IAA production (93µg/ml), Ammonia production (80 µg/ml) and able to produce siderophore and HCN under optimized growth conditions and Trehalose, as carbon source. Present research revealed that *Stenotrophomonas maltophilia* AVP 27 is a promising plant growth promoting rhizobacteria with wide variety of mechanisms. First time report of using *Stenotrophomonas maltophilia* AVP 27 as plant growth promoting rhizobacteria from chilli rhizosphere and use of this isolate AVP27 as Plant growth promoting rhizobacteria offers attractive way to replace chemical fertilizers, pesticides and supplements.

KEYWORDS: Plant growth promoting rhizobacteria, Multiple PGPR traits, 16s rRNA partial sequence, *Stenotrophomonas maltophilia*.

INTRODUCTION

The term Rhizobacteria is used to describe a subset of rhizosphere bacteria capable of colonizing the root environment [1,2]. Beneficial, root colonizing, and plant growth promoting (PGP) rhizobacteria, are defined by three intrinsic characteristics: (1) must be able to colonize the root (2) must survive and multiply in microhabitats associated with the root surface, in competition with other micro biota, and (3) must promote plant growth. Plant growth promoting rhizobacteria when applied to seeds/soil or crops; enhance the growth of the plant directly by providing nutrients to plants or indirectly by reducing the damage from soil-borne plant pathogens [3]. The Concept of rhizosphere was a narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities[4]has now been extended to include the soil surrounding a root in which physical, chemical and biological properties have been changed by root growth and activity[5]. Since bacteria are the most abundant microorganisms in the rhizosphere, it is highly probable that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization [6,7,8]. Plants select those bacteria

contributing most to their fitness by releasing organic compounds through exudates [9] creating a very selective environment where diversity is low [10]. Rhizobacteria inhabit plant roots and exert a positive effect ranging from direct influence mechanisms to an indirect effect. So, the bacteria inhabiting the rhizosphere and beneficial to plants are termed PGPR [3]. In the last few years, the number of PGPR that have been identified has seen a great increase, mainly because the role of the rhizosphere as an ecosystem gained importance in the functioning of the biosphere. Various species of bacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance the plant growth[11,12]. There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism; suppression of plant disease (Biocontrol), improved nutrient acquisition (Bio fertilizers), or phytohormone production (Bio stimulants), induction of systemic resistance, and production of siderophore or antibiotics. Exposure to the PGPR triggers a defense response by the crop as if attacked by pathogenic organisms. Siderophore produced by some PGPR scavenge heavy metal

micronutrients in the rhizosphere (e.g. iron) starving pathogenic organisms of proper nutrition to mount an attack of the crop. Antibiotic producing PGPR releases compounds that prevent the growth of the pathogens.

Phosphates and other nutrient are also solubilized by PGPR strains to increase the availability of Phosphorus for plants in soil with large amount of precipitated phosphates [13] and nitrogen fixation. These bacteria are also capable to suppress the growth of deleterious microorganisms by production of siderophore, β 1, 3 glucanases, chitinases and antibiotics [14]. Siderophore producing bacteria promote plant growth indirectly by sequestering the limited iron in the rhizosphere and reduce availability for growth of phytopathogens [15]. Several fluorescent *Pseudomonas* and *Bacilli* have been used as seed or root inoculants for higher growth yield of various crops [11]. The common traits of growth promotion includes production or changes in the concentration of plant hormones such as Auxin, gibberellins, cytokinins and ethylene. Indole acetic acid (IAA) is one of the most physiologically active auxin. IAA is released as secondary metabolite because of rich supplies of substrates exuded from the roots [16,17]. Microbial biosynthesis of IAA in soil is enhanced by tryptophan secreted from roots or decaying cells [18]. Gibberellins are implicated in promotion of root growth, root hair abundance and inhibition of floral bud differentiation in woody angiosperms, regulation of vegetative and reproductive bud dormancy and delay of senescence in many organs of a range of plant species [19,20,21]. *Bacillus* is the most abundant genus in the rhizosphere, and the PGPR activity of some of these strains has been known for many years, resulting in a broad knowledge of the mechanisms involved [22,23]. There are a number of metabolites that are released by these strains [24]. Which strongly affect the environment by increasing nutrient availability of the plants [25]. *Stenotrophomonas maltophilia* is a common microorganism in the rhizosphere of cruciferous plants, and has also been found in association with corn and beets [26]. Excretion of sulphur-containing amino acids such as methionine by roots of cruciferous plants may favour the growth of this species. However, *S. maltophilia* is also quite dominant in the rhizosphere of cereal crops [27]. *Stenotrophomonas maltophilia* can even colonize and persist inside tissues of potato plants [28]. However, *S. maltophilia* has not been evaluated yet for its potential to control potato brown rot. However, it was proven to be an effective biocontrol agent for the control of various fungal and oomycetous plant pathogens [29,30,31,32,33]. *Stenotrophomonas maltophilia* inhibited the growth of *Rhizoctonia solani* and *Verticillium dahliae* in vitro, possibly as a result of antibiosis and production of lytic enzymes [34]. Three antifungal compounds, designated xanthobaccins A, B and C were isolated from the culture filtrate of a strain of *Stenotrophomonas* isolated from sugar beet that suppressed damping-off of beet seedlings caused by *Pythium* spp. [32]. Xanthobaccins were in vitro not effective against three bacterial species (*R. solanacearum* was not included). *Stenotrophomonas maltophilia* can take up iron (to a limited extent) from the siderophore pseudobactin [35], but it is unknown if it produces a siderophore. However much attention has not been paid so far on *Stenotrophomonas maltophilia*. In the present investigation multiple potential of rhizobacteria *Stenotrophomonas maltophilia* isolated from chilli rhizosphere has been characterized in terms of phosphate solubilization, PGP traits and Acid phosphatase.

MATERIALS AND METHODS

ISOLATION AND CHARACTERIZATION

A bacterium (AVP 27) was isolated from chilli rhizosphere peddakurapadu, Guntur district of Andhra Pradesh in India, on normal nutrient agar medium with Glucose at pH 7.0, Temperature 37°C with incubation period 48 hrs. The bacterial isolate was characterized by its cultural conditions, morphological and biochemical characteristics [36].

IN VITRO SCREENING OF PHOSPHATE SOLUBILIZATION

The isolate was screened for phosphate solubilization [37]. On modified Pikovskaya agar with insoluble Tricalcium phosphate (TCP). A loop full of culture was placed on the center of agar plate and incubated at 30 ± 0.1 °C for 5 days. The Solubilization zone was determined by subtracting the diameter of bacterial colony from the diameter of total zone.

QUANTITATIVE ESTIMATION OF PHOSPHATE:

Inorganic phosphate Solubilization was Quantitative estimation [38]. Bacterial isolate was grown in National Botanical Research Institute's Phosphate (NBRIP) broth containing 0.5% Tricalcium phosphate (TCP). 500 μ l of bacterial inoculum was added to 50 ml of medium and incubated at 30 ± 0.1 °C at 180 rpm for 5 days in Incubator Shaker and uninoculated medium was taken as control. The culture was centrifuged at 10,000 rpm for 10 min. Inorganic phosphate present in supernatant was estimated by vanado-molybdate-yellow color method by using Barton's reagent and 0.5 ml of the supernatant was added to 2.5 ml Barton's reagent and volume was

made to 50 ml with de-ionized water. After 10 min of incubation the absorbance was read at 430 nm in UV/Visible Spectrophotometer and the total soluble phosphorous was calculated from the regression equation of standard curve. And values were expressed in ppm. The pH of culture supernatants were also measured. The experiment was repeated in triplicates.

GROWTH OPTIMIZATION:

Growth optimization of isolate was studied at different temperatures (25°C, 37 °C, 50 °C), pH ranging from (3, 5, 7, 9, 12), NaCl (0.3%, 0.5%, 0.7%, 0.9%, 1%), Carbon sources (Sucrose, Maltose, Lactose, Dextrose)

IN VITRO SCREENING OF BACTERIAL ISOLATE FOR PLANT GROWTH PROMOTING

ACTIVITIES:

IAA PRODUCTION

IAA production was detected [39]. Quantitative analysis of IAA was performed using the method of Loper and Scot at a concentration of 1000 µg/ml of tryptophan. Bacterial culture grown for 48hrs on the respective media at 30±1 °C. Fully grown culture was centrifuged at 10,000 rpm for 10 minutes. The supernatant (1 ml) was mixed with 4 ml of Salkowski reagent (50ml, 35% of perchloric acid, 1 ml 0.5M FeCl₃ solution) with few drops of Orthophosphoric acid. Development of pink color indicates IAA production. Optical density was taken at 535nm in UV/Visible Spectrophotometer. Concentration of IAA produced by culture was measured with the help of standard graph of IAA obtained in the range of 10-100 µg /ml.

AMMONIA PRODUCTION

Ammonia production was estimated by Nesslerization reaction. Freshly grown culture was inoculated into 4ml of peptone water and incubated for 48hrs at 37 °C

c. Broth was collected, centrifuged and 1ml Nessler's reagent was added to 1ml of supernatant and the volume of this mixture was made up to 10ml by addition of ammonia free distilled water. Development of brown to yellow color was a positive test for ammonia production and optical density was measured by spectrophotometer at 450nm [40]. The concentration of ammonia was estimated based on a standard curve of ammonium sulfate ranging from 0.1 to 1 µmol/ml

PHOSPHATASE ACTIVITY

Acid phosphatase activity was also estimated [41]. Cells grown overnight in citrate salt medium (g/l) [trisodium citrate, 3 ; K₂HPO₄, 10.5; KH₂PO₄, 5.4; (NH₄)₂SO₄, 1.2; MgSO₄, 0.4; CaCl₂, 0.15 (pH 7.0)] were harvested by centrifugation at 8,000 rpm for 8 min. Cell pellets were suspended in normal saline (O.D of 1.0 at 600 nm). Incubation mixture for acid phosphatase enzyme assay contained 50 µl of cell suspension, 50 µl of 0.12 M pNPP (p-nitrophenyl phosphate) and 500 µl of 50mM Tris-Malate buffer (pH 5.3). The suspended cells were incubated for 30 min at 30 °C. After centrifugation, 1 volume of 0.5 M NaOH was added to 1 volume of supernatant, and O.D₄₂₀ was measured. Results were expressed in µmol units of product formed per OD₆₀₀ of cells. One unit was defined as the hydrolysis of 1 µM of pNPP to pNP (p-nitrophenol) per minute at 30 °C.

CATALASE

Presence of catalase was checked qualitatively using the method [42]. Six percent hydrogen peroxide was added on the colonies grown on nutrient agar plates; effervescences of O₂ released from the bacterial colonies indicate the presence of catalase activity.

HCN PRODUCTION

Qualitative estimation of HCN production was done by Picrate assay [43]. Nutrient agar medium was amended with 4.4g glycine L-1 and bacterium was streaked on plate. A Whatman filter paper no.1 soaked in 2% sodium carbonate in 0.5% picric acid solution was placed between the base and the lid of the petri dish. Plates were sealed with Para film and incubated at 27±2 °C for 4 days. After incubation, the color change of filter indicates the release of cyanide from bacterial isolate.

MOLECULAR IDENTIFICATION OF BACTERIAL ISOLATE

Pure culture of AVP 27 bacterial isolate was grown until log phase achieved and genomic DNA was isolated essentially according to Bazzicalupo[44].The amplification of 16S rRNA gene was done by using universal bacterial primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3') and 27F (5' AGAGTTTGATCMTGGCTC AG- 3') as per the conditions described by Pandey[45]. The PCR product was sequenced at Macrogen South Korea. The sequences obtained were compared with those from the GenBank using the BLAST program[46]and Phylogenetic trees reconstructions were obtained by the Neighbor joining method 1000 bootstrap replicates were performed to assess the statistical support for each branch in the tree[46,47].

RESULTS:

ISOLATION AND CHARACTERIZATION:

Out of 55 bacteria isolated from chilli rhizosphere, one of the colonies AVP27 showed mucoid and water bubble morphology (Plate-1A).On the basis of culture, morphological, biochemical and molecular characteristics, the bacterial isolate was identified as *Stenotrophomonas maltophilia* AVP 27.

Plate-1A

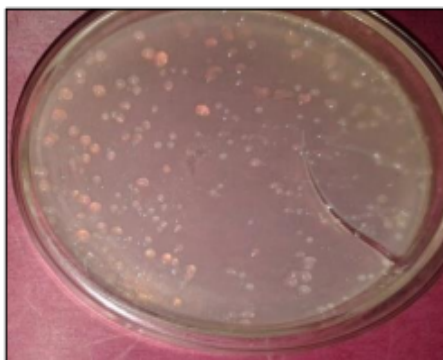
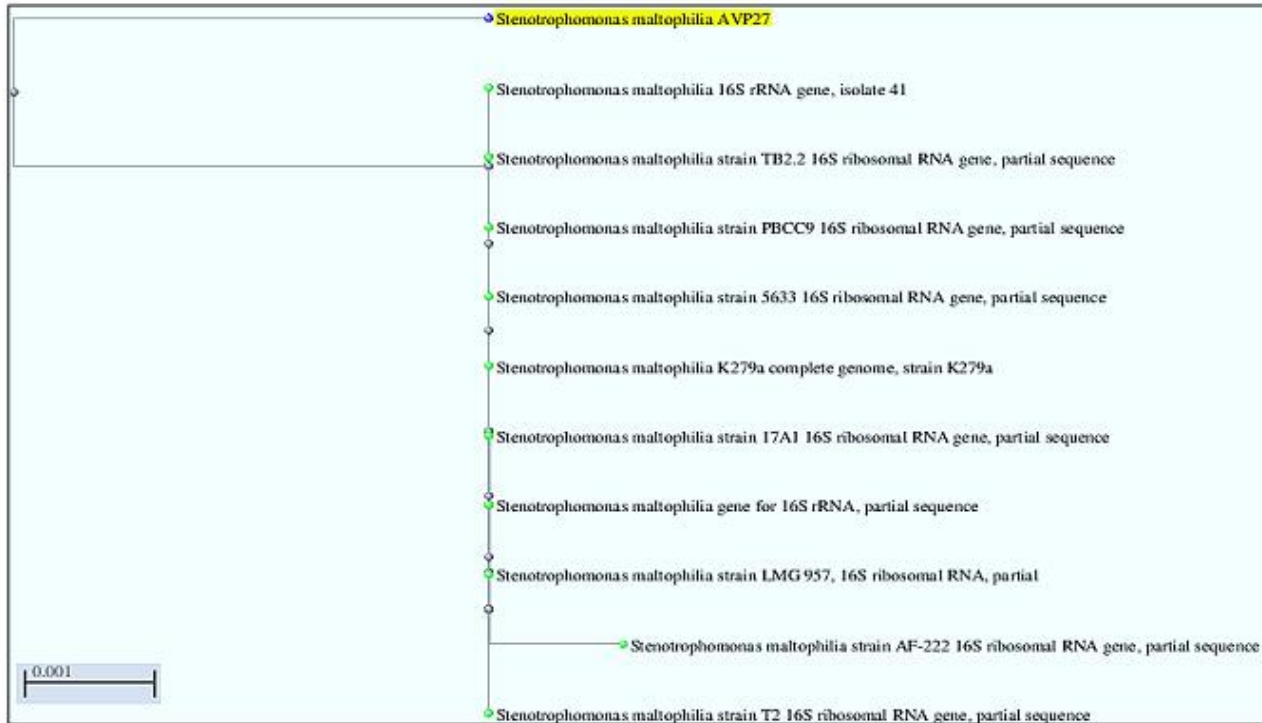


Table 1: Morphological physiological and Biochemical characteristics of AVP27

Morphological and physiological characteristics								
Test	Morphology Arrangement	Gramstaining/ Pigmentation	Motile	Urease	Starch	Glucose	Lactose	Sucrose
AVP27	Rod& single	Pink and No pigmentation	-ve	-ve	-ve	+ve	+ve	+ve
Biochemical characteristics								
Test	Oxidase	Catalase	H ₂ S	Nitrate reduction	Indole	Methyl red	Voges Proskuaer	Citrate utilization
AVP27	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve

MOLECULAR CHARACTERIZATION

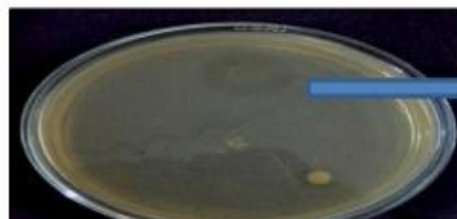
1000bp PCR product of 16SrRNA gene was amplified from genomic DNA of *Stenotrophomonas maltophilia* AVP 27 strain. 16S ribosomal RNA partial gene analysis was done at Macrogen South Korea. Phylogenetic analysis of 1000bp of fasta sequence by BLAST n, NCBI revealed that the strain AVP 27 showed 99% similarity with *Stenotrophomonas maltophilia* 2DT. Hence the sequence was submitted in GenBank NCBI with a name *Stenotrophomonas maltophilia* AVP 27 (Accession No. KM14433). Fig : 1
PHYLOGENETIC TREE OF *STENOTROPHOMONAS MALTOPHILIA* AVP 27 BASED ON 16S rRNA GENE ANALYSIS



SCREENING OF PHOSPHATE SOLUBILIZATION

Qualitative characterization of AVP 27 isolate of phosphate solubilization showed zone of clearance on Pikovskaya's agar medium after 4 days. (Plate-1B)

Plate-1B



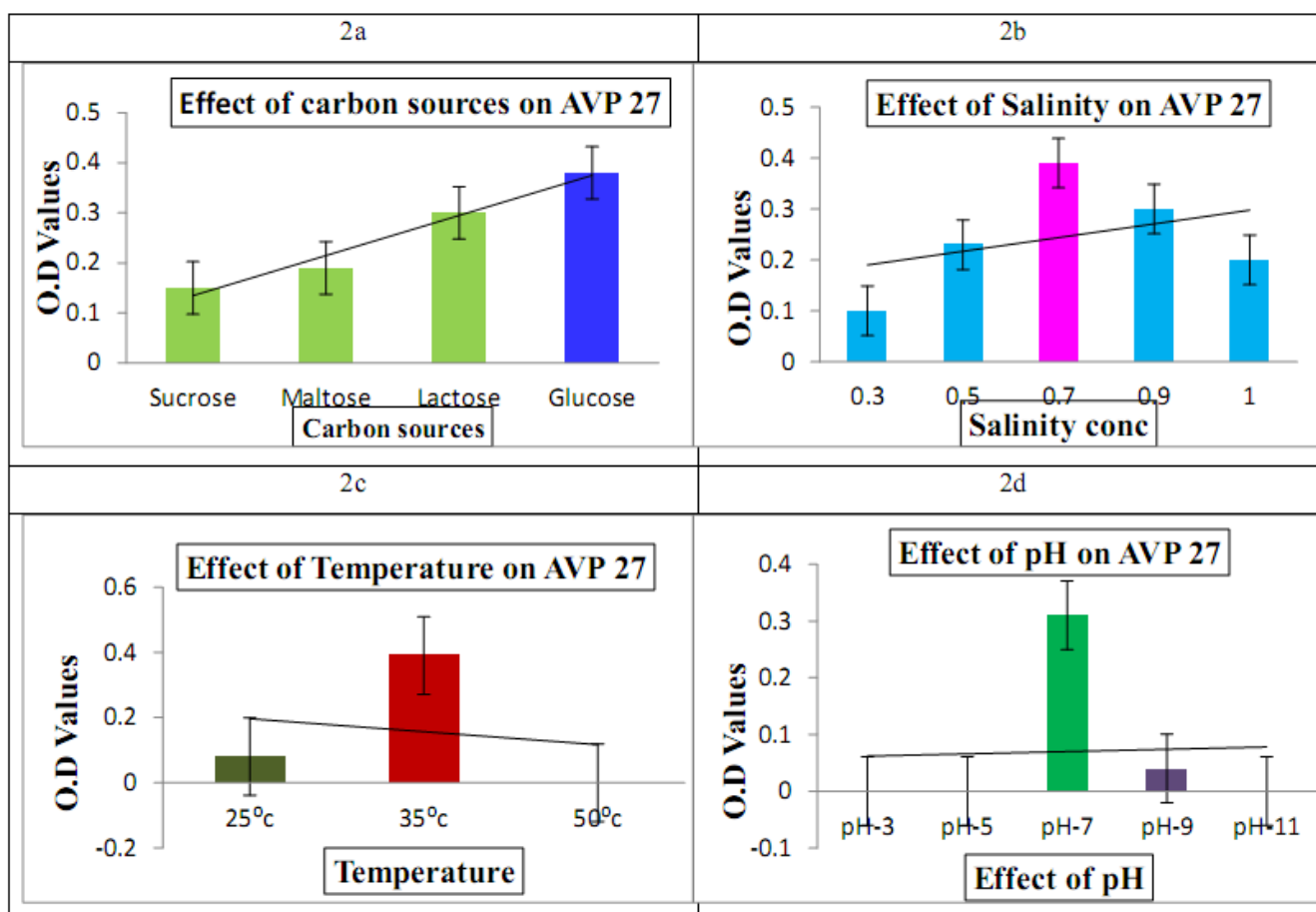
Clear Zone of Inorganic Phosphate solubilization

GROWTH OPTIMIZATION OF AVP27

AVP 27 showed variation in growth in different carbon sources such as Sucrose, Maltose, Lactose and Dextrose (fig 1a). Maximum growth was observed in Glucose and found to be very minimum in Sucrose. It is observed that growth pattern of AVP27 showed variation at different concentrations of NaCl. AVP 27 showed maximum growth rate at 0.7% NaCl and moderately high growth rate at 0.9% NaCl (fig1b). It is also observed that AVP 27 showed growth variation at different temperatures and found to be maximum at 37°C and moderate at 25°C.(fig1c). Growth at different pH ranging from pH3.0- pH 12 also showed variation and observed to be high at pH7 and relatively moderate at pH9 (fig1d). Based on the results cited in fig1, growth of AVP-27 was optimized in a medium containing Sucrose, 0.7%NaCl, pH7 at 37°C temperature.

Figure: 2

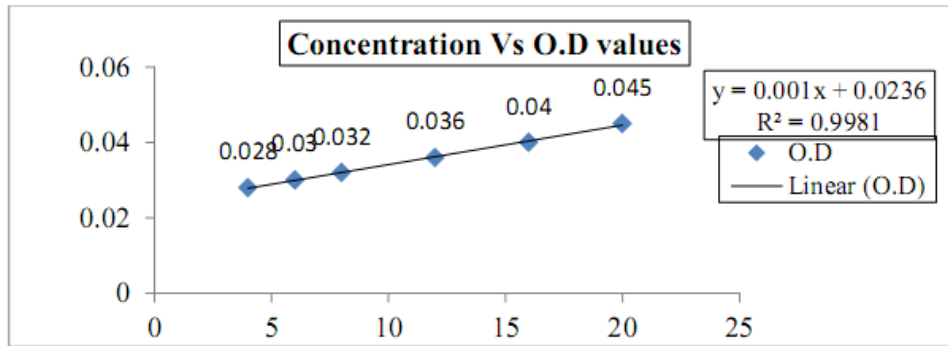
QUANTITATIVE ANALYSIS OF PHOSPHATE SOLUBILIZATION Results revealed that the solubilization



of tricalcium phosphate was progressively increased for 7 days and gradually declined in 10th day and 13th day.

.Phosphate solubilization was observed to be maximum (818 ppm) on 7th day and also noted that the solubilization was gradually decreased with rapid decline of pH from 7 to 4(fig 2).

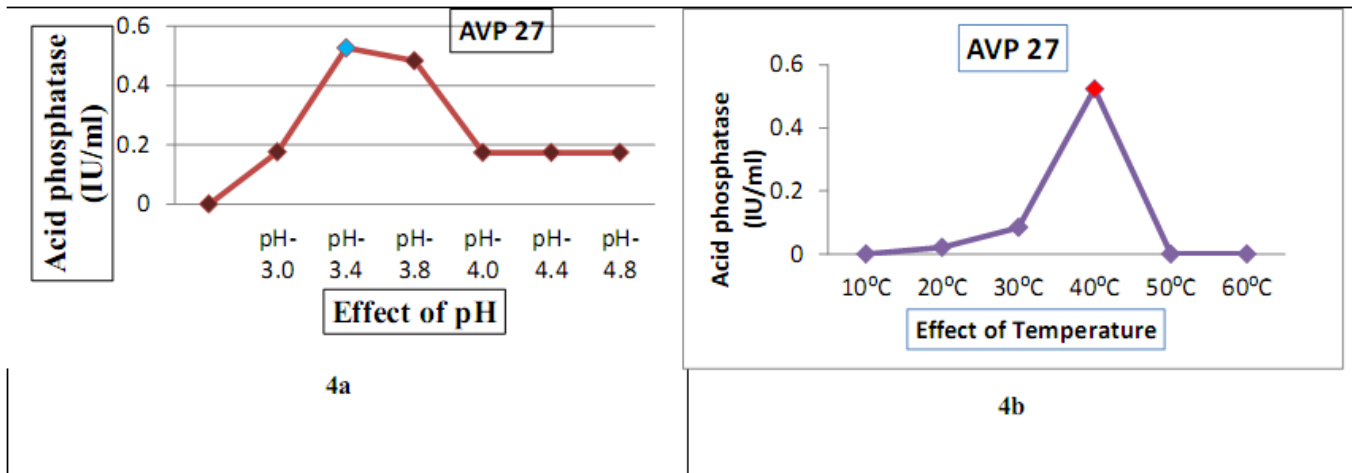
Figure 3: STANDARD GRAPH OF PHOSPHATE SOLUBILIZATION AT VARYING CONCENTRATIONS OF TRI CALCIUM PHOSPHATE

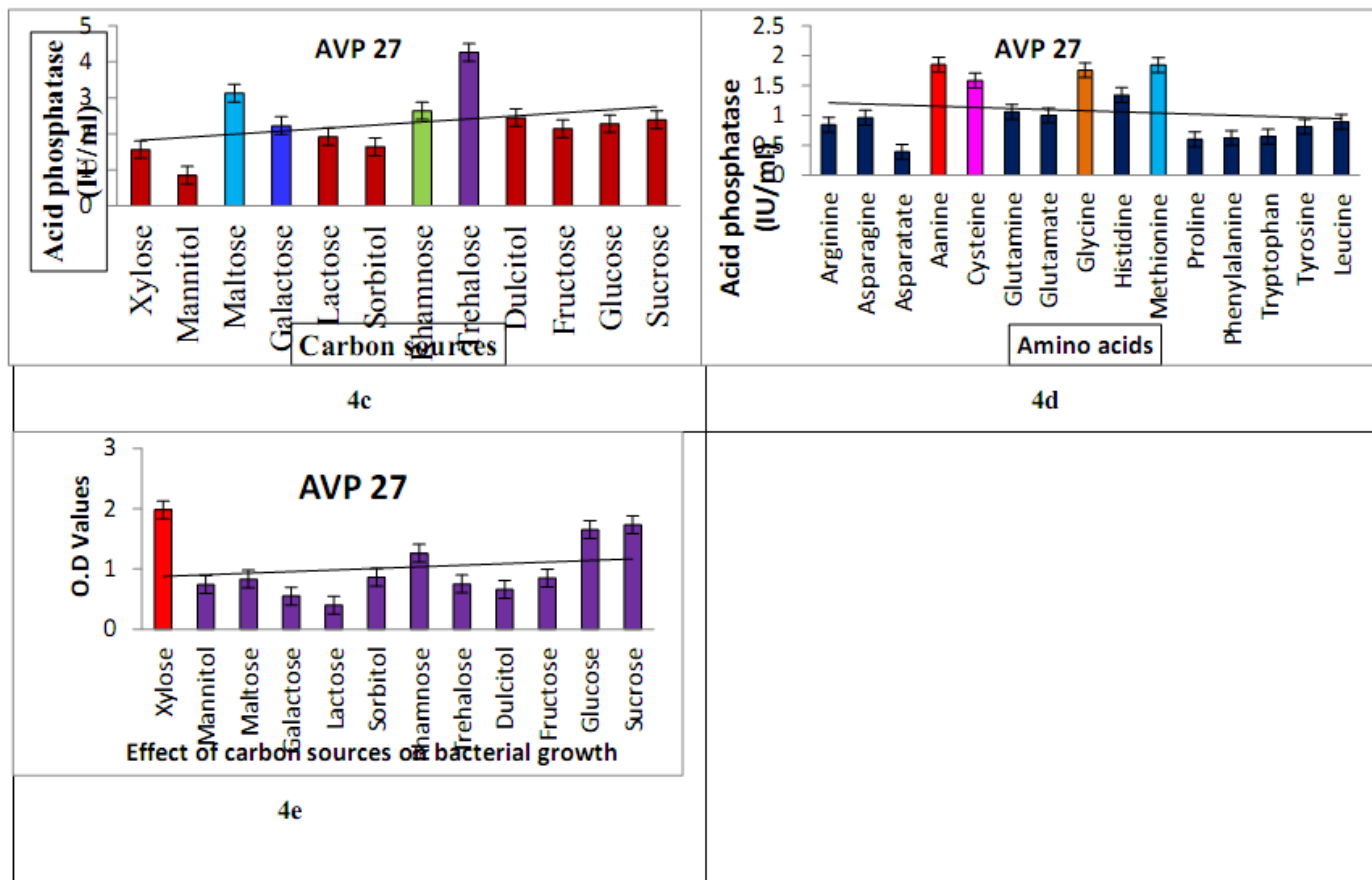


QUANTITATIVE ANALYSIS OF ACID PHOSPHATASE

Results revealed that AVP 27 showed high acid phosphatase activity (0.527 IU/ml) at pH3.4 and temperature 40°C (Fig3). For the first time, acid phosphatase activity of AVP 27 was also estimated quantitatively at 12 different sugars and 15 amino acids and relative activity was measured in international units (IU). It has been observed that the isolate showed acid phosphatase activity in all 12 different types of sugars. Figure 4 revealed that the activity was very high in Trehalose (4.260 IU/ml), Maltose (3.128 IU/ml), Rhamnose (2.640 IU/ml), Dulcitol (2.450 IU/ml) and Sucrose (2.390 IU/ml). AVP 27 isolate also showed acid phosphatase activity in all 15 different types of amino acids. Significant increase of acid phosphatase activity was observed (fig 5). The isolate showed acid phosphatase activity high in Alanine (1.852 IU/ml), Methionine (1.844 IU/ml), Glycine (1.760 IU/ml), Cysteine (1.584 IU/ml) and Histidine (1.340 IU/ml). Growth and acid phosphatase production of AVP27 was studied at 12 different sugars and 15 different amino acids. It was observed that Rhamnose and Sucrose proportionately enhances the enzyme activity along with growth. Enzyme activity was not proportionately increased along with growth in presence of Trehalose. It clearly indicates that sugars like Fructose and Mannitol act as inducers for enzyme acid phosphatase. Effect of sugars on growth of AVP9 was also studied (fig-6) and observed that the growth is very high in sugars like Xylose, Mannitol, Maltose, Trehalose, Glucose and sucrose and also found that the growth was maximum in Trehalose.

Figure 4: QUANTITATIVE ANALYSIS OF ACID PHOSPHATASE





QUALITATIVE AND QUANTITATIVE ANALYSIS OF PLANT GROWTH PROMOTING TRAITS OF AVP27

Bacterial Isolate	IAA (µg/ml) production	Ammonia (µg/ml) Production	Siderophore production	HCN Production	Catalase production
AVP27	93	80	Positive	Positive	Positive

DISCUSSION

Several factors viz. root morphology, the stage of plant growth, root exudates, and the physical and chemical properties of the soil are reported to influence the occurrence and distribution of microbial communities in the soil and rhizosphere. Previous isolations of nitrogen fixing bacteria have revealed a broad diversity of diazotrophs to inhabit the crop rhizosphere [48] and this study surveyed the rhizosphere soil of agriculturally important crops widely cultivated in Peddakurapadu, Guntur A.P, India for the presence of plant growth promoting bacteria.

Nitrogen and phosphorus are two most limiting nutrients in the soil as well as plant enhancing nutrients [49]. Phosphate fertilizer represents a high cost to the farmer and most of the soils are poor in available phosphorus contents and therefore it is of interest to take advantage of soil microorganisms for the mobilization of phosphorus in the soil [50]. In present investigation *Stenotrophomonas maltophilia* AVP 27 was screened invitro for

phosphate solubilization and Acid phosphatase activity both qualitatively and quantitatively. After seven days of incubation the isolate AVP 27 showed high phosphate solubilization. 37°C Temperature, 0.7%, NaCl (salinity), pH 7 and Glucose were identified as influencing factors for optimization of growth and maximum phosphate solubilization. In the present study AVP 27 showed significant production of ammonia and strong phosphate solubilization. This infers that AVP 27 isolate in the rhizosphere makes ammonia and phosphorus available to the plant by which nutritional needs of the plant can be fulfilled.

The morphological and biochemical analyses indicated highest (98%) similarity of the isolate with the genus *Stenotrophomonas* when compared with Bergey's Manual of Determinative Bacteriology [51]. In addition the phenotypic characteristic of this species correlate well with the molecular analyses based on 16S rRNA partial sequence analyses. Naz et al., (2010) [52] identified phosphate solubilizing bacteria belonging to genera *Stenotrophomonas maltophilia* by 16S-rRNA. The PGPR strains have been reported to produce IAA either with or without the tryptophan supplement in culture media [53,54]. Greater production of IAA obtained during the present study might be due to the presence of tryptophan deaminase enzyme in *Stenotrophomonas maltophilia* that utilized tryptophan as precursor of IAA. Auxin biosynthesis is wide spread among soil and plant associated bacteria[55] including *Stenotrophomonas maltophilia*[56].

Microbial IAA has been implicated in the stimulation of growth or pathogenesis of plants. A diverse group of microbes, including soil, epiphytic and tissue colonizing bacteria have been found to synthesize IAA [57]. In this study bacterial strains produced considerable amount of IAA, which is comparable with earlier studies on various bacteria including *Pseudomonas* and *Stenotrophomonas* [58,56]. This study reports the isolation and characterization of the strain *S. maltophilia* AVP 27 from the rich rhizosphere soils of Chilli crop confirming their plant growth potential. Bacteria from this genera was generally regarded as good phosphate solubilizers and as biofertilizers [59,56,48]. So it clearly reveals that apart from the normally encountered rhizosphere microflora: *Azospirillum*, *Azotobacter*, *Herbaspirillum*, *Klebsiella*, etc., other species may also possess diazotrophy. *S. maltophilia* has an ambivalent character, first as a biocontrol and bioremediation agent and second as a multiresistant pathogen in nosocomial infections. There are numerous reports on the isolations from diverse rhizospheres. The clinical isolates are separated from the rhizosphere isolates by 16S rDNA analysis [60]. However it requires further studies on the virulence of the rhizosphere isolates before recommending it as a bioinoculant. Conventionally, insoluble phosphates are chemically processed by reacting with sulphuric acid or phosphoric acid into soluble P. However, this process increases P fertilizer cost, and has environmental implications. In view of environmental concerns and current developments in sustainability, research efforts are concentrated on the development of a technique that uses phosphatesolubilizing microorganisms to solubilize insoluble phosphates [61,62]. In this work, a phosphate solubilizing bacterium, which is identified as *S. maltophilia*, was isolated from Chilli rhizosphere soil (Pedakuraadu, Guntur, A.P, India). Generally, the action of microorganisms leading to the solubilization of minerals is recognized as direct and indirect actions [63]. On one hand, for the direct action, microorganisms utilize minerals as their growth substrate, the growth cause the solubilization of minerals. On the other hand, for the indirect action, microorganisms produce some metabolites during the solubilizing periods, such as organic acids, which also solubilize minerals. It has been well established that, as a common strategy to release soluble P from insoluble phosphates, phosphate solubilizing microorganisms reduce the pH of the surroundings by the production of organic acids[64]. In this work, *S. maltophilia* AVP 27 presents high growth rate in NBRIP growth medium containing 0.1 g TCP, and a positive correlation between the concentration of soluble P and the population of the isolate in the culture medium is observed. The results show that the isolate can solubilize TCP as its growth substrate. It was reported that acid phosphatase produced in this process, thus also cause TCP solubilization[65]. In this study we also made an attempt to characterize acid phosphatase enzyme activity at different pH, different carbon sources and amino acids. Best of our knowledge, relation between growth of isolate and acid phosphatase activity has not been reported so far. First time an attempt was made to correlate growth of isolate with acid phosphatase activity at different carbon sources. Our reports revealed that there is no direct correlation between bacterial growth and enzyme activity in presence of carbon sources. However some of the carbon sources such as Mannitol and Fructose act as inducers and enhance the activity of acid phosphatase. No significant enhancement of acid phosphatase activity was observed in presence of amino acids. To harness the dual potential of AVP27 in the field of Agriculture and Industry, future studies should be carried out.

This indicates that the direct action by *S. maltophilia* AVP 27 is one of the reasons for TCP solubilization. In addition, a decrease of pH is presented, depletion of pH in the broth was due to the production of organic acids that cause TCP solubilization are detected in the culture medium inoculated with *S. maltophilia* AVP 27 during the solubilizing experiment.

The results indicate that the indirect action by *S. maltophilia* AVP 27 is another reason for TCP solubilization. It further affirms that phosphate solubilization by microorganisms is involved with the production of organic acid released by microorganisms and followed by a decrease in the pH of the culture medium[66]. However, the mechanism of phosphate solubilization by microorganisms is also a subject of controversy today. Therefore, it needs further studies to understand the characteristics and mechanisms of phosphate solubilization by phosphate solubilizing microorganisms. Moreover, the role of phosphate solubilizing microorganisms on plant growth under field conditions is also important and necessary to be studied.

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CONCLUSION:

It is expected that this report will prompt further screenings of phosphate solubilizing microorganisms so as to enhance agronomic value of soils and benefit crop growth. Evaluation of this isolate under the field condition and thorough investigation of *Stenotrophomonas maltophilia* AVP 27 use as a plant growth promoting rhizobacterial agent constitute future research. This shows that multiple potential of AVP 27 can help in plant protection and enhance plant growth.

REFERENCES:

- [1] Kloepper, JW, R.M. Zablotowics, E.M. Tipping and R. Lifshitz. "Plant growth promotion mediated by Bacterial Rhizosphere Colonizers", In: *The Rhizosphere and Plant Growth*, Keister, D.L. and P.B. Cregan (Eds.) Kluwer Academic Publishers, USA, 315-326, 1991.
- [2] Kloepper, JW. "Plant growth promoting Rhizobacteria", (Other systems). In: *Azospirillum/Plant Associations* Okon, Y. (Eds.) CRC Press, Boca Raton, FL, USA, 111-118, 1994.
- [3] Kloepper, JW, Leong, J, Teintze, M, Schroth, MN, "Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria" *Nature* 286: 885-886, 1980.
- [4] Hiltner L. "Über neuere erfahrungen und probleme auf dem gebiet der boden bakterilogie und unter besonderer berucksichtigung der grundung und branche". *Arb. Deut. Landw. Ges.* 98: 59-78, 1904.
- [5] McCully M. "The rhizosphere: the key functional unit in plant/soil/microbial interactions in the field. Implications for the understanding of allelopathic effects". In *Proceedings of the 4th World Congress on Allelopathy: 21-26 August 2005*; Charles Sturt University, Wagga Wagga, NSW, Australia. International Allelopathy Society. Edited by Harper J, An M, Wu H, Kent J.
- [6] Antoun H, Kloepper, JW, "Plant growth promoting rhizobacteria" (PGPR). In *Encyclopedia of Genetics*. Academic Press, New York. Edited by Brenner S, Miller JH, 1477-1480, 2001.
- [7] Kloepper, J.W., Shaw, J.J., and Chalifour, F.-P., "Root colonization of faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) by *Rhizobium leguminosarum* bv. *viciae* in the presence of nitrate nitrogen". *Can. J. Microbiol.* 47:1068-1074, 2001.
- [8] Barriuso, J., Solano, BR., Lucas, JA., Lobo, AP., Villaraco, AG., Mañero, FJG., "Ecology, Genetic Diversity and Screening Strategies of Plant Growth Promoting Rhizobacteria" (PGPR). WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Edited by Ahmad I, Pichtel J, Hayat S, 1-17, 2008.
- [9] Lynch, JM., "The Rhizosphere". John Wiley & Sons Ltd, Chichester, Edited by Lynch JM, 458, 1990.
- [10] García, JL., Probanza, A., Ramos, B., Mañero, FJG., "Ecology, genetic diversity and screening strategies of plant growth promoting rhizobacteria". *Journal of Plant Nutrition and Soil Sciences*, 164: 1-7, 2001.
- [11] Glick, BR., "The enhancement of plant growth by free living bacteria". *Canadian Journal of Microbiology*, 41 (Suppl 2): 109-114, 1995.
- [12] Joseph, B., Patra, RR., Lawrence, R., "Characterization of plant growth promoting Rhizobacteria associated with chickpea" (*Cicer arietinum* L). *International Journal of Plant Production*, 1 (Suppl 2): 141-152, 2007.
- [13] Goldstein, A.H., "Bacterial solubilization of mineral phosphate: Historic perspective and future prospects". *Am. J. Altern. Agric.* 1: 51-57, 1986.
- [14] Cattelan, A.J., Hartel, P.G., Fuhrmann, J.J., "Screening for plant growth promoting Rhizobacteria to promote Early Soybean Growth". *Soil Sci Soc Am J* 63:1670-1680, 1999.
- [15] Alexander, B.D., Zeeber, D.A., "Use of chromazurol S to evaluate siderophore production by rhizosphere bacteria". *Biol. Fertil. Soils*. 2:39-54, 1991.
- [16] Ahmed, F., Ahmad, I., Khan, M.S., "Indole acetic acid production by the indigenous isolated of *Azotobacter* and *Fluorescent Pseudomonas* in the presence and absence of tryptophan". *Turk. J. Biol.* 29:29-34, 2005.
- [17] Srrzelczyk, E., Pokojaska-Burdzej, A., "Production of auxin and gibberellin like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and mycorrhizosphere of pine (*Pinus silvestris* L.)". *Plant and Soil*. 81:185-194, 1984.
- [18] Benezri, E., Courtade, A., Picard, C. & Guckert, A., "Role of maize root exudates in the production of auxins by *Pseudomonas fluorescens* M.3.1". *Soil Biol and Biochem* 30:1481-1484, 1998.
- [19] Bottini, R., Luna, V., "Bud dormancy in deciduous fruit trees". *Curr Top Plant Physiol* 1:147-159, 1993.
- [20] Fulchieri, M., Lucangeli, C., Bottini, R., "Inoculation with *Azospirillum lipoferum* affects growth and gibberellin status of corn seedling roots". *Plant Cell Physiol* 34: 1305-1309, 1993.
- [21] Rienoso, H., Dauria, C., Luna, V., Pharis, R. & Bottini, R. "Dormancy in peach (*Prunus persica* L.) flower buds VI. Effects of gibberellins and an acylcyclohexanedione (Cimectacarb) on bud morphogenesis in field experiments with orchard trees and on cuttings". *Can J Bot* 80, 656-663, 2002.
- [22] Probanza, A., Lucas García, JA., Ruiz Palomino, M., Ramos, B., Gutiérrez Mañero, F.J., "Pinus pinea L seedling growth and bacterial rhizosphere structure after inoculation with PGPR *Bacillus* (*B. licheniformis* CECT 5106 and *B. pumilus* CECT 5105)". *Applied Soil Ecology*, 20 (Suppl 2): 75-84, 2002.
- [23] Gutiérrez Mañero, FJ., Probanza, A., Ramos, B., Colón Flores, JJ., Lucas García, JA., "Ecology, Genetic Diversity and Screening Strategies of Plant Growth Promoting Rhizobacteria (PGPR)", *Journal of Plant Nutrition*, 26 (Suppl 5): 1101-1115, 2003.
- [24] Charest, MH., Beauchamp, CJ., Antoun H., "Effects of the humic substances of de-inking paper sludge on the antagonism

between two compost bacteria and *Pythium ultimum*”, *FEMS Microbiology Ecology*, 52(Suppl 2): 219–227, 2005.

[25] Barriuso, J., Solano, BR., Lucas, JA., Lobo, AP., Villaraco, AG., Mañero, FJG., “Ecology, Genetic Diversity and Screening Strategies of Plant Growth Promoting Rhizobacteria (PGPR)”, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim, Edited by Ahmad I, Pichtel J, Hayat S, 1-17, 2008.

[26] Debette, J., & Blondeau, R. “Presence of *Pseudomonas maltophilia* in the rhizosphere of several cultivated plants”, *Canadian Journal of Microbiology*, 26(4), 460–463, 1980.

[27] Lambert, B., & Joos, H. “Fundamental aspects of rhizobacterial plant growth promotion research”. *Trends in Biotechnology*, 7, 215–219, 1989.

[28] Garbeva, P., van Overbeek, L. S., van Vuurde, J. W. L., & Van Elsas, J. D, “Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments”, *Microbial Ecology*, 41, 369–383, 2001.

[29] Berg, G., Eberl, L., & Hartmann, A, “The rhizosphere as a reservoir for opportunistic human pathogenic bacteria”, *Environmental Microbiology*, 7, 1673–1685, 2005.

[30] Dal Bello, G. M., Moñaco, C. I., & Simón, M. R, “Biological control of seedling blight of wheat caused by *Fusarium graminearum* with beneficial rhizosphere microorganisms”, *World Journal of Microbiology and Biotechnology*, 19, 627–636, 2002.

[31] Dunne, C., Crowley, J. J., Moeñne-Loccoz, Y., Dowling, D. N., de Bruijn, F. J., & O’Gara, F, “Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity”, *Microbiology*, 143, 3921–3931, 1997.

[32] Nakayama, T., Homma, Y., Hashidoko, Y., Mizutani, J., & Tahara, S, “Possible role of xanthobaccins produced by *Stenotrophomonas* sp. Strain SB-K88 in suppression of sugar beet damping-off disease”, *Applied and Environmental Microbiology*, 65, 4334–4339, 1999.

[33] Zhang, Z., & Yuen, G. Y, “Biological control of *Bipolaris sorokiniana* on tall fescue by *Stenotrophomonas maltophilia* strain C3”, *Phytopathology*, 89, 817–882, 1999.

[34] Berg, G., Marten, P., & Ballin, G, “*Stenotrophomonas maltophilia* in the rhizosphere of oilseed rape – occurrence, characterization and interaction with phytopathogenic fungi”, *Microbiological Research*, 151, 19–27, 1996.

[35] Jurkevitch, E., Hadar, Y., & Chen, Y, “Differential siderophore utilization and iron uptake by soil and rhizosphere bacteria”, *Applied and Environmental Microbiology*, 58, 119–124, 1996.

[36] Cappuccino, J.C., Sherman, N., “In Microbiology” A Laboratory Manual third ed. Benjamin/Cummings Pub.Co., New York, pp:125-179, 1992.

[37] Gupta, R., S. Rekha, S. Aparna. and R.C. Kuhad, “A modified plate assay for screening phosphate solubilizing microorganisms”, *J. Gen. Appl. Microbiol.*, 40: 255–60, 1994.

[38] Nautiyal, C.S., Mehta, S and Jackson, “An Efficient Method for Qualitative Screening of Phosphate-Solubilizing Bacteria”, *Microbiol.* 43:51-56, 2001 .

[39] Brick, J.M., Bostock, R.M., Silverstone, S.E., “Rapid in situ assay for Indole acetic acid production by bacteria immobilized on nitrocellulose membrane”, *Appl. Environ. Microbiol.* 57, 535-538, 1991.

[40] Demutskaia LN, Kalinichenko IE, “Photometric determination of ammonium nitrogen with the nessler reagent in drinking water after its chlorination”, *J Water Chem Tech.* 32(2):90-94, 2010.

[41] Gugi, B., Orange, N., Hellio, F., Burini, JF., Guillou, C., Leriche, F., Guespin-Michael, JF., “Effect of growth temperature on several exported enzymes activities in the psychrotrophic bacterium *Pseudomonas fluorescens*”, *J bacteriol*, 173, 381, 1991.

[42] Clarke H, Cowan ST, “Biochemical methods for bacteriology”, *J Gen Microbiol* 6:187-197, 1952

[43] Kang Y, Cheng J, Mei L, Yin S, “Screening and identification of plant growth promoting rhizobacteria”, *Acta microbiologica Sinica*, 50 (7):853, 2010.

[44] Bazzicalupo, M., Fani, R., Humana Press., Inc., Totowa, NJ., 112-124, 1995.

[45] Pandey, P., Kang, SC., Maheswari, DK., *Curr Sci*, 89(1): 177-180, 2005.

[46] Astschul, SF., Gish, W., Miller, W., Myers, EV., Lipman, D., *J Mol Biol*, 215: 403-410, 1990.

[47] Tamura, K., Dudley, J., Nei, M., Kumar, Sm., *Mol Biol Evol*, 24, 1596-1599, 2007.

[48] Vessey, J.K., “Plant growth promoting rhizobacteria as biofertilizers”, *Plant Soil* 255, 571–586, 2003. [49] Graham PH, Vance CP, “Nitrogen fixation in perspective : An overview of research and extension needs”, *Field Crops Res.* 65:93-10, 2000.

[50] Richardson AE, “Prospects for using soil microorganisms to improve the acquisitions of phosphorous by plants”, *Aust J. Plant Physiol.* 28:897-906, 2001.

[51] Holt, J.G., N.R. Kreig, P.H.A Sneath, J.T. Staley and S.T. Williams, “Bergey’s Manual of Determinative Bacteriology”, Williams and Wilkins, Baltimore, USA, pp. 40-169, 1994.

[52] Naz I., A. Bano and Tamoor-ul-Hassan, “Morphological, biochemical and molecular characterization of rhizobia from halophytes of Khewra salt range and Attock”, *Pak. J. Bot.*, 41(6): 3159-3168, 2009.

[53] Fatima, Z., M. Saleemi, M. Zia, T. Sultan, M. Aslam, Riaz-Ur- Rehman and M.F. Chaudhary, “Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat”, *Afr. J. Biotechnol.*, 8(2): 219-225, 2009.

[54] Mehnaz, S., M.S. Mirza, J. Haurat, R. Balley, P. Normand, A. Bano and K.A. Mali, “Isolation and 16S-rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice”, *Can. J. Microbiol.*, 47: 110-117, 2001.

- [55] Verma, S.C., J.K. Ladha and A.K. Tripathi, "Evaluation of plant growth promoting and colonizing ability of endophytic diazotrophs from deep water rice", *J. Biotech.*, 91: 127-141, 2001.
- [56] Suckstorff, I. and G. Berg, "Evidence for dose dependent effects on plant growth by *Stenotrophomonas* strains from different origins", *J. Appl. Microbiol.*, 95: 656-663, 2003.
- [57] Patten, C.L., Glick, B.R., "Bacterial biosynthesis of indole-3-acetic acid", *Can. J. Microbiol.* 42, 207-220, 1996.
- [58] Malik, K.A., Bilal, R., Mehnaz, S., Rasul, G., Mirza, M.S., Ali, S., "Association of nitrogen fixing and plant growth promoting rhizobacteria (PGPR) with kallar grass and rice", *Plant Soil* 194, 37-44, 1997.
- [59] deFreitas, J.R., Banerjee, M.R., Germida, J.J., "Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of Canola (*Brassica napus* L.)", *Biol. Fert. Soils* 24, 358-364, 1997.
- [60] Minkwitz, A., Berg, G., "Comparison of antifungal activities and 16S ribosomal DNA sequences of clinical and environmental isolates of *Stenotrophomonas maltophilia*", *J. Clin. Microbiol.* 39, 139-145, 2001.
- [61] Biswas D R, Narayanasamy G. "Rock phosphate enriched compost: An approach to improve low-grade Indian rock phosphate [J]", *Bioresour Technol*, 97: 2243-2251, 2006.
- [62] Sahu S N, Jana B B. "Enhancement of the fertilizer value of rock phosphate engineered through phosphate-solubilizing bacteria [J]", *Ecol Eng*, 15: 27-39, 2000,
- [63] Tributsch H. "Direct versus indirect bioleaching" [J]. *Hydrometallurgy*, 59: 177-185, 2001
- [64] Chen Y P, Rekha P D, Arun A B, Shen F T, Lai W A, Young C C. "Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities" [J]. *Appl Soil Ecol*, 34: 33-41, 2006.
- [65] Rodríguez H, Fraga R. "Phosphate solubilizing bacteria and their role in plant growth promotion", [J]. *Biotechnol Adv*, 17: 319-359, 1999.
- [66] Rashid M, Khalil S, Ayub N, Alam S, Latif F. "Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions" [J]. *Pakistan J Biol Sci*, 7: 187-196, 2004