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A Review: Phosphate Solubilizing Microorganisms and their role in plant growth promotion

[1]Gamit Kinjal B., [2] Trupti Pandya J.

[1]M.sc microbiology student, [2]Teaching assistant

[1]Department of microbiology

[1]Bhagwan Mahavir college of basic and applied sciences, Bhagwan Mahavir University, 395007.

Abstract:Phosphorus is the second most important macronutrient after nitrogen required for metabolism, growth, and development of plants. Phosphate deficiency prevents plants from growing and developing properly. Although total P is present in soil in the form of organic and inorganic compounds, the majority of these molecules are inactive and so unavailable to plants. Phosphate solubilizing microorganisms can solubilize insoluble phosphates in soils through a variety of mechanisms, including the secretion of organic acids, the production of enzymes, and the excretion of siderophores that can chelate metal ions and form complexes, allowing for plant uptake. Seeds /crop and soil inoculating with phosphate solubilizing microorganisms is a viable technique for increasing food production without causing environmental hazard. The physical qualities of the soil can be improved using biofertilizers, as well as the water holding capacity.

Key word: Biofertilizer, Growth promoting factors, Phosphate solubilization, Phosphate solubilizing microorganisms (PSM)

I. INTRODUCTION

A plant's health depends on its ability to absorb nutrients. Minerals in the soil are classified as macronutrients or micronutrients, depending on the amount required. NPK(nitrogen, phosphorus, potassium) are primary macronutrients that must be consumed in vast quantities, where as copper, boron and iron are examples of micronutrients that must be consumed in minute amounts (Zakaria *et al.*,2009). Phosphorus is a plant macro nutrient that play important role in photosynthesis, energy storage and transformation of starches and sugars, respiration, cell division in living plant (Sharma *et al.*,2013;kalayu *et al*.,2019,Ahemad *et al.*,2009;Mitra *et al.*,2020).For the promotion of early root development and growth, sufficient phosphate is required. Phosphate promotes crop quality and is required for seed production as well as flower and fruit development (Tirado *et al.*,2012; Krishnaraj *et al.*,2014).When Phosphate is insufficient or deficient however, it affects leaf surface area, number of leaves, shoot and root growth, delayed blossoming and maturity, reduced forage, vegetable, fruit and grain crop quality, and decreased disease resistance. Phosphate deficient leaves turn purple or turn a dark blush green color in some plant such as corn and tomatoes (Sawyer *et al.*,2000; Ahemad *et al.*,2009).

Soil is a natural medium that helps plants grow by supplying nutrients and other vital ingredients. Rhizosphere refers to the thin layer of soil that surrounds a plant's roots. It is directly influenced by root exudates and so has a high concentration of soil-associated microorganisms (Baetz *et al.*,2014;suyal *et al.*,2021). It is generally recognized that a large variety of bacterial species, primarily those found in the plant rhizosphere, have the ability to promote plant growth (Glick *et al.*,1995; Rodriguez *et al.*, 1999). A large portion of soil phosphate is present in form of insoluble phosphate and plant can not utilize insoluble phosphate. Organic matter, pH, active sesquioxides, lime, and the nature and quantity of clay all affect Phosphate solubility (Kour *et al.*,2019;Suyal *et al.*,2021).The root absorb phosphorus in a variety of forms, the majority of which is taken in the form of $HPO4^{2-}$ and H_2PO_4 depending on the soil pH. The majority of Phosphate will be present as monovalent H_2PO_4 below pH 6.0. Plant absorption is likewise strong in the pH range of 5.0–6.0, indicating that monovalent P is largely taken up by plants (Furihata *et al.*,1992; Pradhan *et al.*,2017).Some bacterial species have ability to mineralize and solubilize organic and inorganic phosphorus, making it available to plants (Khaiari *et al.*,2005;Jena *et al.*,2013).

II. PHOSPHATE SOLUBILIZING MICROORGANISMS

Microbes are tiny, but they are extremely potent and useful (Nath *et al.*, 2018; soumare *et al.*,2020).These soil microbes play an important role in soil through their metabolic activities, and they are an important aspect of integrated nutrient management in the soil because they help plants get nutrients from the soil (Rajwar *et al.*,2018; Rawat *et al.*,2021). Phosphate solubilization and mineralization abilities are found in a wide range of microorganisms, including bacteria, fungi, algae and actinomyces (Alori *et al.*,2017;Dandessa *et al.*,2018).

Table 1 Diversity of Phosphate solubilizing Microorganisms

Diversity of Phosphate solubilizing Microorganisms	References
Bacteria *Alcaligenes, Acinetobacter, Arthrobacter, Flavobacterium, Bacillus, Salmonella, Enterobacter, Azotobacter, Pseudomonas, Agrobacterium, Burkholderia, Kushneria, Paenibacillus, Ralstonia, Rhizobium, Rhodococcus, Bradyrhizobium, sinomonas, Erwinia, Serratia, Thiobacillus, Klebsiella*	Rodriguez *et al.*,1999; Krishnaraj *et al.*,2014, Sharma *et al.*,2013; Alori *et al.*,2017
Fungi *Achrothcium, Alternaria, Arthrobotrys, Aspergillus, Cephalosporium, Cladosporium, Curvularia, Cunninghamella, Chaetomium, Fusarium, Glomus, Helminthosporium, Micromonospora, Mortierella, Myrothecium, Oidiodendron, Penicillium, Phoma, Pichia fermentans, Populospora, Pythium, Rhizoctonia, Rhizopus, Saccharomyces, Schizosaccharomyces, Schwanniomyces, Sclerotium, Torula, Trichoderma, Yarrowia*	Alori *et al.*,2017; Dandessa *etal.*,2018
Cyanobacteria *Calothrix braunii, Nostoc sp., Scytonema spp., Anabaena variabilis, Westiellopsis prolifica*	Sharma *et al.,2013;* Alori *et al.,2017*, Suyal *et al.,2021*
Vesicular arbuscular mycorrhiza *Glomus fasciculatum, Entrophospora colombiana, Rhizophagus irregularis MUCL 43194*	Sharma *et al.*,2013
Actinomycetes *Streptomyces thermocarboxydus, Streptomyces werraensis, streptomyces ambifaria, Streptoverticillium, Microbacterium lacusdiani sp*	Hamim *et al.*,2019; Zhang *et al.*,2017

The phosphate Solubilizers *Pseudomonas, Bacillus and Rhizobium* are among the most potent in the soil (Rodriguez *et al.*,2014; Krishnaraj *et al.*,2014). *Penicillium* spp. And *Aspergillus* spp. Are the most frequent phosphonate-solubilizing filamentous fungus (Wakelin *et al*.,2004; Mitra *et al*.,2020).Soil fungi have been shown to be able to travel longer length within the soil than bacteria, suggesting that they are more significant for solubilization of inorganic phosphate in soil (Kucey *et al*.,1983; Sharma *et al.*,2013). The nematofungus *Arthrobotrys oligospora* can also dissolve phosphate rocks (Duponnois *et al.*,2006; Khan *et al*.,2009).

The growth of saline-alkali soil base agriculture is aided by salt- tolerance or halophilic soil microorganisms that also possess the ability to solubilize insoluble phosphorus (Zhu *et al.*,2011;Alori *et al.*, 2017). Under drought conditions, *Pseudomonas* sp. And *Azospirillum* sp. Solubilized phosphate. Cold tolerant phosphate solubilizing ability has been demonstrated in *Pseudomonas* sp., *Pantoea* sp.,*Mycobacterium* sp., *Mycoplasma* sp., And *Acinetobacter* sp. At low temperatures ranging from 4 to 16°c (Pandey *et al.*,2006, Gulati*et al.*,2009).Under saline conditions, *Aerococcus* sp., *Arthrobacter* sp.,*Bacillus* sp., *Pantoea* sp., And*Pseudomonas* sp., Solubilized phosphate (1% to 10% nacl)(Srinivasan *et al.*,2012; Prabhu *et al.*,2019). *Cupruavidus basilensis*, which was previously Known to be capable of copper chelation, was recently discovered to have the ability to mineralize both inorganic and organic phosphorous for the first time (Qian *et al.*,2010; Krishnaraj *et al.*,2014).

Phosphate solubilizing microorganisms is isolated and characterized in laboratories using a variety of growing media. Pikovskaya was the first to describe a reliable method for preliminary screening and separation of potential phosphate solubilizing microbes. Inorganic phosphate solubilizing microorganisms form a clear zone surrounding the colonies on pikovskaya's agar plate (Gupta *et al.*,1994; Prabhu *et al.*,2019).

III. MECHANISMS OF PHOSPHATE SOLUBILIZATION BY MICROORGANISMS

Microorganisms use three primary phosphate solubilization mechanism:1) release of complexing or mineral dissolving substances such as organic acid anions, protons, carbon dioxide and siderophores. 2) extracellular enzyme liberation such as acid phosphatase and alkaline phosphatase. 3) P release during substrate break down (McGill *et al* ,1981;Sharma *et al*.,2013; nath *et al*.,2018).

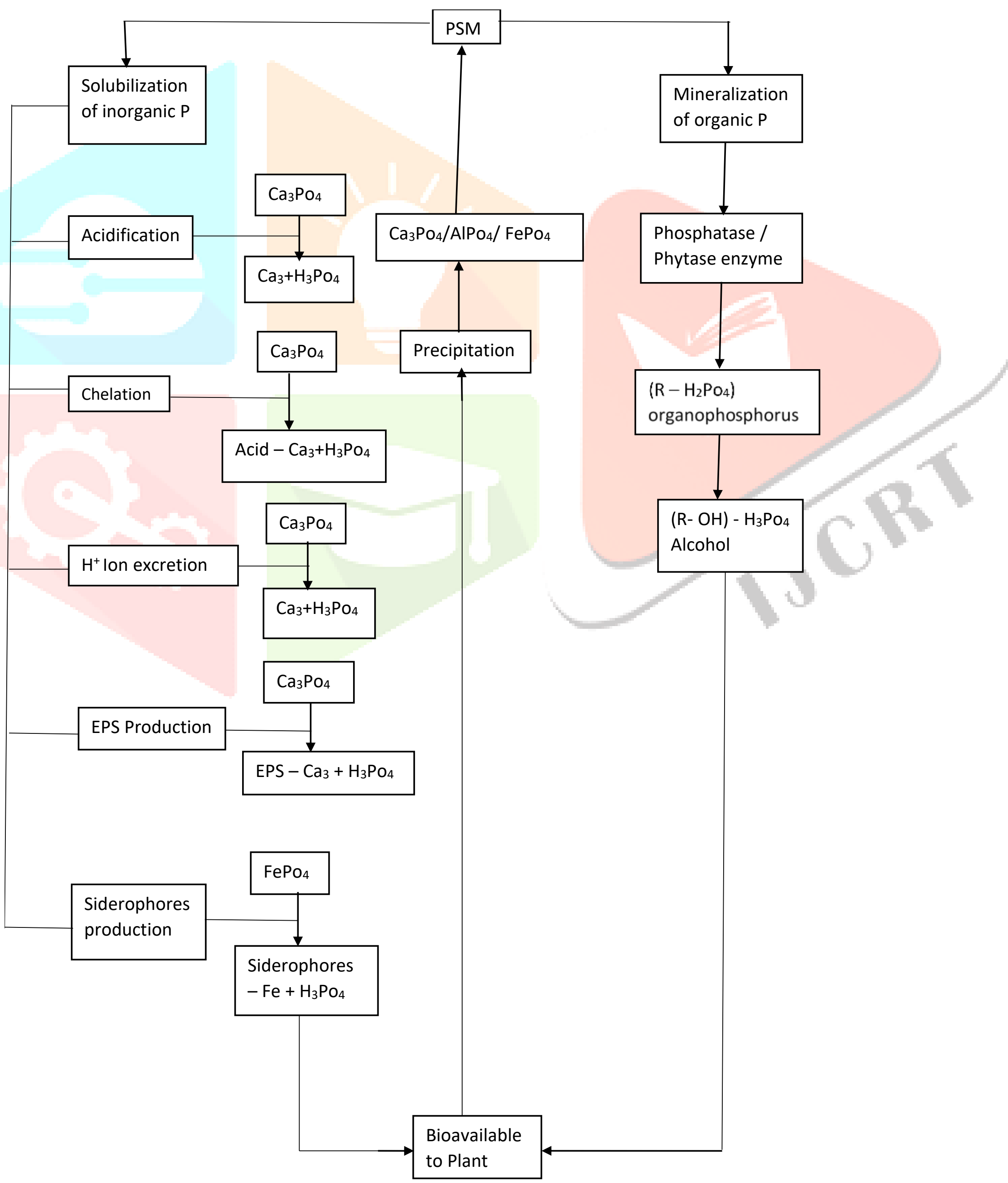

Figure:Mechanism of Phosphate solubilization by microorganisms

1. Inorganic phosphate solubilization

1.1organic acid production

Cation complex in the soils precipitate nearly 75-90% of appliedphosphatic fertilizers (walpola *et al.*,2012; Kalayu *et al.*,2019).In normal or calcareous soils, cation such as Ca_2^+ immobilize Phosphate to create a complex calcium phosphate, while in acidic soils Al_3^+ and Fe_3^+ immobilize Phosphate to form aluminium phosphate and ferrous phosphate (Kumar *et al.*,2018; satyaprakash *et al.*,2018; Kalayu *et al.*,2019). To solubilize inorganic phosphate compounds, Phosphate solubilizing microorganisms have been found to produce monocarboxylic acid (acetic, formic), monocarboxylic hydroxy (lactic,gluconic, glycolic), tricarboxylic hydroxyl (Citric)acids. The decreased pH indicate phosphate solubilizing microorganisms are secrete organic acids (whitelaw *et al.*,1999; Rashid *et al.*,2004; Ingle *et al.*,2017). Phosphatesolubilizing microbes produces organic and inorganic acids that chelate cations and compete with phosphate for adsorption site in the soil. The acid's hydroxyl and carboxyl groups chelate the phosphate bound cations, turning it to soluble forms. These acids may operate as fixation site for AL and Fe insoluble Oxides, stabilizing them when they react with them. Ketogluconic acid is a calcium chelating agent (Walpola *et al.*,2013; Kalayu *et al.*,2019).

1.2 Inorganic acid production

Phosphate solubilizing microbes have been shown to produce hydrochloric acid, sulfuric acid, nitric acid, and carbonic acid, which have been shown the solubilize Phosphate, albeit with limited effectiveness when compared to organic acids.*Nitrobacter and Thiobacillus* spp., respectively use the generation of inorganic acids like nitric acid and sulfuric acid to dissolve Phosphate (Shrivastava *et al.*,2018; Kim *et al.*,1997; *Rawat et al.*,2021).

1.3 Siderophores production

Microorganisms produce siderophores, which have a great affinity for chelating iron (Gamit *et al.*,2014; Jyothi *et al*.,2020). Siderophores have been found in a variety of phosphate solubilizing microorganisms. The solubility of iron phosphate in soil is influenced by siderophores (Gaonkar *et al.,* 2013; Vassilev *et al.*,2006; Prabhu *et al*.,2019). Several Phosphate solubilizer including *Bacillus megaterium*, *Bacillus subtilis*, *Rhizobiumradiobacter*, *Pantoes allii,*were found to create siderophores in the range of 80 to 140 μmol L^{-1}, which helped organisms survive in stressful environment and increased Phosphate solubilization (Ferreira *et al.*,2019; Rawat *et al.*,2021).

1.4 Exopolysaccharide production

Microorganisms release exopolysaccharides, which are homo or heteropolymers of carbohydrates with an organic or inorganic component, beyond their cell wall. Microbes create exopolysaccharides in response to stress or the production of biofilms. Phosphate is solubilized by exopolysaccharides secreting microorganisms by forming complex with metal ions (Al3+, Fe3+, cu2+) found in soil. Exopolysaccharides generation and Phosphate solubilization efficiency were both seen in *Paenibacillus polymyxa* GOL0202(Sutherland*et al.*,2001; Ochoa *et al.*,2001; Cherchali *et al.*,2019; Rawat *et al.*,2021).

1.5 Proton extrusion

Proton extrusion is a method for bacteria to dissolve Phosphate in soil. Phosphate solubilizing microbes use ammonium (NH_4^+) from the soil to synthesis amino acids. NH_4^+ is transformed to ammonia inside the microbial cell and the excess proton H^+ is discharged into cytoplasm. This causes the media around the microbial cell to become acidic, which aids in the break down of insoluble Phosphates. When media provided with $(NH_4)_2SO_4$ as nitrogen source, a *Bacillus subtilis* BPM12 strain was reported to solubilize maximal Phosphate in vitro (Park *et al.*,2011; Gaind *et al*.,2016; Wang *et al*.,2020; Rawat *et al*.,2021). When NH_4^+ was employed as a nitrogen source instead of NO3- the amount of P dissolved was higher (Sharan *et al.*,2008; Prabhu *et al.*,2019).

2. Organic phosphate solubilization

The soil contains a variety of organic substances that can provide plant growth with P. Nucleic acid, phospholipids, phosphonates, phytic acid, polyphosphonates, and sugar phosphates are all examples of organic phosphate molecules found in soil (Sharma *et al.*,2013; Kalayu *et al*.,2019,Ozanne *et al.*,1980; Anand *et al.*,2016). Microbial enzymes such as phosphatase, phytase, phosphonates, and C-P lyase help to dissolve organic phosphate in soil. Phosphatase enzymes hydrolyze ester-phosphate bonds to release phosphate ions, converting high-molecular weight organic phosphate to low- molecular weight molecules. Based on their pH, these enzymes are classified as acid or alkaline phosphatase. The phytase enzyme breaks down phytic acid molecules. Phosphonates and C-P lyase hydrolyze phosphonate ester linkages and convert phosphonates into hydrocarbons and phosphate ions for absorption (Cabugao *et al.*,2013;Wanner *et al.*,1996;Prabhu *et al*.,2019). The most effective way to mineralize organic phosphonate is to use mixed cultures of phosphate solubilizing microbes (*Bacillus, Pseudomonas, streptomyces* etc)(Molla *etal*.,1984; Khan *et al.*,2009).

IV. ROLE OF PSM ON CROP PRODUCTION

Phosphate rock minerals are frequently insoluble, making it difficult for crop to absorb enough phosphate. Plant have a wide range of phosphorus requirements. Tree have low phosphorus needs, with critical values ranging from 0.12 to 0.25 %. Grass required more phosphorus, with critical amounts ranging from 0.20 to 0.25 %. Plants have a high initial P concentration that decreases with age, and because phosphorus is a relatively mobile element, insufficiency is more common in older tissue. Legumes and some vegetable crops require more phosphorus, with essential values ranging from 0.25 to 0.30 % or slightly more (Saha *et al.*,2009; Krishnaraj *et al.*,2009, Pradhan *et al.*,2017). High amounts of plant phosphate can sometimes lead to imbalances and deficiencies in other elements including zn, cu and Fe and others. Proper P fertilization and microbially mediated soil transformation are required to keep plant P levels within the sufficiency range (Krishna Raj *et al.*,2009, Pradhan *et al.*,2017).

Crop yields can be increased by up to 70% when using phosphate solubilizing Microorganisms. Inoculation with arbuscular mycorrhiza and Phosphate solubilizing microorganisms improves native phosphatic rock P uptake. PSB solubilizer fixed soil P as well as administered phosphate, resulting in higher crop yields. Phosphate solubilizing microorganismsalso improve nitrogen fixation. In the soybean crop *Pseudomonas* spp. Increased the number of nodules, nodule dry weight, yield components, nutrient availability and uptake (son *et al.*,2006; cabello *et al.*,2005; Khan *et al.*,2009). Rice grain yield, root length, leaf length, and plant weight were all greatly boosted by *Serratia marcescens* and *Azospirillum* (Da silva aragujo *etal*.,2013; Billah *et al.*,2019).Phosphate Solubilizing *Pseudomonas* and *Bacillus* species were inoculated into wheat resulting in improved phosphorus uptake and grain production (Afzal *et al.*,2005;Walpola *et al*., 2012). *Bacillus megaterium* is powerful Phosphate solubilizer, and its use improved banana bunch weight, length and number of hands per bunch, as well as lentin nodulation and grain yield (Kumar *et al*.,2008;Billah *etal*.,2019).

Table2 list of Phosphate solubilizing microbes, their host plant, organic acids production, Phosphatesolubilization and role of plant growth promotion

Organisms	**Host plant**	**Organic acids production**	**Amount of Phosphate solubilized (µg/mL)**	**Growth promotion**	**References**
Pseudomonas fluorescens BIHB 740	*Zea Mays*	Gluconic acid, 2-ketogluconic acid, succinic acid, formic acid, citric acid, malic acid	768.3	Increased plant height, shoot dry weight and root length.	Vyas *et al.*,2009
Bacillus megaterium cs22	*Brassica napus*	Succinic acid, oxalic acid, citric acid	119.37	Increasedin phosphorous content and biomass production	Zheng *et al.*,2019
Klebsiella varricola	*Helianthus tuberosus* L	Gluconic acid, oxalic acid, lactic acid, acetic acid	371.00	IAA production, improvement in root length, shoot height	Nacoon *et al.*,2020
Acinetobacter rhizosphaerae BIHB723	*Zea Mays*	Gluconic acid, formic acid, oxalic acid	750.00	In treated plants, plant height and root shoot length increased and root shoot phosphate content increased	Gulati *et al.*,2010
Penicillium oxalicum	*Triticum aestivum*	Oxalic acid, gluconic acid, malic acid, acetic acid	586.00	When compared to control, the shoot Height of inoculated wheat plants increased 1.5 times,resulting in 42% increase in wheat production.	Singh *et al.*,2011
Azotobacter sp. PSRB7	*Lycopersicum esculentum*	Gluconic acid, oxalic acid	143.00	Higher root and shoot biomass and increased in Phosphate uptake	Hariprasad *et al.*,200
Pantoea spp.	*Solanum lycopersicum*	Acetic acid, formic acid, gluconic acid, propionic acid	956.00	Increased in shoot and root biomass, as well as total dry matter yield.	Sharon *et al.*,2016
Pseudomonas sp. AZ5	*Cicer arietinum L.*	Acetic acid, oxalic acid, gluconic acid	103.00	IAA production, improvement in grain yield, straw weight, number and dry weight of nodules, and P uptake.	Zaheer *et al.*,2019
Serratia sp. 5D	*Cicer arietinum*	Citric acid, acetic acid, gluconic acid, malic acid, succinic acid, lactic acid, oxalic acid	119.94	IAA production, increased nodule dry weight, straw yield and grain yield.	Zaheer *et al.*,2016
Klebsiella sp. MR13	*Oryza sativa* L.	Acetic acid, citric acid, gluconic acid, oxalic acid	392.00	Improved grain yield of crop and dry matter production	Rasul *et al.*,2019
Micrococcus sp. NII0909	*Vigna unguiculata*	Malic acid, fumaric acid	122.4	Root and shoot length increased by 100 and 39.2% respectively, IAA production, siderophores production	Dastager *et al.*,2010
Enterobacter sp. Fs-11	*Helianthus annuus* L.	Malic acid, gluconic acid	43.46	IAA production, In comparison to control there was a 64.8% increase	Shahid *et al.*,2012

				in root length and 91.9% rise in shoot length.	
Pseudomonas fluorescens CB501	*Zea Mays*	Oxalic acid, citric acid, tartaric acid, gluconic acid, malic acid, succinic acid, fumaric acid	99.80	Increased in plant height and yield, improved in shoot and seed P content	Henri *et al.*,2008
Alcaligenes faecalis PSB-26	*Arabidopsis thaliana*	Oxalic acid, succinic acid, acetic acid, malic acid, citric acid	48.00	Improved number of leaves and flowers, improved shoot and root length	Behera *et al.*,2017

V. APPLICATION

1. Biofertilizer and Biocontrol

Biofertilizers, which are products that include beneficial microorganisms that have the potential to boost soil fertility and crop output, are helpful to environment since they minimize reliance on chemical fertilizers (Orhan *et al.*,2006; Billah *et al*.,2019*)*. Chemical based phosphate fertilizers are commonly used to boost crop yield, but their long-term use in Arabic lands has resulted in a variety of stresses, including salinity stress due to salt build-up induced by cation-bound insoluble phosphate complexes. Phosphate solubilizing bacteria and phosphate Solubilizing fungi strains will be exploited as a Biocontrol and Bio- fertilizers for crop enhancement in agriculture (Tamilarasi*et al.*,2007; Mitra *et al.*, 2020). Alternative source such as biofertilizer or microbial inoculants are used, both cost effective and environmentally beneficial (Anand *et al*.,2016; Dandessa *et al.*,2018).

Pathogens have posed a significant danger to plant health by lowering plant out put and lowering the quality of food (Fallahzadeh *et al.*,2009; Mitra *et al*.,2020). *Bacillus subtilis* is has been used to manage a variety of phytopathogenic fungi andbacteria. Plant are additionally protected by PSMs, which produce antibiotics, hydrogen cyanate (HCN) and antifungal metabolites, which help them escape phytopathogens. Plant growth is aided by PSMs (phosphate solubilizing microbes) that produce phytohormones such as auxins, gibberellins, cytokinins and polyamides (Yousefi *et al.*,2011; Kalayu *et al.*, 2019).

2. Bioremediation

Metal concentrations that are too high results in reduced plant biomass and stunted growth. Phosphate solubilizing bacteria, such as *Pseudomonas* sp., *Klebsiella* sp., *Enterobacter* sp., And other, have been shown to be useful in metal bioremediation and in influencing phytoremediation in metal- contaminated soil via phytoextraction or phytostabilization. Bioremediation is aided by phosphate solubilization mechanism such as organic acid generation, H excretion, Siderophores production and exopolymeric substance creation (Ahemad *et al.*,2015; Prabhu *et al.*,2019).

VI. CONCLUSION

Phosphate solubilizing microorganisms have capacity to transform insoluble phosphates into soluble form through their organic acid production and phosphatase secretion. PSM promotes plant development by producing siderophore and improving nitrogen fixation efficiency. Furthermore, through producing antibiotics, hydrogen cyanate (HCN), and antifungal metabolites, phosphate solubilizing microorganisms works as a biocontrol agent against plant infections. PSM use as a biofertilizer that improve the fertility of the soil and increase crop productivity. The Phosphate solubilizing bacteria such as *Pseudomonas, Enterobacter and Bacillus* are the most effective Phosphate solubilizing bacteria for increasing plant accessible P in soil, as well as crop development and yields.

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