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A Review on the Role of Phosphate Solubilizing Microorganisms with a Brief Focus on its Genetic Aspects.

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ABSTRACT

Phosphorus is one of the most vital elements required for plant growth and when humanity is still dealing with the food crisis in several parts of the earth, to provide food for ever-growing population one needs ample amount of fertilizers which helps plants to fill the need of essential nutrients but chemically made fertilizers have several disadvantages and have been proved bane for mankind instead of a boon. Phosphorus is one of the most limiting elements in soil for plants which are essential for its growth and biofertilizers supplementing these essential nutrients are in huge demand. There are several microorganisms that can solubilize insoluble phosphate via various mechanisms, which can be further made effective by strain improvement. The role of Phosphate solubilising bacteria for phytoremediation has been well studied. Several PSB can produce siderophores that is very important to prevent infection of plants from different Microorganisms. There are several genes responsible for solubilising insoluble phosphate which can be infectively done via genetic engineering which we shall be discussing in this paper.

Keywords: - Phosphorous, Biofertilizers, Phytoremediation, Siderophores, Genetic engineering.

INTRODUCTION

Phosphorus (P), is inarguably one of the most growth-limiting macronutrients required for proper plant growth, as it is one of the least available plant nutrients in the rhizosphere. It makes up about 0.2% to 0.8% of the dry weight of plants and is one of the major constituents of DNA, RNA, enzymes, coenzymes, and phospholipids[1]. It is essential for cell division and development of the growing tip of the plant, it also participates in metabolic processes such as photosynthesis, energy production, energy transfer, and synthesis and breakdown of carbohydrates[2]. In determining the quality of the crop, it is very crucial to maintain a concentration of phosphorous. Additionally, for the primordial development from which different parts of plant organs can be generated, phosphorous is one of the vital factors. Hence, starting from the molecular level to ultimately plant development, the role of phosphorous is enormous[1][2].

"Phosphorus is the second most important macronutrient after nitrogen but unlike for nitrogen, there is no large atmospheric source that can be made biologically available for phosphorus availability". Only 0.1% of phosphorus is available to the soil, with the total amount of Phosphorus in soil being 0.5%, due to its poor solubility and its fixation as insoluble phosphates of other metallic elements in soil such as Ca, Al, Fe to form Calcium phosphate, Aluminium phosphate & Ferrous phosphate[3]. To address this problem, a large number of phosphate fertilizers can be supplemented to soil with low available phosphorus such as tricalcium phosphate, but most of the chemical phosphorus fertilizer is precipitated by metal-cation complexes and rapidly become fixed in soils and has a long term impact on the environment such as eutrophication and soil fertility depletion[4]. So, an alternative method is needed and phosphorus biofertilizers in the form of microorganisms can help in the availability of accumulated phosphates for plant growth through various mechanisms and make them available so that plants can uptake. Additionally, by enhancing the number of trace elements, soil nitrogen, potassium, and plant growth-promoting hormones by phosphate solubilizing bacteria it is essential to increase plant growth. Phosphate solubilizing microorganisms play an important role in supplementing phosphorus to plants[5]. PSB as bio-inoculants play a vital role in maintaining the soil nutrient status, structure and this review is interested to provide a brief on different aspects of phosphorus nutrition, its availability in soil, diversity of PSM, mechanism of PSM, and how PSM induces plant growth and it's crucial role as biofertilizers.

SCREENING & ISOLATION

Phosphate Solubilizing Microorganisms (PSM) are routinely screened by Pikovskaya (PVK) media[6]. In this process, 1 ml of serially diluted rhizospheric soil suspension is plated on a sterilized PVK media supplemented with insoluble tricalcium phosphate (TCP)/ hydroxyapatite as the sole phosphate source. After incubation, colonies forming holo/clear zone on media are screened as PSM. These clear zones are formed due to the production of organic acids by the PSM into the surrounding media[6][7]. However, a novel microbiological growth medium NBRIP (National

Botanical Research Institutes Phosphate growth medium) is more efficient than PVKmedia[8]. NBRIP medium is more preferred as it excludes the use of yeast extract which enhances the phosphate solubilizing activity. The presence of yeast extract in excess in PVK medium is inhibitory to phosphate solubilization. Isolation of phosphate solubilizers is better observed in the liquid medium than in the agar plate technique. Many isolates which didn't show any clear zones on agar plates, solubilized insoluble inorganic phosphate in liquid medium. Therefore microbes from soil screened in broth assay for identification of phosphate solubilizers is a better technique. NBRIP broth assay is the most preferred technique as it not only excludes yeast extract in the medium but also it is more efficient in a broth assay compare to PVK[1][6][7][8].

The most preferred technique for isolating PSM

NBRIP broth assay > PVK broth assay > NBRIP agar media assay > PVK agar media assay[8]



Flowchart of isolation of PSM [1][8]

Media composition

Name of the media	Composition (in g/l)
Pikovskaya (PVK) media [17]	Yeast extract 0.500
	Dextrose 10.000
	Calcium phosphate 5.000
	Ammonium sulphate 0.500
	Potassium chloride 0.200
	Magnesium sulphate 0.100
	Manganese sulphate 0.0001
	Ferrous sulphate 0.0001
National Botanical Research Institute's phosphate growth	Glucose 10
medium (NBRIP) [9]	Calcium phosphate 5
	Magnesium chloride hexahydrate 5
	Magnesium sulfateheptahydrate0.25
	Potassium chloride 0.2
	Ammonium sulfate 0.1

ESTIMATION OF SOLUBILIZATION INDEX

The ability of P solubilization of a particular PSM can be estimated as solubilization index (SI) and the same can be calculated by the formula given below [10]:-

 $SI = \frac{Colony \ diameter \ + Halozone \ diameter}{Colony \ diameter}$

The percent of change in Pi fractions in the soil can be calculated by the formula given below[11]:-

% of $\Delta p = [(Pi_2 - Pi_1)/Pi_1] \times 100$

Where Δp is the percent of change in Pi fractions in soil, Pi₂ is the concentration of inorganic phosphate (mg·kg⁻¹) in the soil after application of PSM and Pi₁ is the concentration of inorganic phosphate (mg·kg⁻¹) in the soil before application of PSM.

ESTIMATION OF SOLUBLE PHOSPHATE RELEASE

The quantitative estimation of phosphate solubilization by PSM can be measured by the Vanadomolybdate Phosphoric Acid Colorimetric method or Ascorbic Acid method[12].

Vanadomolybdate Phosphoric Acid Colorimetric Method

In this method the dilute Orthophosphate solution, Ammonium Molybdate reacts under acidic conditions to form a Heteropoly Acid, Molybdo Phosphoric acid. In the presence of Vanadium yellow Vanadomybdo Phosphoric acid is formed. The intensity of the yellow color is proportional to the Phosphate concentration [12][13].



Flowchart of the method

Extraction:-

50 ml Benzene-isobutanol solvent along with 15 ml Molybdate reagent II is added to 40 ml of sample in a separatory funnel.

The funnel is closed and shook for about 15 seconds. 25 ml of the separated organic layer is removed from the funnel and transferred to 50 ml of volumetric flask.

After that, 15 ml of alcoholic H₂SO₄ is added to the organic layer and is swirled.

0.50 ml of dilute Stannous chloride reagent II is added and swirled and diluted to the mark with alcoholic H₂SO₄ and mixed thoroughly.

Reading of solution against the blank is taken at 625 nm between 10 to 30 minutes in which the blank is prepared by using 40 ml water, replacing the 40 ml sample in the same process[13].

Calculation

Direct procedure :

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mg \ P/L = \frac{mg \ P \ (in \ approximately \ 104.5 \ mL \ final \ volume \ ) \times 1000}{mL \ sample}
\underline{Extraction \ procedure :}
mg \ P/L = \frac{mg \ P \ (in \ approximately \ 50 \ mL \ final \ volume \ ) \times 1000}{mL \ sample}
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Ascorbic Acid Method

In this method, ammonium molybdate and potassium antimonyltartarate react in acidic solution with orthophosphate to form a heteropolyic acid i.e, phosphomolybdic acid which can be reduced to intensely colored molybdenum blue by ascorbic acid[12][13].



Flowchart of the method

Calculation

 $mg \ P/L = \frac{mg \ P \ (in \ approximately \ 58 \ mL \ final \ volume \) \times 1000}{mL \ sample}$

MECHANISMS OF PHOSPHATE SOLUBILIZATION

Inorganic phosphate solubilization

Chelation by Organic and Inorganic acid:-Phosphate solubilizing microorganism produces some organic and inorganic acids which dissolve insoluble soil phosphate by chelation of cation and competing with phosphate for adsorption site in the soil. The organic acid converts tricalcium phosphate to di and monobasic phosphate, enhancing the availability of phosphorus to the plant[1][14]. Organic acids such as acetic, citric, lactic, propionic, oxalic, glycolic, gluconic, 2-ketogluconic, malonic, tartaric, and fumaric acid are produced by phosphate solubilizers. Gluconic acid and 2-ketogluconic acid are the most frequent agent of mineral phosphate solubilization by PSM. Organic acids do so by lowering the soil pH as they dissociate in a pH-dependent equilibrium, into their respective anion(s) and proton(s), which are consumed by the soil. Depending upon the strength and nature of acid efficiency of solubilization depends such as aliphatic acids are found to be more effective in phosphate solubilization compared to phenolic, citric, and fumaric acid[1][14]. Not only organic acids but also inorganic acids such as nitric and sulfuric acids produced by nitrifying bacteria and *Thiobacillus*during the oxidation of nitrogenous or inorganic compounds of sulfur reacts with calcium phosphates and convert them into soluble forms[1][14]. These acids may also compete for fixation sites of Al and Fe, on reacting with them, stabilize them and are called chelates. The chelation processes is also one of the important phosphate solubilization processes. 2-ketogluconic acid is a powerful chelator compound and those PSMs which

excrete it also solubilize various forms of hydroxyapatite, fluorapatite, and aluminum phosphate. Humic and Fulvic acid release during microbial degradation of plant debris are also good chelators of calcium, iron, and aluminum present in insoluble phosphates [14][15].

Proton release from NH_4^+ : Phosphate solubilizing microorganisms assimilate ammonium present in the soil for the synthesis of amino acid, reducing the cytosolic pH as inside the microbial cell, ammonium (NH_4^+) is converted to ammonia (NH_3) and the excess proton (H^+) is released into the cytoplasm of the microbial cell. This results in lowering the soil pH which leads to the dissolution of insoluble phosphate[15][16].

Indirect mechanism:-Large amount of phosphorus is assimilated indirectly from the soil by the rhizospheric microbes. During stressed conditions, microbial cells can lyse and can release this phosphorus into the soil. Plants can take up this phosphorus as a nutrient source[18].

Exopolysaccharide production:-It has been observed that some phosphate solubilizing microorganisms can solubilize phosphate via exopolysaccharide (EPS) production. In soil microbial EPS can improve aggregation of the particle and benefit plants by maintaining environmental moisture and trapping nutrient. These compounds convert insoluble inorganic phosphate into soluble form via their ionizable functional groups such as carboxyl, carbonyl, and amino. The addition of EPS in the medium increases the amount of phosphorus solubilized by organic acids. EPS with the ability of phosphorus holding is the important factor in the microbial dissolution of tricalcium phosphate. Three phosphates solubilizing bacteria-*Enterobacter* sp. EnHy-401, *Arthrobacter* sp. ArHy505, *Azotobacter*sp. AzHy510 which have EPS producing capacity have also a stronger ability for phosphate solubilization compared to *Enterobacter* sp.EnHy-402(without EPS producing capacity). *Enterobacter*sp.EnHy-401 with the highest EPS production had a stronger capacity for P-solubilization than the other three.

Siderophore production:-Siderophores are secondary metabolites produced by different organisms in order to scavenge iron from their surrounding environment making this essential element available to the cell. The production of siderophore can be determined by plating bacteria on CAS agar (Chrome Azurole S) [19]. A color change from blue to purplish-red indicates positive results for siderophore production.

Even under alkaline conditions, several phosphate solubilizing bacteria releases siderophore such as *Bacillus megaterium* followed by *B. subtilis*[20]. Releasingofsiderophores is a strategy to chelate iron from Fe–P complexes to make the phosphate available for the plant.

Organic phosphate solubilization

Mineralization:-Mineralization is another mechanism via which phosphorus solubilization occurs. Organic phosphate is converted into a utilizable form by PSM through this process, which occurs in the soil at the expense of plants and animal remains, which contain a large amount of organic phosphorus compounds such as nucleic acid, phospholipid, sugar phosphate, phytic acid, polyphosphate, and phosphonates. Phosphorus can be released in the soil from organic compounds by several groups of enzymes such as-

Non-specific phosphatases - It dephosphorylates phosphoester or phosphoanhydride bonds in the organic matter[21].

Phytases-It releases phosphorus in the form of phytic acid[14].

Phosphonatases and C-P lyases- These are the phosphonate degrading enzymes that perform C-P cleavage in organophophate[21].

Apart from these, several other extracellular enzymes like phosphoesterases, phosphodiesterases, and phospholipases are released by several phosphate solubilizing microbes such as *Bacillus* and *Streptomyces* spp. which leads to the mineralization of very complex organic matter. Mixed cultures of PSMs (*Bacillus, Streptomyces,* and *Pseudomonas* are most effective in mineralizing organic phosphate. Several physicochemical and biochemical properties of the molecules affect the degradability of organic phosphorus compounds. Microbial action to degrade phosphorus is influenced by moisture content, pH of the soil, weather condition, chemical nature, particle size, and degree of solubility of phosphate in water[22][23].

Genetics of PSM

Several genes of phosphate solubilizing microorganisms correlate with phosphate solubilization. Soil labile phosphate is negatively correlated with phosphatase activity and *phoC* and *phoD* gene abundance[24][25].

Several genes such as gcd, pqqE, pqqC genes are responsible for glucose dehydrogenase (gcd) mediated phosphate solubilization[26][27].

eno gene has been also found to solubilize phosphate. Mutant bacterial strain 71-2 showed reduced phosphate solubilizing activity compared to a wild type strain 71-2. The disrupted gene in 71-2-MT51 shares 65.26% identity to protein sequences of enolase from *E. coli* suggesting that the gene codes the enzyme enolase and is responsible for phosphate solubilizing capacity of *Burkholderiacenocepacia* strain 71-2[28].

Translation of *acp* genes and *pho* genes produces acid phosphatase and alkaline phosphatase respectively which helps in phosphorus transport into the PSM cell. These *acp* genes are only active when soil is deprived of soluble phosphate[29].

*phy*A gene from *Aspergillusniger*has been genetically transformed to Arabidopsis plant, resulting in improving phosphorus nutrition due to release of phytase enzyme as this enables organic phosphorus assimilation to plants[30][31].

gabY and pcc genes are also concerned with phosphate solubilization. gabY gene is involved in phosphate solubilization without organic acid release[31].

The increased expression level of the ppk gene is correlated with the content of extracellular phosphorus[31].

Role of pqq gene

Transformation of insoluble tricalcium phosphate into soluble phosphorus involves a strong expression of the pyrroloquinolinequinone biosynthesis gene (pqq gene). Expression of the pqq gene cluster is essential for the phosphate solubilization capacity of most of the inorganic phosphate solubilizing microorganisms[34]. Mutation in any pqq gene cluster would reduce the inorganic phosphate solubilization capacity [35]. The pyrroloquinolinequinone, a small redox-active molecule, serves as a cofactor for glucose dehydrogenase enzyme in the release of gluconic acid and 2-keto-D-gluconic acid from glucose such as in *Enterobacterintermidium*(60-2G)[36]. PQQ is encoded by pqq operon which consist of six core genespqqA, B, C, D, E, F. Among them pqqA, pqqC, pqqD and pqq are essential in PQQ biosynthesis[32].

PQQA is a small peptide of 22-24 amino acids that acts as a substrate for PQQE.

PQQC catalyzes the final step of PQQ biosynthesis. It is a cofactorless oxygen-activating enzyme.

PQQD can interact with PQQE but the function of this is not very clear.

PQQE is an enzyme that can cleave S-Adenosyl-L-methionine (SAM) to methionine and 5'-deoxyadenosine [33]

PQQB and PQQF are Metallo-β-lactamase and metalloendopeptidase respectively but both of them are not crucial in PQQ production because of greater genetic variation[32].

Determination of gluconic acid (GA) is carried out using HPLC (High-Performance Liquid Chromatography). It is observed that the more the level of GA is produced, the higher the concentration of phosphate is released. Thus, acidification seems to be one of the main strategies for solubilizing phosphorus which is confirmed by HPLC analysis of supernatants experimentally[37].

Genetic analysis shows that the endophytic strain of *Pseudomonas fluorescens*(L321 strain) possesses a full *pqq* operon as well as *gcd* and *gad* genes. When this endophytic strain is inoculated into the rhizosphere of *P.sativum*L. plants under soluble phosphorus limiting conditions, strains that were capable of producing medium-high levels of GA, resulted in greater plant growth i.e, Endophytic*Pseudomonasfluorescens* can solubilize the insoluble phosphate compound, and hence significant growth of *P.sativum* with L321 inoculation is observed compared to uninoculated*P.sativum*. These inocula produce GA in the rhizosphere of the inoculated plants, which results in the release of soluble phosphorus and this soluble phosphorus is subsequently assimilated by the plant. Also, the production release of PQQ by inoculated strains may enhance the phosphorus solubilizing activity of other indigenous microflora.

However, knockdown of *the pqq* gene results in the reduction of inorganic phosphorus solubilizing capacity such as in mutant *Serratia* sp. RSL. (*pqqE*). Complementation with synthetic PQQ factor restores phosphate solubilization by gluconate production reaching the levels produced by wild type strains. This shows the role of *the pqq* gene in phosphate solubilization [35].

Cloning of mps genes

E.coli can produce PQQ-dependent GDH but are unable to produce PQQ. Phosphate solubilizing activity can be conferred to the DH5 α strain of *E. coli* by cloning and placing a *pqq* gene cluster producing pqq from phosphate solubilizing bacteria. As a result of the cloning of the pqq gene into DH5 α , PQQ produced from it acts as a co-factor resulting in the activation of an endogenous glucose dehydrogenase, permitting gluconic acid secretion that solubilizes the insoluble phosphate[<u>36</u>]. Some strains of *E. coli* such as K12 synthesize apo-GDH but not the cofactor PQQ essential for the formation of the hollow enzyme. Therefore in the absence of exogenous PQQ, these strains cannot produce gluconic acid and hence cannot solubilize phosphate. However, the expression of a single 396- base *P. capacia*open reading frame (designated as gabY) in *E. coli* JM109 (a K12 derivative) induces mineral phosphate solubilization ______[40]. Similarly, *napA* phosphatase gene, containing plasmid pPM9 can be transferred from *Morganellamorganii*into*Burkholderiacapacia*IS16 using the broad host range vector pRK293. Here also, recombinant construction was transformed and expressed in *E. coli* (*E. coli* MC1061) first after which transformed clones were selected and characterized and with the help of Zymograms, acid phosphatase activity was detected and then ultimately genetic constructions were transformed to *B. cepacia* by IS16 by conjugation [41].

pqq gene has a very important role in conferring mineral phosphate solubilization in many microorganisms and so when it is cloned in different microorganisms, those microorganisms too attained the capacity of phosphate solubilization. Till now pqq genes have been successfully isolated from several phosphate solubilizing bacteria such as *E. entermedium, Bacillus licheniformis, Bacillus Mycoides*Gnyt1, etc.[36][38][39]



Flowchart of process for cloning of *pqq* gene[38][39]

Detection of phosphate solubilizing bacteria

This strain of recombinant phosphorus solubilizing genes produces small phosphorus solubilizing circles on the culture medium and phosphorus solubilizing characteristics of linked expression vector is identified via liquid chromatography. Xiaomey and Yao et.al. Liquid chromatography is a separation technique in which the mobile phase is liquid where the sample molecules are dissolved.

Organic acid contains secreted by recombinant strains, standard solutions of different concentrations are purified and enriched by column mobile phase and analyzed under specific chromatographic conditions.

Recombinant phosphorus solubilizing gene strains have been shown to secrete organic acid, thus solubilizing phosphate. [39]

Symbiosis between Nitrogen fixers, Phosphate solubilizers, and Arbuscular Mychorrhizal Fungi

Nitrogen fixers, Phosphate solubilizers & arbuscular mycorrhizal fungi are involved in mutual symbiosis and during the inter-generic interaction, nitrogen-fixing microorganisms provide nitrogen to the plants, improving the nitrogen status of the soil, while PSM enhances plant growth by providing it with phosphates [42]. If both nitrogen and phosphorus are limiting, arbuscular mycorrhizal fungi comes into the picture, improving phosphate uptake for plants [43]. It has been observed that combined inoculation between nitrogen fixers and PSM contributes to a high yield production rather than applying a single inoculation as when plants are inoculated with both nitrogen fixers and phosphorus was inoculated along with *Rhizobium tropici*, *Phaseolus vulgaris* showed an increase in growth [44][47].

Also, nodulation and Nitrogen fixation was enhanced [42][44]. Increased growth, nodulation, and grain yield in common bean and chickpea in comparison to control when co-inoculated with phosphate solubilizers and rhizobia. Rhizobium and phosphate-solubilizing fungi (*Aspergillusawamori*), when used as seed inoculants have been shown to increased the grain yield of chickpea under field conditions[42][45].

In association with nitrogen fixers, arbuscular mycorrhizal fungi increase nitrogen and phosphatic nutrients of plants, especially in phosphorus-deficient soil. Simultaneous dual inoculation of arbuscular mycorrhizal fungi and PSM stimulates plant growth more than the inoculation of either microorganism alone in certain situations when the soil is phosphorus-deficient. Beyond the root hair zone, where phosphate is depleted mycorrhizal external hyphae reaches and helps in improved uptake of nutrients improving plant growth and not only this, root exudation and plasticity can also be changed by PSM inoculation which in turn affect the arbuscular mycorrhizal development and these also produces plant hormones which ultimately increase the activity of the nitrogen-fixing organism at the root zone [42][45][46].

When *Centrosemamacrocarpum* plants were inoculated with Rhizobium strains and the arbuscular mycorrhizal fungi *Glomus manihotis* or *Acaulosporalongula*, a significantly greater mineral absorption, nodulation, and infection by arbuscular mycorrhizal fungi were recorded [42][46].

Very few reports are available on the effect of combined inoculation of crop plants with nitrogen fixers and PSM in the presence of arbuscular mycorrhizal fungus. Inoculation of rhizobium, *Bacillus polymyxa*, and *Glomus faciculatum* resulted in significantly greater PO⁴⁻ uptake as compared with single or double inoculation [42][47].

Role of PSM in phytoremediation

Phytoremediation basically refers to the use of plants and associated soil microbes to reduce the concentration and toxic effects of contaminants in the environment. Among existing strategies to remediate metal contaminants in soil phytoremediation approach using metal accumulating plants is much convincing in terms of metal removal efficiency but it has many limitations because of slow plant growth and decreased biomass owing to metalinduced stress. In addition, constrain of metal bioavailability in the soil is the prime factor to restrict its applicability. Phytoremediation of metals in association with PSM considerably overcomes the practical drawbacks impost by metal stress on plants. Several phosphate solubilizing bacteria have been implicated in the phytoremediation of metalliferous soils due to their metal detoxifying traits and plant growth-promoting activities. Wherever metal concentration is extremely elevated, the phytoremediation (phytoextraction/phytostabilisation) approach has limitations. Under high metallic stress their physiological activities, growth, and development are severely hampered and resistance mechanisms are weakened and in turn, they become prone to phytopathogen attack. Further, their metal phytoremediating efficiency is affected and the process of metal decontamination is protected depending upon several factors. Various plant growth-promoting characteristics of PSB such as organic acid production, secretion of siderophores, IAA production, and ACC deaminase activity enhances the phytoremediation capability of plants[48]. Several phosphate solubilizing strains belonging to the genera of Bacillus, Paenibacillus, Brevibacterium, and Staphylococcus have been reported to solubilize phosphate as well as dissolves the Pb mineral. PSM in addition to chelating agent EDTA (Ethylenediaminetetraacetic acid) when added to Arsenic contaminated soil, phytoremediation property of Echinochloafrumentacia is enhanced and it has been reported within 85 days from the seeding of Echinochloafrumentacia, arsenic contamination in the soil decreases when compared with initial concentration [49]. Inoculation with both BrevibacteriumfrigoripoleransYSP40 and Bacillus paralicheniformisYSP151 and their consortium have been reported to enhance the growth and Pb uptake of Brassicajunceaplants grown in metal contaminated soil. Hence, PSM has an effective role to play in phytoremediation[50].

Conclusion and Future Prospects

The efficient use of phosphate solubilizing microorganisms provides a remarkable success in increasing crop productivity and opens up a new horizon in tackling food security. Therefore, there is a need to characterize more and more phosphate solubilizing microorganisms, and not only that but we also need to increase solubilizing capacity of PSMs either via strain improvement or cloning the genes responsible for phosphate solubilization such as pqq to other plants too. To conclude, phosphorus is an essential element required for plants for growth. We can say that without phosphorus, there are nucleic acids and no life.

References:-

GirmayKalayu, "Phosphate Solubilizing Microorganisms: Promising Approach as Biofertilizers", *International Journal of Agronomy*, vol. 2019, Article ID 4917256, 7 pages, 2019.https://doi.org/10.1155/2019/4917256

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. *et al*.Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus* 2, 587 (2013). https://doi.org/10.1186/2193-1801-2-587

Satyaprakash, M., T. Nikitha, E.U.B. Reddi, B. Sadhana and Satya Vani, S. 2017. A Review on Phosphorous and Phosphate Solubilising Bacteria and Their Role in Plant Nutrition. *Int.J. Curr. Microbiol. App. Sci.* 6(4): 2133-2144. doi: <u>https://doi.org/10.20546/ijcmas.2017.604.251</u>

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H. *et al*.Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *SpringerPlus* 2, 587 (2013). https://doi.org/10.1186/2193-1801-2-587

Bioremediation of phosphate solubilising bacteria isolated from the nearby areas of Sundarban; IJESM ; ISSN-2320-0294; Vol-7, special issue 4(3), April 2018,Impact factor- 6.765

Zheng, BX., Ibrahim, M., Zhang, DP. *et al.* Identification and characterization of inorganic-phosphate-solubilizing bacteria from agricultural fields with a rapid isolation method. *AMB Expr* 8, 47 (2018). https://doi.org/10.1186/s13568-018-0575-6

Qaramaleki, S. V.; Villamil, J. A.; Mohedano, A. F.; Coronella, C. J. Factors Affecting Solubilization of Phosphorus and Nitrogen through Hydrothermal Carbonization of Animal Manure. ACS Sustainable Chem. Eng. 2020, 8, 12462–12470, DOI: 10.1021/acssuschemeng.0c03268C. ShekharNautiyal, An efficient microbiological growth medium for screening phosphate solubilizing microorganisms, *FEMS Microbiology Letters*, Volume 170, Issue 1, January 1999, Pages 265–270, https://doi.org/10.1111/j.1574-6968.1999.tb13383.x

Sane, S. A., and S. K. Mehta. "Isolation and evaluation of rock phosphate solubilizing fungi as potential biofertilizer." *J FertilPestic* 6.2 (2015): 156-160.

Afzal, Aftab&Bano, Asghari. (2008). Rhizobium and Phosphate Solubilizing Bacteria Improve the Yield and Phosphorus Uptake in Wheat (Triticumaestivum). Int. J. Agri. Biol. 10. 1560-8530.

Phosphate Solubilizing Bacteria and Arbuscular Mycorrhizal Fungi Impacts on Inorganic Phosphorus Fractions and Wheat Growth Abdol Amir Yousefi, KazemKhavazi, Abdol Amir Moezi, FarhadRejali and Habib Allah Nadian ; World Applied Sciences Journal 15 (9): 1310-1318, 2011 ISSN 1818-4952 © IDOSI Publications, 2011

Swaminathan, A. G., K. P. Hariprasad, A. V. Suryawanshi, G. B. Kadam and Vilas S. Ghule. 2020. Effect of Phosphorus Solubilizing Bacteria on Growth, Yield, Quality and Nutrient Uptake in Nanasaheb Purple Grapes Grown under Semi-Arid Condition.Int.J.Curr.Microbiol.App.Sci. 9(7): 1274-1283. doi: https://doi.org/10.20546/ijcmas.2020.907.146

FerdousNawar: Determination of phosphorus: Ascorbic acid procedure. protocols.io <u>https://dx.doi.org/10.17504/protocols.io.ggcbtsw</u>

Walpola, Buddhi& Yoon, Min-Ho. (2012). Prospectus of phosphate solubilizing microorganisms and phosphorus availability in agricultural soils: A review. African journal of microbiology research. 6. 6600-6605. 10.5897/AJMR12.889.

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springerplus*. 2013;2:587. Published 2013 Oct 31. doi:10.1186/2193-1801-2-587

Edwin J Parks, Gregory J Olson, Frederick E Brinckman, Franco Baldi, Characterization by high performance liquid chromatography (HPLC) of the solubilization of phosphorus in iron ore by a fungus, *Journal of Industrial Microbiology*, Volume 5, Issue 2-3, 1 April 1990, Pages 183–189, <u>https://doi.org/10.1007/BF01573868</u>

H.O. Halvorson, A. Keynan, H.L. Kornberg, Utilization of calcium phosphates for microbial growth at alkaline pH,Soil Biology and Biochemistry, Volume 22, Issue 7,1990, Pages 887-890, ISSN 0038-0717, https://doi.org/10.1016/0038-0717(90)90125-J.

C.R. Butterly, E.K. Bünemann, A.M. McNeill, J.A. Baldock, P. Marschner, Carbon pulses but not phosphorus pulses are related to decreases in microbial biomass during repeated drying and rewetting of soils, Soil Biology and Biochemistry, Volume 41, Issue 7,2009, Pages 1406-1416, ISSN 0038-0717, https://doi.org/10.1016/j.soilbio.2009.03.018.

URL=https://www.frontiersin.org/article/10.3389/fenvs.2019.00133 DOI=10.3389/fenvs.2019.00133 ISSN=2296-665X

Yi, Y., Huang, W. & Ge, Y. Exopolysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. *World J MicrobiolBiotechnol* 24, 1059–1065 (2008). https://doi.org/10.1007/s11274-007-9575-4

Ferreira, C.M.H., Vilas-Boas, Â., Sousa, C.A. *et al.* Comparison of five bacterial strains producing siderophores with ability to chelate iron under alkaline conditions. *AMB Expr* 9, 78 (2019). https://doi.org/10.1186/s13568-019-0796-3

Tarafdar, J.C., Yadav, R.S. and Meena, S.C. (2001), Comparative efficiency of acid phosphatase originated from plant and fungal sources. Z. Pflanzenernähr. Bodenk., 164: 279-282. <u>https://doi.org/10.1002/1522-2624(200106)164:3<279::AID-JPLN279>3.0.CO;2-L</u>

Nannipieri P., Giagnoni L., Landi L., Renella G. (2011) Role of Phosphatase Enzymes in Soil. In: Bünemann E., Oberson A., Frossard E. (eds) Phosphorus in Action. Soil Biology, vol 26. Springer, Berlin, Heidelberg. <u>https://doi.org/10.1007/978-3-642-15271-9_9</u>

Rawat, Pratibha& Das, Sudeshna&Shankhdhar, Deepti&Shankhdhar, Shailesh. (2020). Phosphate-Solubilizing Microorganisms: Mechanism and Their Role in Phosphate Solubilization and Uptake. Journal of Soil Science and Plant Nutrition. 10.1007/s42729-020-00342-7.

Tandra D. Fraser, Derek H. Lynch, Elizabeth Bent, Martin H. Entz, Kari E. Dunfield, Soil bacterial phoD gene abundance and expression in response to applied phosphorus and long-term management, Soil Biology and Biochemistry, Volume 88, 2015, Pages 137-147, ISSN 0038-0717, https://doi.org/10.1016/j.soilbio.2015.04.014.

Tandra D. Fraser, Derek H. Lynch, Jonathan Gaiero, Kamini Khosla, Kari E. Dunfield, Quantification of bacterial non-specific acid (phoC) and alkaline (phoD) phosphatase genes in bulk and rhizosphere soil from organically managed soybean fields, Applied Soil Ecology, Volume 111, 2017, Pages 48-56, ISSN 0929-1393, https://doi.org/10.1016/j.apsoil.2016.11.013.

Maria Rasul, Sumera Yasmin, M. Suleman, Ahmad Zaheer, Thomas Reitz, Mika T. Tarkka, Ejazul Islam, M. SajjadMirza, Glucose dehydrogenase gene containing phosphobacteria for biofortification of Phosphorus with growth promotion of rice, MicrobiologicalResearch, Volumes 223–225, 2019, Pages 1-12, ISSN 0944-5013, https://doi.org/10.1016/j.micres.2019.03.004.

Suleman M, Yasmin S, Rasul M, Yahya M, Atta BM, Mirza MS. Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat. PLoS One. 2018 Sep 21;13(9):e0204408. doi: 10.1371/journal.pone.0204408. PMID: 30240432; PMCID: PMC6150522.

Liu C, Mou L, Yi J, Wang J, Liu A, Yu J. The Eno Gene of Burkholderiacenocepacia Strain 71-2 is Involved in Phosphate Solubilization. CurrMicrobiol. 2019 Apr;76(4):495-502. doi: 10.1007/s00284-019-01642-7. Epub 2019 Feb 23. PMID: 30798378.

Richardson AE, Hadobas PA, Hayes JE. Extracellular secretion of Aspergillus phytase from Arabidopsis roots enables plants to obtain phosphorus from phytate. Plant J. 2001 Mar;25(6):641-9. doi: 10.1046/j.1365-313x.2001.00998.x. PMID: 11319031.

Rodriguez, Hilda & Fraga Vidal, Reinaldo & González, Tania & Bashan, Yoav. (2007). Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. 10.1007/978-1-4020-5765-6_2.

SonaliBhawsar, Some Genetic Manipulations of Phosphate Solubilizing Bacteria, Biotech Research, <u>https://www.biotecharticles.com/Biotech-Research-Article/Some-Genetic-Manipulations-of-Phosphate-Solubilizing-Bacteria-734.html</u>

Maria Rasul, Sumera Yasmin, M. Suleman, Ahmad Zaheer, Thomas Reitz, Mika T. Tarkka, Ejazul Islam, M. SajjadMirza, Glucose dehydrogenase gene containing phosphobacteria for biofortification of Phosphorus with growth promotion of rice, MicrobiologicalResearch, Volumes 223–225, 2019, Pages 1-12, ISSN 0944-5013, https://doi.org/10.1016/j.micres.2019.03.004.

Zheng, BX., Zhang, DP., Wang, Y. *et al.* Responses to soil pH gradients of inorganic phosphate solubilizing bacteria community. *Sci Rep* 9, 25 (2019). https://doi.org/10.1038/s41598-018-37003-w

Ludueña, L.M., Anzuay, M.S., Angelini, J.G. *et al.* Role of bacterial pyrroloquinoline quinone in phosphate solubilizing ability and in plant growth promotion on strain *Serratia* sp. S119. *Symbiosis* 72, 31–43 (2017). <u>https://doi.org/10.1007/s13199-016-0434-7</u>

Puehringer S, Metlitzky M, Schwarzenbacher R. The pyrroloquinoline quinone biosynthesis pathway revisited: a structural approach. *BMC Biochem*. 2008;9:8. Published 2008 Mar 27. doi:10.1186/1471-2091-9-8

An, Ran, "STUDIES ON REGULATION OF PQQ-DEPENDENT PHOSPHATE SOLUBILIZATION AMONG RHIZOSPHERE DWELLING BACTERIA" (2016). *Theses and Dissertations--Plant and Soil Sciences*. 79. <u>https://uknowledge.uky.edu/pss_etds/79</u>

Pyrroloquinoline Quinone Is a Plant Growth Promotion Factor Produced by Pseudomonas fluorescens B16 - Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Comparison-of-the-pqq-gene-clusters-of-P-fluorescens-B16-with-those-from-Pfluorescens_fig1_5791784 [accessed 11 Aug, 2021]

yang xiaomei, Tuo Yao. Cloning and Functional Analysis of pqq Genes Phosphorus Solubilizing From Bacillus Mycoides Gnyt1, 23 July 2020, PREPRINT (Version 1) available at Research Square [https://doi.org/10.21203/rs.3.rs-41467/v1]

Cloning of a mineral phosphate-solubilizing gene from Pseudomonas cepacia, Babu-Khan S, Yeo T C, Martin W L, Duron M R, Rogers R D, Goldstein A H, 1995, Applied and Environmental Microbiology, American Society for Microbiology ,doi: 10.1128/aem.61.3.972-978.1995 https://doi.org/10.1128/aem.61.3.972-978.1995 2021/08/10

Fraga Vidal, Reinaldo & Rodriguez, Hilda & González, Tania. (2001). Transfer of the Gene Encoding the NapA Acid Phosphatase of Morganellamorganii to a Burkholderiacepacia Strain. ACTA BIOTECHNOL. 21. 359-369. 10.1002/1521-3846(200111)21:4<359::AID-ABIO359>3.0.CO;2-B.

Khan, Mohammad & Zaidi, Almas & Wani, Parvaize. (2007). Role of Phosphate Solubilizing Microorganisms in Sustainable Agriculture - A Review. http://dx.doi.org/10.1051/agro:2006011. 27. 10.1051/agro:2006011.

Meng L, Zhang A, Wang F, Han X, Wang D and Li S (2015) Arbuscular mycorrhizal fungi and rhizobium facilitate nitrogen uptake and transfer in soybean/maize intercropping system. Front. Plant Sci. 6:339. doi: 10.3389/fpls.2015.00339

FatmaTajini, Mustapha Trabelsi, Jean-Jacques Drevon, Combined inoculation with Glomus intraradices and Rhizobium tropici CIAT899 increasesphosphorus use efficiency for symbiotic nitrogen fixation in common bean (Phaseolus vulgaris L.), Saudi Journal of Biological Sciences, Volume 19,Issue2,2012,Pages157-163,ISSN1319-562X,https://doi.org/10.1016/j.sjbs.2011.11.003.(https://www.sciencedirect.com/science/article/pii/S1319562X11000726)

G. S. Tagore, S. L. Namdeo, S. K. Sharma, Narendra Kumar, "Effect of Rhizobium and Phosphate Solubilizing Bacterial Inoculants on Symbiotic Traits, Nodule Leghemoglobin, and Yield of Chickpea Genotypes", International Journal of Agronomy, vol. 2013, Article ID 581627, 8 pages, 2013. https://doi.org/10.1155/2013/581627

Kafle, Arjun, "Tripartite Interactions of Legumes with Arbuscular Mycorrhizal Fungi and Rhizobial Bacteria: Insight into Plant Growth, Seed Yield, and Resource Exchange" (2018). Electronic Theses and Dissertations. 2947.https://openprairie.sdstate.edu/etd/2947

Tajini F, Trabelsi M, Drevon JJ. Combined inoculation with Glomus intraradices and Rhizobium tropici CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (Phaseolus vulgaris L.). Saudi J Biol Sci. 2012;19(2):157-163. doi:10.1016/j.sjbs.2011.11.003

Ahemad, M. Phosphate-solubilizing bacteria-assisted phytoremediation of metalliferous soils: a review. *3 Biotech* 5, 111–121 (2015). https://doi.org/10.1007/s13205-014-0206-0

Park, Ji-Yeon& Kim, Kyoung-Woong& Kim, Juyong& Lee, Byung-Tae & Lee, Jin-Soo & Bae, Beom-Han. (2008). Enhanced Phytoremediation by Echinochloafrumentacea Using PSM and EDTA in an Arsenic Contaminated Soil. The International Symposia on Geoscience Resources and Environments of Asian Terranes, 4th IGCP 516, and 5th APSEG.

Yahaghi Z, Shirvani M, Nourbakhsh F, de la Peña TC, Pueyo JJ, Talebi M. Isolation and Characterization of Pb-Solubilizing Bacteria and Their Effects on Pb Uptake by *Brassica juncea*: Implications for Microbe-Assisted Phytoremediation. J MicrobiolBiotechnol. 2018 Jul 28;28(7):1156-1167. doi: 10.4014/jmb.1712.12038. PMID: 29975995.

Yahaghi Z, Shirvani M, Nourbakhsh F, de la Peña TC, Pueyo JJ, Talebi M. Isolation and Characterization of Pb-Solubilizing Bacteria and Their Effects on Pb Uptake by Brassica juncea: Implications for Microbe-Assisted Phytoremediation. J MicrobiolBiotechnol. 2018 Jul 28;28(7):1156-1167. doi: 10.4014/jmb.1712.12038. PMID: 29975995.