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Review of genetic analysis and mechanisms of phosphate solubilization by phosphate solubilizing bacteria

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Abstract

Phosphorous (P) is one of the most common metallic element in the earth's crust. Phosphorous is a component of important compounds, including nucleic acids, phospholipids, and ATP. Plants uptake only inorganic P as phosphate anions (HPO_4 or H_2PO_4) from the soil. Phosphate solubilizing bacteria (PSB) are present in every ecological habitat; these bacteria are more effective than fungi at phosphate solubilization in soil. A variety of PSB has been isolated and identified from various ecological habitats. Numerous media have been utilized to screen and isolate PSB, such as Pikovskaya's (PVK) agar/broth, bromophenol blue dye method, and National Botanical Research Institute's phosphate growth medium (NBRIP). PSB employs various mechanisms to make phosphorus available to absorb by plants depending on the type of insoluble P source. PSB promotes the growth and production of many crops by direct or indirect mechanisms. The genetics of phosphate solubilization is studied to understand the mechanism of phosphate solubilization at the molecular level. The genes involved in the solubilization of insoluble phosphate have been isolated and identified. Genetic engineering and gene manipulation are promising methods for developing PSB strains with improved phosphate solubilizing capacity.

Keywords: Phosphate solubilization, Phosphate solubilizing bacteria, Acid phosphatase, Phosphate-related genes, Biofertilizer.

Introduction

Phosphorous (P) is one of the most common metallic element in the earth's crust, and can exist in soils in both inorganic and organic forms (Gyaneshwar *et al.*, 2002). It is essential for plant growth and crop yield after nitrogen. Still, the availability of P for plants is limited by different chemical reactions that can be made P available for utilization by plants (Sharma *et al.*, 2013).

Phosphorous is a component of essential compounds, including nucleic acids, phospholipids, and ATP; plants cannot grow without a regular supply of this element. It is also involved in various metabolic processes such as photosynthesis, energy transfer reactions, cell division, and enzyme activity regulation (Khan *et al.*, 2014).

The total concentration of P in the dry weight of crops generally ranges from 0.1 to 0.5 %. P deficiency is one of the most important chemical variables limiting crop yield and significantly adversely impacting crop performance and biodiversity (Alori *et al.*, 2017).

Plants uptake only inorganic P as phosphate anions (HPO_4 or H_2PO_4) from the soil, and the amount of inorganic P in the soil is very low because most of P exists in insoluble forms. Phosphate anions form

a complex with Al, Fe, and Mn in acidic soils, while in alkaline soils, it reacts with Ca or Mg, which are insoluble in water and unavailable to plants. Al and Fe-complexed P become more soluble in basic soil with higher pH values, while Ca-complexed P becomes more soluble in acidic soil with lower pH values (Yadav and Verma 2012; Khan *et al.,* 2014).

The majority of dissolved inorganic phosphate is applied to agricultural land in chemical fertilizers, which rapidly immobilize, become insoluble, and are no longer usable by plants. The overuse of chemical fertilizers has resulted in environmental hazards; therefore, there are working hard to find environmentally-friendly substitutes to avoid the adverse effects and keep soil health (Viruel *et al.,* 2011; Wang *et al.,* 2017).

Soil microorganisms play a significant role in making P available in the soil to plants via solubilizing and mineralizing complex P molecules from inorganic and organic sources (Khan *et al.,* 2014). Phosphate solubilizing bacteria (PSB) are a group of microorganisms has increasingly attracted the attention that can dissolve insoluble phosphate so easily absorbed by plants and promote plants growth and increase crop yield (Wang *et al.,* 2017).

Some inorganic and organic phosphate solubilization genes have been isolated and identified. The optimization and improvement of these genes by using genetic manipulation and modification for obtaining modified bacterial strains with improved phosphate solubilizing capacity; as a result, these bacteria can be used more effectively as agricultural biofertilizers (Rodrı´guez *et al*., 2006).

Phosphate solubilizing bacteria (PSB)

Phosphate solubilizing bacteria (PSB) are present in every ecological habitat. However, their numbers are substantially high in the rhizospheric soil, rhizoplane, phyllosphere, and phosphate rock deposit areas (Zaidi *et al.,* 2009).

Bacteria are more effective than fungi at phosphate solubilization in soil. Generally, Phosphate solubilizing bacteria constitute 1- 50 % of the overall microbial population in the soil, while P solubilizing fungi constitute 0.1 to 0.5 % of the total population (Sharma *et al.,* 2013).

Phosphate solubilizing bacteria are ubiquitous with different types and populations in different soils. Its population is affected by soil properties such as physical and chemical features, organic matter, P content, and cultural activities (Khan *et al.,* 2009). Populations of PSB are found in more significant numbers in agricultural and rangeland soils.

A variety of PSB have been isolated and identified from various habitats to solubilize Ca3(PO4)2, $CaHPO_4$, $Ca_{10}(PO_4)_6(OH)_2$, rock phosphate, and organic phosphates (Espinosa-Victoria *et al.,* 2009).

The most common reported PSB belongs to *Bacillus*, *Pseudomonas*, *Enterobacter*, *Burkholderia*, *Azotobacter*, *Klebsiella*, *Erwinia*, *Mycobacterium*, *Rhizobium*, *Serratia*, *Agrobacterium*, *Acetobacter*, *Mesorhizobium*, *Corynebacterium*, *Flavobacterium*, *Azospirillum* and *Aerococcus* genera (Sharma *et al.,* 2013; Kaur and Kaur, 2018).

Al-Ansari *et al.* (2015) isolated and identified ten isolates of phosphate solubilizing bacteria from the rhizosphere of wheat, barley, and tomato plants grown in different areas of Basrah governorate south of Iraq. All the obtained isolates were identified as *Bacillus Polymyxa*. The bacteria isolated from the rhizosphere of wheat plants were most effective in dissolving phosphate.

Phosphate solubilizing bacteria were utilized in various applications, such as combining PSB with biochar as heavy metal remediation. PSB was used as

fertilizer by inoculating them with animal bone waste, rocks phosphate, and eggshells. PSB could be used as a biopesticide and bioinoculant, whereas PSB with silicon can be mixed with nitrogen fixation bacteria, montmorillonite, and hydroxylapatite clay mineral. In addition, PSB combined with a variety of other fertilizers can be used instead of chemical fertilizers (Kumari *et al.,* 2018; Sudewi *et al.,* 2020).

Isolation and detection of PSB

Several bacterial species have been isolated and characterized in detail for their capabilities of phosphate-solubilization; such bacteria have been isolated using conventional cultural methods.

Numerous methods and media have been utilized for screening and isolating PSB, such as Pikovskaya's (PVK) agar/broth, bromophenol blue dye method, and National Botanical Research Institute's phosphate growth medium (NBRIP) (Nautiyal, 1999; Sharma *et al.,* 2013; Kalayu, 2019).

Pikovskaya's agar medium is supplemented with insoluble tricalcium phosphate (TCP) or hydroxyapatite as a sole source of insoluble phosphate to isolate PSB. PVK agar medium forms an opaque white gel in Petri plates in the presence of TCP. Colonies of PSB can solubilize insoluble phosphate sources in PVK agar medium, forming a visible halo around each colony of PSB after incubation at optimum conditions (Sharma *et al.,* 2013; Kalayu, 2019).

Some PSB could solubilize different types of insoluble phosphate compounds in PVK agar medium but did not form any clear halo around their colonies due to the ability of PSB to produce metabolites such as oxalic acid, which forms insoluble crystal of calcium oxalate around the colonies. PVK agar medium was modified by adding bromophenol blue dye to enhance the visibility of the halos around colonies. The solubilization of insoluble phosphates via the secretion of organic acids leads to declines in the pH of the medium around colonies. The color of bromophenol blue dye changes to form yellow halos around colonies (Mehta and Nautiyal, 2001).

NBRIP medium is demonstrated efficiency about three-fold more sensitive than PVK for screening and detection of PSB, especially in a broth assay. Another advantage of NBRIP medium is that it can be used as a defined medium because the use of yeast extract is excluded from NBRIP medium. The concentrations of glucose and $MgCl_2$ in NBRIP media played an important role in the ability of phosphate solubilization. NBRIP-BPB is NBRIP media supplemented with bromophenol blue dye (BPB). It is designed to be one of the efficient methods for qualitative screening of PSB (Nautiyal, 1999; Alaylar *et al.,* 2020).

Different molecular methods are being used to identify and discover phosphate solubilizing bacterial isolates, and these methods can be used to detect PSB from soil habitats. The most important and valuable molecular methods for investigating and identifying bacteria are Fatty Acid Methyl Ester (FAME) analysis, Denaturing Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE), DNA polymorphism-based methods, In situ hybridization methods, metagenomics and DNA microarrays (Alaylar *et al.,* 2020).

Mechanisms of phosphate solubilization

PSB employs various mechanisms to make phosphorus available to absorb by plants depending on the type of insoluble P source; the mechanisms involved in phosphate solubilization by PSB are outlined in figure 1 and as follows:

Lowering of Soil pH

The principal mechanism for solubilization of soil P is the lowering of

soil pH by microbial production of organic acids or the release of a proton.

The lowering in pH of soil and medium has often been due to the release of organic acids such as citric acid, malic acid, keto-gluconic acids, glutamic acid, tartaric acid, succinic acid, malonic acid, lactic acid, oxalic acid by PSB to dissolve insoluble P as a result of phosphate anion exchange by acid anion. The organic acids are the product of microbial metabolism, mostly through an oxidation pathway on the outer face of the cytoplasmic membrane or fermentation of organic carbon sources (Zaidi et al., 2009). The efficiency of P solubilization depends on the strength and nature of acids produced by bacteria. Furthermore, tri- and dicarboxylic acids are more efficient as compared to monobasic and aromatic acids; also, aliphatic acids are appeared to be more efficient in P solubilization compared to phenolic fumaric and citric acids. The amount of organic acid produced and their types differ with different microorganisms (Walpola & Yoon, 2012; Kalayu, 2019).

The organic and inorganic acids convert tricalcium phosphate to di and monobasic phosphates with the net result of enhanced availability of the element to the plant (Song *et al.,* 2008; Walpola & Yoon, 2012).

Gram-negative bacteria have been shown to release various organic acids into the surrounding soil; therefore, it is more effective than Gram-positive bacteria at dissolving mineral phosphates (Kumar *et al.,* 2018).

The organic acids help reduce the pH of soil and medium by reacting with the insoluble P. H2PO4- (The monovalent anion phosphate), a major soluble form of inorganic phosphate that occurs at lower pH levels. Increasing the soil pH led to the form of the divalent (HPO4 -2) and trivalent (HPO4 -3) of Pi, respectively. So, the synthesis and release of organic acids by PSB into the surrounding environment and media resulted in the acidification of the cells and their surroundings, ultimately leading to releasing of P ions from the P mineral via H^+ substitution for the cation linked to phosphate (Arcand & Schneider, 2006; Sharma *et al.,* 2013).

Another mechanism to produce acidity and is considered effective for increasing the solubilization of inorganic P is CO_2 production by plant roots and microorganisms. However, this mechanism of acidification occurs insolubilization of calcium phosphates ($Ca_3(PO_4)_2$), whereas the water charged with CO_2 reacts with calcium phosphate to release soluble P (Rawat *et al.,* 2018).

Chelation

The organic and inorganic acids produced by PSB can dissolve the insoluble soil P by chelating Fe, Al. The Ca ions complexed with P. The chelation of metal ions connected with complex forms of soil P, enabling the release of adsorbed P via ligand exchange processes (Pradhan & Sukla, 2005).

The carboxyl and hydroxyl groups of the acids can replace phosphates from sorption complexes by ligand exchange and chelating the Fe, Al. The Ca ions are connected with phosphate, thereby converting insoluble phosphates into soluble phosphates forms (Walpola & Yoon, 2012). Carboxylic acids were mainly in charge of the solubilization of Al-P and Fe-P, while 2- ketogluconic acid is a strong chelator of Ca-P (Jones, 1998; Kalayu, 2019). Inorganic acids, such as hydrochloric acid (HCl), can also dissolve phosphate, but they are less effective at the same pH than organic acids (Kim *et al.,* 1997; Jha *et al.,* 2014)

Mineralization

The solubilization of organic phosphatic compounds into utilizable form is called mineralization of organic phosphates mediated by different enzymes produced by PSB and plays a major role in phosphorus

cycling in the agricultural soil (Sharma *et al.,* 2013).

The mineralization process takes place in soil that contains residues of plant, animal, and microorganisms, consisting of large quantities of organic phosphatic compounds like nucleic acids, phospholipids, proteins, and sugar phosphates. The enzymes that PSB produces are mediated mineralization processes of organic phosphatic compounds, including phosphatases, phytases, phosphonatases phosphoesterase, phosphodiesterase, and phospholipase (Behera *et al.,* 2013; Kalayu, 2019).

The phosphatase enzymes are non-specific exoenzymes that are secreted outside of the cells, which carry out dephosphorylation of phospho-ester or phosphoanhydride bonds in organic phosphatic compounds as a substrate to transform them into inorganic substances (Abd-Alla and Omar 2001; Yadav & Verm, 2012).

According to their pH optima, phosphatase enzymes are classified as acid or alkaline phosphatase. Phosphatase enzymes are synthesized in all living things, but only microorganisms can produce them extracellularly. Acid phosphatases are dominated in acidic soils, while alkaline phosphates are prevalent in neutral to alkaline soils. Acid phosphatases are common enzymes and an extensively distributed in fungi (Behera *et al.,* 2013; Sharma *et al.,* 2013).

Phytases (myo-inositol hexa kis phosphate phosphohydrolase) are phytate hydrolyzing enzymes produced in nature by microorganisms, plants, and animals. Phytase-producing bacteria are mostly found in the rhizospheric soil of crops, including gram-positive and gram-negative bacteria such as *Pseudomonas* sp, *Aerobacter* sp, *Citrobacter sp, Bacillus* sp, *Erwinia sp, Enterobacter* sp, and *Klebsiella* sp (Singh *et al.,* 2013; More *et al.,* 2014). Phytase enzymes have mediated the degradation of phytate and further cause the release of soluble inorganic phosphate (PO_4^{3-}) to be available for plants (Yadav & Verma, 2012).

Phytate (inositol hexa-phosphate) is a major component of organic phosphates in soil and is the principal source of inositol and the main stored form of phosphate in plant seeds and pollen (Yadav and Tarafdar, 2011; Singh *et al.,* 2013). High levels of phytates in soils can harm the environment by potentially seeping into water bodies during heavy rains, causing eutrophication and algae blooms (Liu *et al.,* 2018).

Phosphonatases enzymes are called phosphonate hydrolases and C-P lyases, capable of releasing P by hydrolysing C-P bonds of phosphonate compounds (Rodríguez *et al.,* 2006). Phosphonates are organophosphorus compounds that contain carbon–phosphorus (C–P) bonds as a characteristic feature. Some bacteria such as *Pseudomonas fluorescens, Burkholderia cepacia,* and *Bacillus cereus* can hydrolyze C-P bonds of phosphonate compounds by phosphatase enzymes and utilize the products as a carbon and phosphorus sources (Kamat & Raushel, 2013).

Genetics analysis of PSB

The genetics of phosphate solubilization is studied to understand the mechanism of phosphate solubilization at the molecular level and how precisely the PSB brings out insoluble phosphate solubilization. The genes involved in the solubilization of insoluble phosphate have been isolated and identified.

There are three gene families: phoA, phoD, and phoX, which code for alkaline phosphatases, common among prokaryotic organisms. Alkaline phosphatase releases a free soluble inorganic phosphate from many phosphate-containing organic compounds and provides bacteria with the soluble inorganic phosphate as a nutrient (Neal *et al.,* 2017).

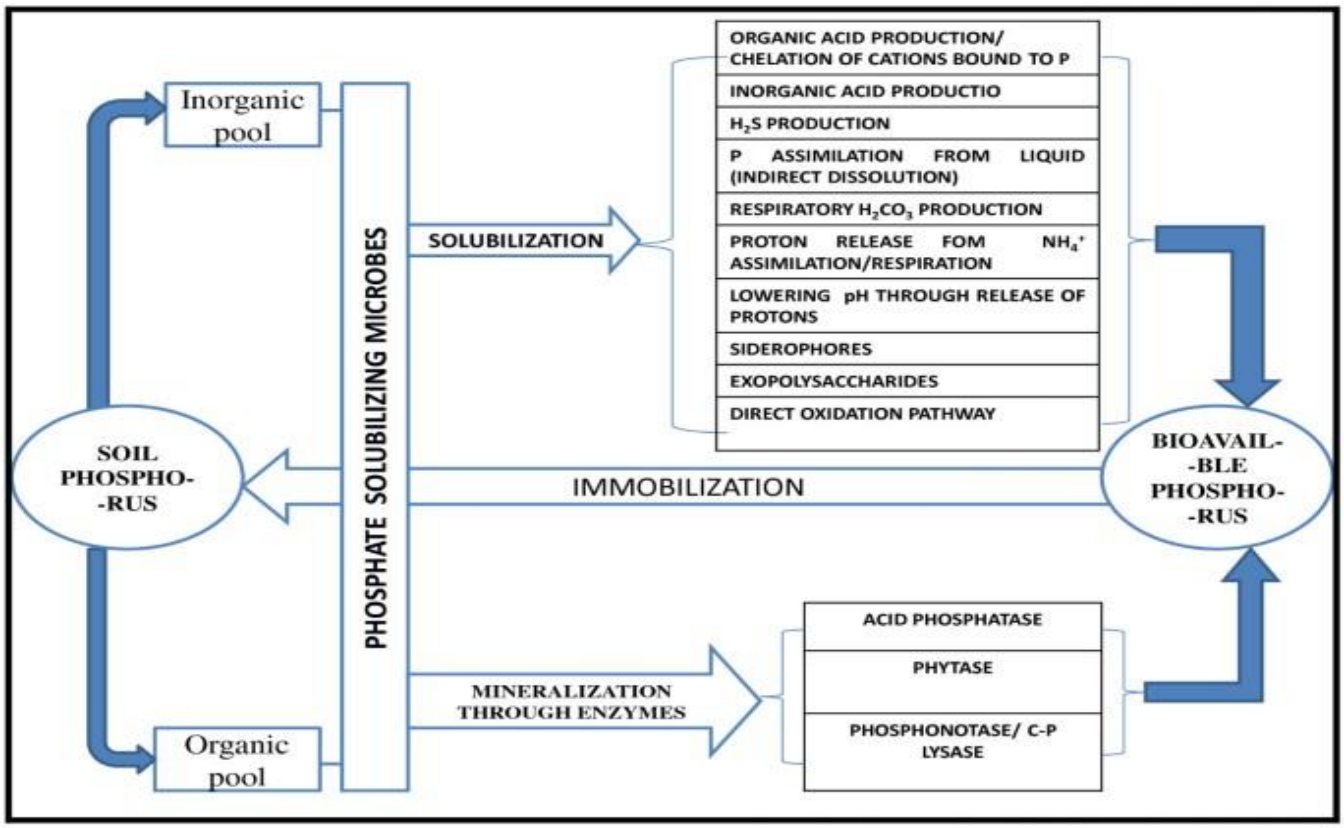

Figure 1. Schematic diagram of soil phosphates solubilization, mineralization, and immobilization by PSB (Sharma *et al.*, 2013).

There is evidence that alkaline phosphatase genes *phoD* and *phoX* are more prevalent than *phoA* in aquatic ecosystems. *phoD* gene is the dominant alkaline phosphatase gene in both terrestrial and aquatic ecosystems. *PhoD* is widespread in terrestrial and aquatic ecosystems (Ragot *et al.*, 2015).

The *phoA* gene is found in many bacteria, such as *E. coli*, which code for alkaline phosphatase, these alkaline phosphatases produced by different bacteria have similar functional important domains (Wu *et al.*, 2007).

The *phoD* gene is mostly found in Actinobacteria, Cyanobacteria, *Deinococcus*, *Thermus*, Planctomycetes, and Proteobacteria. The *phoX* gene is mainly found in the bacterial phyla Proteobacteria and Cyanobacteria but has also been found in Actinobacteria, Bacteroidetes, Chloroflexi, and Lentisphaerae (Ragot *et al.*, 2016).

Genes encoding non-specific acid phosphatases in the bacterium *Morganella morganii* are usually classified into two classes, class A (*PhoC*) and class B (*NapA*) (Rodríguez *et al.*, 2006). The *olpA* gene encodes acid phosphatase isolated from *Chryseobacterium meningosepticum* and is classified as a class C (Passariello *et al.*, 2003). The two genes (*napD* and *napE*) were isolated and characterized from *Sinorhizobium meliloti* encode for nonspecific periplasmic acid phosphatases, which are involved in the solubilization of insoluble phosphatic compounds and transport phosphate to the bacterial cells (Deng *et al.*, 2001).

The *acpA* gene encodes acid phosphatase isolated from *Francisella tularensis* with a wide range of substrate specificity and an optimal pH of 6 (Mohapatra *et al.,* 2006).

The pyrroloquinoline quinone (PQQ) is an active redox cofactor used by several microorganisms. PQQ has been shown to be necessary for phosphate solubilizing in several bacterial species. Genetics analysis of PQQ synthesis has been studied in many genera, including *Acinetobacter*, *Gluconobacter*, *Hyphomicrobium*, *Klebsiella*, *Paracoccus*, *Pseudomonas*, *Methylobacillus*, *Methylobacterium*, *Mycobacterium*, *Thiobacillus*, and *Xanthobacter* (Ogut *et al.,* 2010, Anzuay *et al.,* 2013). The genes encoding PQQ are regulated in pqq operon consisting of at least 5–7 genes such as pqqA, pqqB, pqqC, pqqD, and pqqE genes, as reported among bacterial species like *Klebsiella pneumonia* and *Rahnella aquatilis*, these genes are highly conserved in several bacteria (Han *et al.* 2008; Anzuay *et al.,* 2013). The pqq genes (pqq A-E) of *Gluconobacter oxydans* showed homology to the five-pqq genes (pqq I-V) from *Acinetobacter calcoaceticus* (Felder *et al.,* 2000). Genes responsible for PQQ production are involved in the expression of phosphate solubilizing phenotype via the activation of the direct oxidation pathway of glucose, which leads to the production of gluconic and 2-ketogluconic acids. These acids result from the extracellular (periplasmic space) oxidation pathway of glucose via a membrane-bound pyrroloquin-oline-quinone-dependent glucose dehydrogenase (PQQ-GDH), and the intracellular phosphorylative process involves active absorption of glucose and its subsequent oxidation to 6-phosphogluconate (Rodríguez *et al.,* 2000). The gcd gene responsible for glucose dehydrogenase (GDH) enzyme production mediates the solubilization of inorganic phosphatic compounds (Liang *et al.,* 2020). The gene *gabY* is involved in gluconic acid (GA) production and mineral phosphate solubilization (MPS); therefore, MPS has been involved in organic acid production. Gluconic acid is the principal organic acid produced by bacteria via direct oxidation of glucose and plays important role solubilization of phosphate in soil (Rodríguez *et al.,* 2006).

Several phytase enzymes have been identified in various bacteria, and corresponding genes have been isolated, cloned, and expressed in various hosts (Roy *et al.,* 2016). The phytase gene *appA* was obtained and isolated from various bacteria such as *Buttiauxella* sp. GC21, *E. coli* and *Bacillus subtilis* (Gerlach *et al.,* 2004; Pengjun *et al.,* 2008).

The *phnX* gene encode phosphatase enzyme, which cleaves the C–P bond of phosphonate substrate to produce acetaldehyde and Pi. The expression of the phnX gene is induced under the condition Pi starvation. The phosphatase pathway is a part of the Pho regulon that has been characterized in *Enterobacter aerogenes* and *Salmonella typhimurium* (Villarreal-Chiu *et al.,* 2012).

The products of seven genes (*PhnGHIJKLM*) constitute the core components of the C–P lyase enzyme, where the PhnJ gene encodes a catalytic component of the C–P lyase enzyme, and its product is known to catalyze the central reaction to release free soluble phosphates (Kamat *et al.,* 2011; Villarreal-Chiu *et al.,* 2012).

Some other genes such as *pst* (Pi-specific transporter), *glpQ* (glycerophosphoryldiester phosphodiesterase), and *ushA* (nucleotidase) are involved in the solubilization of organic phosphatic compounds and phosphate transport was stimulated by phosphate starvation (Zeng *et al.,* 2017).

Genetic engineering of PSB

Genetic engineering is considered the most promising method to enhance the potentialities of bacteria as growth promoters of crops. The achievements in manipulating the genes involved in the solubilization of mineral and organic phosphates using genetic manipulation and molecular biotechnology, then expressing them inappropriate bacterial strains. Point to a potential future for developing PSB strains with improved phosphates solubilizing capacity and more effective than a wild strain to use as agricultural inoculants (Sharma *et al.,* 2013). Genetic engineering could also improve the inoculant strain survival by making them more capable of utilizing certain nutrients than the rest of the microbial population (Saikia & Baishya, 2017).

The introduction or overexpression of genes involved in soil organic and inorganic phosphatic compounds solubilization in natural rhizosphere bacteria is a promising strategy for increasing bacteria's ability to function as biofertilizers. The insertion of genes involved in soil organic and inorganic phosphatic compounds solubilization into bacteria that do not have this ability may be possible to avoid the present need to mix two groups of bacteria (nitrogen fixers and phosphate-solubilizers) when utilized as biofertilizers (Bashan *et al.*, 2000; Sharma *et al.,* 2013). The successful gene insertions into recipient strains should overcome barriers like the metabolic machinery dissimilarity and various regulating mechanisms (Sharma *et al.,* 2013).

Fraga *et al.* (2001) were transferred the *napA* phosphatase gene from the soil bacterium *Morganella morganii* using the broad-host-range vector pRK293 to *Burkholderia cepacia* IS-16 for increasing the heterologous expression of *napA* phosphatase gene and improvement of phosphate solubilization capacity in agriculturally important bacteria. A considerable increase in the extracellular phosphatase enzyme activity was achieved in the recombinant strains to improve the growth promotion.

Isolation, cloning, and heterologous expression of a novel phytase gene (*phy*) from *Bacillus* sp. in *Escherichia coli* was achieved by Rao *et al.* (2008). The novel phytase enzyme was successfully developed with wide pH and temperature ranges, high renaturation capabilities, and substrate specificity. This is considered the first study on *Bacillus* phytase purification and effective in vitro refolding from the inclusion bodies synthesized in the transformed *E. coli.*

The phosphate dissolving gene is a promising model for investigating the mechanism of phosphate solubilization by specific phosphate dissolving bacteria, which makes it an attractive option for biotechnology applications. Four full-length DNA encoding *pqqA, pqqB, pqqC* and *pqqE* genes cloned from *Bacillus mycoides* Gnyt1by xiaomei (2020) and expressed in *E. coli*. They were named *pBI-pqqA, pBI-pqqB, pBI-pqqC* and *pBI-pqqE* and four recombinant phospholytic genes were obtained, which proved that *PBI-pqqA, PBI-pqqB, PBIpqqC* and *PBI-pqqE* have phosphate dissolving properties.

Role of PSB as promoters of plant growth and crop production

Phosphate solubilizing bacteria promote the growth and production of many crops by direct or indirect mechanisms, as outlined in figure 2. The direct mechanism can attribute to converting insoluble soil P to an accessible form, enhancing biological nitrogen fixation, and phytohormones and Plant growth regulators such as gibberellins, auxins, cytokinin, and polyamides make the nutrients available to plants.

The indirect mechanism can be due to the production of beneficial crop metabolites like antibiotics that prevent or reduce harmful effects of pathogenic microorganisms and siderophores that increase the accessibility of trace elements (Zaidi *et al.,* 2009, Sharma *et al.,* 2103; Khan *et al.,* 2014; Kumar, 2016).

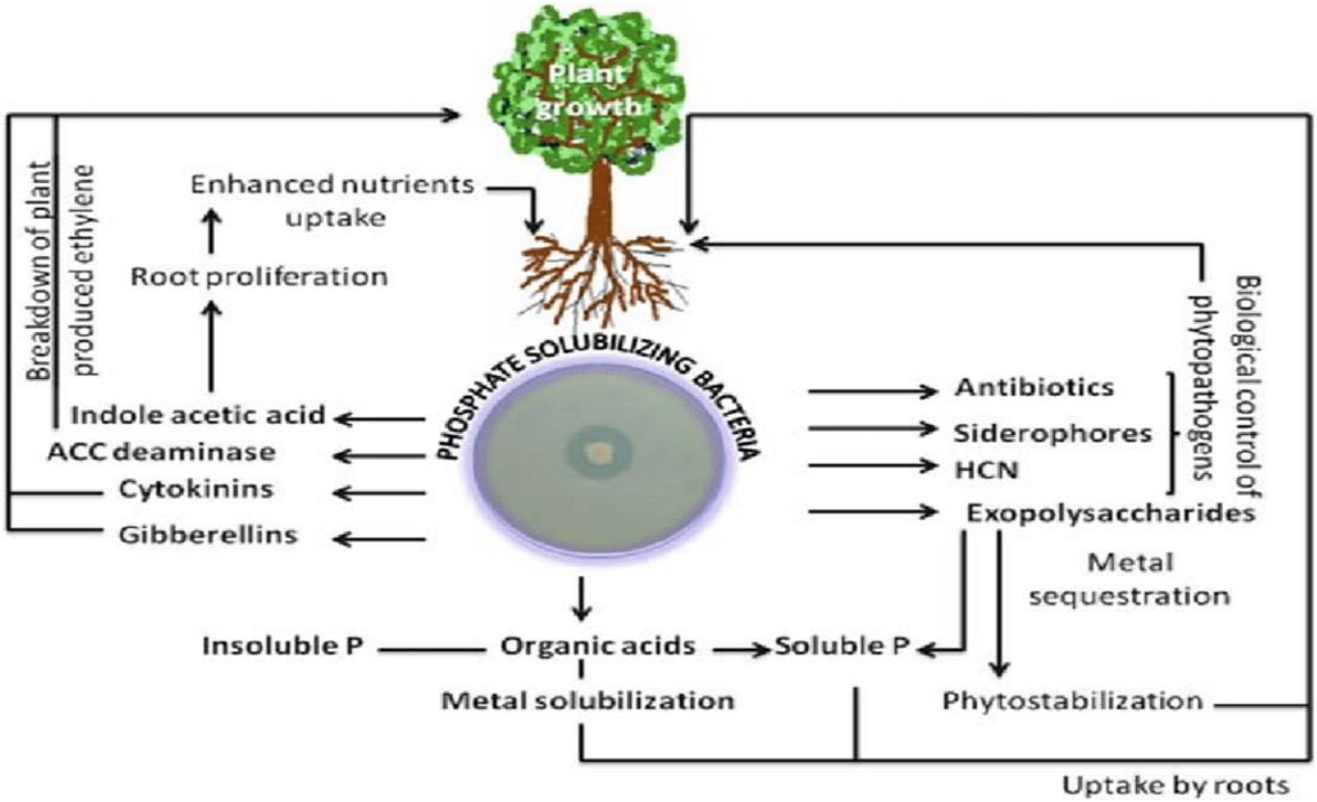

Figure 2. Mechanism of plant growth promotion by PSB (Zaidi et al., 2009).

Different PSB was studied to improve crop productivity in various parts of the world. The yield of green gram seeds was improved by 24 % by triple inoculation of bacteria including *Bradyrhizobium*, *Glomus fasciculatum* and *Bacillus subtilis* (Zaidi & Khan, 2006).

The impact of dual inoculation of an arbuscular mycorrhizal fungus (*Glomus etunicatum*) and an indigenous PSB strain (*Burkholderia cepacia* BAM-6) was determined on growth, yield and P concentration of wheat crop grown in pots containing loam soil with low available P. The two strains were enhanced biomass and phosphorous uptake by wheat crop (Saxena *et al.,* 2013).

Two bacterial strains (*Pseudomonas* sp. MS16 and *Enterobacter* sp. MS32) were isolated from the rhizosphere of wheat (*Triticum aestivum*) cultivated on soils in Peshawar and the southern Punjab region of Pakistan. In contrast, the isolates showed a variety of plant growth promotions. The soil with low P content was inoculated with *Pseudomonas* sp. MS16 strain enhanced wheat grain yield by 38.5% and 18% in pot and field trials, respectively (Suleman *et al.,* 2018).

Experiments carried out by Alzobi *et al.* (2006) to study the effect of *Bacillus megaterium* and cows' manure on solubilization of Syrian phosphate rock in the soil and its effect on cotton crop productivity. The same amount added the amounts of phosphate rock and superphosphate fertilizer to the soil. Some experimental plots were also fertilized with urea and others with cow's manure. The results showed a significant increase of the available phosphate in inoculated soil with bacteria and cow's manure compared to the non-inoculated soil. The number of PSB in

the cotton plant rhizosphere also increased. These bacteria effectively solubilized phosphate rock and increased the productivity of the cotton crop, especially in the soil that was amended with cow's manure and phosphate rock.

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دراسة مراجعة حول التحليل الوراثي وآليات إذابة الفوسفات بواسطة البكتيريا المذيبة للفوسفات

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المستخلص

الفوسفور هو أحد العناصر المعدنية الشائعة في قشرة الأرض. يعد الفوسفور أحد المكونات الهامة للمركبات الحيوية مثل الأحماض النووية ، الفوسفوليبيد و ATP. تمتص النباتات الفوسفور غير العضوي فقط كأيونات الفوسفات (HPO_4 أو H_2PO_4) من التربة. توجد البكتيريا المذيبة للفوسفات (PSB) في كل المواطن البيئية ، وهذه البكتيريا أكثر فعالية من الفطريات في إذابة الفوسفات في التربة. تم عزل مجموعة متنوعة من البكتيريا المذيبة للفوسفات وتشخيصها من مختلف المواطن البيئية. تم استخدام العديد من الطرق والتقنيات لفحص وعزل البكتيريا المذيبة للفوسفات مثل وسط Pikovskaya (PVK) وطريقة صبغة البروموفينول الأزرق ووسط النمو التابع للمعهد الوطني للبحوث النباتية (NBRIP). تستخدم البكتيريا المذيبة للفوسفات آليات مختلفة لجعل الفوسفور متاحًا لامتصاصه بواسطة النباتات اعتمادًا على نوع مصدر الفوسفات الغير قابل للذوبان. تعزز البكتيريا المذيبة للفوسفات نمو وإنتاج العديد من المحاصيل من خلال آليات مباشرة أو غير مباشرة. تمت دراسة الجينات الوراثية لإذابة الفوسفات من أجل فهم آلية ذوبان الفوسفات على المستوى الجزيئي ، حيث تم عزل وتشخيص الجينات المشاركة في إذابة الفوسفات غير قابلة للذوبان. تعتبر تقنيات الهندسة الوراثية من أكثر الطرق الواعدة لتطوير سلالات البكتيريا المذيبة للفوسفات ذات قدرة محسنة في إذابة الفوسفات.