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REVIEW

Phosphate solubilization by plant growth promoting rhizobacteria and improvement of their potentials through biofilm formation

NARAYAN CHANDRA MANDAL
Mycology and Plant Pathology Laboratory, Department of Botany, Visva-Bharati, Santiniketan 731235, West Bengal, India

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The major amount of phosphate resource on this earth is remained locked as insoluble- or as rock-phosphate. Different cations, particularly calcium ions play an important role which initially bind with the phosphorus and later form insoluble complexes. Through chemical industrial process such complex phosphates are made soluble for agricultural use but the process has tremendous drawbacks. With the discovery of phosphate solubilizing microrganisms scientists are continuously trying to exploit these tiny organisms to their maximum extent. A number of organisms have been discovered with enormous potentiality to solubilize insoluble tri-calcium phosphate or rock phosphates which are derived mostly from the root environment or rhizosphere. Later on many other beneficial attributes have been discovered from these organisms which make these organisms as super symbionts or 'plant probiotics'. Various scientists again are working to exploit the potentials of these organisms for getting maximum benefits for agricultural improvements. To unravel different mechanisms for the purpose, the importance and aptitude of biofilm formation by these organisms are realized. In this review the quorum sensing based biofilm formation with the help exo-polysachharides and how such biofilms contribute improved phosphate solubilization and other attributes of the plant growth promoting rhizospheric bacteria are discussed.

Key words: Insoluble phosphate, plant growth promoting rhizobacteria, biofilm, quorum sensing, exo-polysaccharides

INTRODUCTION

Phosphorus is an element essential for the existence all living organisms as it participates in their many vital physiological and biochemical processes. It is essential for making nucleotides, the structural units of DNA and RNA that hold and translate genetic information and so control all living processes in plants, animals, humans as well as in microorganisms.

Of all the essential elements needed for the growth and development in plants, next to nitrogen, phosphorus is the most important growth limiting macro-element. It is very important for many major metabolic processes in plant viz., photosynthesis, respiration, energy transfer through ATP/ADP synthesis, macromolecular biosynthesis, signal transduction, metabolic and cell cycle regulation, membrane synthesis and stability, and nitrogen fixation (Khan *et al*., 2010). The P is absorbed through plant root and transported to the shoot portion where it is furtherincorporated into organic compounds through various chemical reactions. It is incorporated energy rich phosphate compounds like adenosine diphosphate (ADP) and adenosine triphosphate (ATP), into nucleic acid (DNA and RNA), phospholipids and sugar phosphates.

Although P is abundant in soils in both inorganic as well as in organic forms, crop plants are unable to use it comfortably as it remains in unavailable form in soil solution (Hinsinger, 2001). In most of the soil the inorganic P (Pi) occurs as insoluble complexes with various cations thus cannot be absorbed by plant-root (Rengel and Marschner, 2005). A large amount of phosphate also remains in organic matter in the soil which also acts as an

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important reservoir of immobilized P. A very little amount of P (approx. 0.1%) presentin the soil in their soluble form for ready use of for plant uptake. Around 30–40% of crop yield on the world's arable land is limited by P availability. The acid-weathered soils of the tropics and subtropics are particularly deficient in P.

A large numbers of cations present in the soil bind with the phosphorus leading to its rapid precipitation or fixation as insoluble complexes. This is the main reason for non-availability of phosphorus to the crop plants. Phosphate ions are strong ligands which form complexes with several metal ions such as Ca, Mg, Al and Fe. On the other hand, P becomes precipitated by aluminum (Al^{3+}) and iron (Fe^{3+}) in the acidic soils whereas in neutral or basic soil it forms insoluble complexes with calcium (Ca^{2+}). Phosphate is primarily fixed by iron and aluminum hydroxides, crystalline and amorphous aluminum silicates and calcium carbonate. Inorganic phosphorus remains in their bound forms as ores in nature (the metal bearing minerals).

Some examples of them are phosphoritis-structure, chlorapatite [$3Ca_3(PO_4)_2,CaCl_2$], fluorapatite [$3Ca_3(PO_4)_2,CaF_2$],wavelite [$4AlPO_4,2Al(OH)_2,9H_2O$], vivianite [$Fe(PO_4)_2,8H_2O$] etc. deposited in nature.

The most usable form of P is Pi (orthophosphate) which is readily accessed by plants, whose concentration in soil solutions rarely exceeds 10 ìM. Variation of soil pH is mainly responsible for the presence of different form of Pi in soil. The dissociation constant values of H_3PO_4 into $H_2PO_4^-$ and then into HPO_4^{2-} are 2.1 and 7.2 respectively. So, below pH 6.0, most Pi remain as the monovalent $H_2PO_4^-$ species, whereas only in minor proportions of H_3PO_4 and HPO_4^{2-} are present at that pH. Except the highly fertilized soils, concentration of the phosphate in most soil solution is very low and this cannot meet the phosphate demands required for many crops like potato, onion, spinach,bean, cotton, pea, or tomato.This is a big problem in tropical and subtropical soils which are thought to be highly weathered as plants cannot utilize the bound forms of phosphates for their nutrition (Gyaneshwar *et al*., 2002).

Because of such problem of soil P, application of readily available chemical P fertilizers is generally recommended for enhancing the levels of available P on most agricultural soil to increase crop yields (Vance *et al*., 2003). Applications of chemical fertilizers has some drawbacks viz., these increase the major cost of agricultural production and also creates environmental problems which ultimately degrade terrestrial, freshwater as well as marine water resources (Tilman *et al*., 2001). Increase of P levels is considered as one of the most important factors for eutrophication of surface water which ultimately leads to algal blooms (Schinder *et al*., 2008). Unnecessary and over-use of chemical P fertilizer is also responsible for loss of soil fertility and reduction of soil health (Gyaneshwar *et al*., 2002). Excessive applications of phosphatic fertilizers disturb soil microbial activities and reduce crop yield. For example application of triple superphosphate in excessive manner reduces microbial respiration and metabolic quotient (qCO_2) (Chandini and Dennis, 2002).

Chemical P fertilizers applied to the agricultural soil also get fixed by several cations present in the soil and form insoluble P complexes which are not utilized by plants (Whitelaw, 2000)). The majority of agricultural soils contain huge reserves of phosphorous (P), of which a major part is accumulated as a consequence of regular applications of phosphatic fertilizers. It has been suggested that, if the total accumulated P in agricultural soil were available, it would be sufficient for maximum crop yield throughout the world for about 100 years (Khan *et al*., 2009). Igual *et al*., (2001) reported that calcium superphosphate fertilizers get fixed by soil minerals like calcium carbonate after application in agricultural fields. Nearly 75% of the soluble P fertilizers applied to crops can get converted to least soluble forms by reacting with the free Ca^{2+} ions in basic soils or with Fe^{3+} or Al^{3+}in acidicsoils.

In chemical fertilizer industry, rock phosphates are processed chemically using various inorganic acids for producing chemical P fertilizers. This is not only an expensive processes that increases the cost of phosphate fertilizers, but also responsible for environmental pollution due to releases of gases as well as industrial wastes (Sharma *et al*., 2013). It has also been estimated that high quality rock phosphate deposits throughout the world may be depleted within the current century due to its high rate of use for the preparation of chemical P fertilizers (Cordell *et al*., 2009).

To overcome these potential problems related with chemical P fertilizers it is necessary to findout environmental compatible and economically reasonable alternative strategies to improve soil P deficiency for ultimate increase of agricultural production. The application of microbial inoculants having P solubilizing activities for increasing available P in agricultural soil is considered as a suitable alternative to avoid the extensive use of chemical P fertilizers.

Phosphate Solubilizing Microorganisms

Microorganisms play a vital role in soil P cycle and are responsible for the transfer of P between different pools of soil P (Sharma *et al*., 2013). In soil there are different kinds of phosphate solubilizing microorganisms which can convert inorganic as well as organic soil P through various mechanisms. The abilities of microorganisms for solubilizing insoluble P were reported. Although various groups of microorganisms including bacteria, fungi, yeast and cyanobacteria were isolated from different sources, but bacteria were considered as most potential members for solubilization of insoluble P.

Phosphate solubilizing bacteria are able to solubilize mineral P by producing several strong organic acids like gluconic acid and 2 ketogluconic acid. These organic acids decrease the soil pH and chelate the metal cations like Ca, Fe, Al etc. from insoluble P complexes and make the bound phosphorus free for plant uptake. Maximum concentration of PSMs (phosphate soluble microorganaism) are found in the plant rhizospheric region although a large number of P solubilizing bacterial strains have been isolated from different sources. In addition to P solubilization plant associated P solubilizing soil bacteria which are also known as plant growth promoting rhizobacteria (PGPR), may help in plant growth and development by various mechanisms. They promote the plant's growth directly by mobilizing different plant nutrition and producing phytohormones or indirectly by controlling different plant pathogens as antagonistic organisms (Fig.1).

In the last few years a large number of PGPR strains have been isolated from rhizosphere of different plants. Besides, they also contribute a number of other plant growth promoting attributes which have great potential to exert role on plant growth and development. There are several commercialized PGPR inoculants that promote crop plant growth by various properties (Saharan and Nehra, 2011). Development of single inoculant with multiple plant growth promoting traits, are now in the focus of agricultural scientists (Zahir *et al*., 2004; Ahmad *et al*., 2008). These requirements can be fulfilled by isolating new PGPR strains with multiple activities, by co-inoculation of PGPR strains (Masciarelli *et al*., 2014) or by introducing multiple PGP traits within a single organism by genetic manipulation.

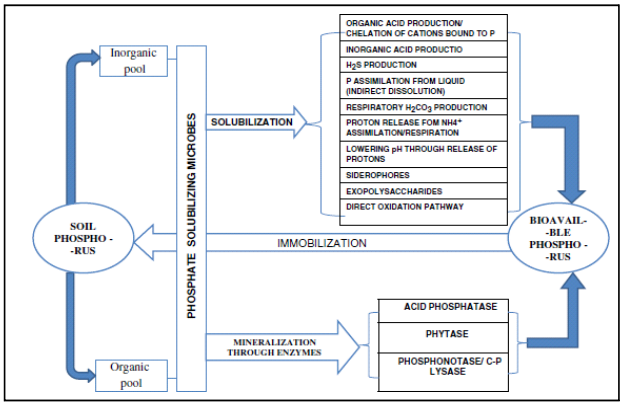

Fig. 1 : Schematic representation of mechanisms of solubilization/ mineralization and immobilization of soil P by PSMs (Adopted from Sharma *et al*., 2013).

Plant Growth Promoting Rhizobacteria

The term "rhizosphere" was used to describe the zone of soil under the influence of exudates of plant roots. The rhizosphere is the narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. It can also be described as the longitudinal and radial gradients occurring with expanding root growth, nutrient and water uptake, exudation, and subsequent microbial growth (Uren 2000). This zone of soil influenced by plants are rich in nutrients in comparison to the bulk soil due to the accumulation of a variety of plant exudates, like sugars, amino acids etc. which provide a rich source of energy and nutrients for associated microorganisms (Gray and Smith, 2005). A number of compounds eg., flavonoids present in the root exudates act as chemical attractants for a large number of actively metabolizing soil microorganisms. As a result of secretion of such compounds, the modified physical and chemical properties of the soil regulate the soil microbial structure in the immediate vicinity of root surface (Dakora and Phillips, 2002). The composition of these exudates

varies along with physiological status and species of plants (Kang *et al*., 2010). The bacteria which are predominant and adapted in rhizospheric region are known as rhizobacteria.

The PGPR organisms primarily help crop plants by providing with nutrients like, nitrogen, phosphorus, iron potassium etc. and produce plant hormones such as IAA and GA_3. They also participate indirectly in plant growth promotion by producing antagonistic metabolites against plant pathogenic organisms or by inducing the resistance power of plant against pathogens. A particular PGPR may promote plant growth and development either by using any one of these, or more mechanisms and PGPR with multifunctional activities are regarded as the most potential in terms of agricultural point ofview.

Solubilization of insouble mineral P by Rhizobacteria

Of the various PSMs present in the rhizosphere, phosphate-solubilizing bacteria (PSB) are considered as most promising biofertilizers because they can very effectively supply soluble P to the plant by solubilizing both organic as well as inorganic insoluble P sources (Zaidi *et al*., 2009). Bacterial genera like *Burkholderia, Enterobacter, Erwinia, Flavobacterium, Azotobacter*, *Bacillus*, *Beijerinckia*, *Microbacterium, Pseudomonas, Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria (Bhattacharyya and Jha, 2012). Some potent strains of *Burkholderia* species have been isolated from the rhizosphere of a fern *Lycopodiella cernuum* from the lateritic soil of Santiniketan, West Bengal with tremendous potential to solubilize tricalcium- and different rock- phosphates (Ghosh *et al*., 2016).

As the rhizospheric niche is metabolically very active, large number of phosphate solubilizing bacteria prefer to reside there and have been isolated from plant rhizosphere (Vazcuez *et al*., 2000; Khan *et al*., 2009). Several scientific literature have suggested the ability of different bacterial species to solubilize different types of insoluble inorganic phosphate compounds, such as tri-calcium phosphate, di-calcium phosphate, hydroxyapatite, and rock phosphate. The pH of themajority of arable soils is above 7.0, so in most of the soil types P is found in the form of poorly soluble calcium phosphates. Therefore solubilizing potential of microorganisms especially for calcium phosphate is considered as one of the important aspect in terms of global Pcycle.

A considerable number of P solubilizing bacterial strains have been isolated from rhizospheric soil of different plants of diverse habitat. Vazquez *et al*. *(*2000) isolated thirteen P solubilizing bacterial strains from rhizosphere of mangroves using culture media containing tri basic calcium. Nautiyal *et al*. (2000) isolated PSB strains from the rhizosphere of chickpea which were able to solubilize mineral P in the presence 10 % salt, pH 12 and temperature 45°C. Chen *et al*. (2006) isolated, screened and characterized 36 strains of PSB from Central Taiwan and checked for their mineral P solubilizing potential. Five PSB strains isolated from rhizosphere of faba bean of Ethiopia showed very good tricalcium phosphate solubilizing potential (Keneni *et al*., 2010). Panhwar *et al*. (2012) isolated a large number of PSB strains from rice rhizosphere with various plant growth promoting attributes. Oteino *et al*.(2015) demonstrated P solubilizing potential of endophytic strains of *Psedomonas*isolated from *Pisum sativum.*

Phosphate solubilizing bacteria (PSB) have the capability of secreting strong organic acids which act upon insoluble phosphates like calcium phosphates, rock phosphates, aluminum phosphate, iron phosphate etc. and brings forth their dissolution. Production of organic acids results in acidification of the bacterial cell and its surroundings which ultimately release the P from its bound form. In case of phosphate solubilizing bacteria the production of various organic acids has been well documented. Among them, gluconic acid seems to be the most frequent acid produced by PSBs during mineral phosphate solubilization. A decrease in the medium from the initial value of 7.0 to a final value of 2.0 was recorded by many workers for different P solubilizing bacterial strains.

Production of gluconic acid as the principal organic acid during mineral P solubilization have been reported for several phosphate solubilizing bacteria such as *Erwinia herbicola*, *Burkholderia cepacia* (Song *et al*., 2008) and *Pseudomonas fluorescence* (de Werra *et al*., 2009). In addition to gluconic acid another organic acid known as 2-ketogluconic acid was also identified in different bacterial strains with phosphate-solubilizing

potential. In *Enterobacter intermedium (*Hwangbo *et al*., 2003*)*, *Pseudomonas poae* (Vyasand Gulati, 2009) and other soil bacteria production of 2 ketogluconic acid was detected during P solubilization. Other organic acids, such as oxalic, malic, citric, formic, malonic, valeric and succinic acid, have also been identified among phosphate solubilizers (Sharma *et al*., 2013). Production of mixtures of lactic, isovaleric, isobutyric, and acetic acids were also being detected in *Bacillus licheniformis* and *Bacillus amyloliquefaciens*(Vazquez *et al*., 2000).

In Gram negative bacteria Gluconic- and 2-keto gluconic- acids are produced in the periplasmic spaces which ultimately acidify the surrounding medium by diffusion. A significant number of evidences suggested the role of direct extracellular oxidative pathway in organic acid production during P solubilization. Through this pathway glucose oxidizes to gluconic acid and 2-keto gluconic acid directly in the periplasmic space (Fig.2). The enzyme glucose dehydrogenase (GDH) plays the

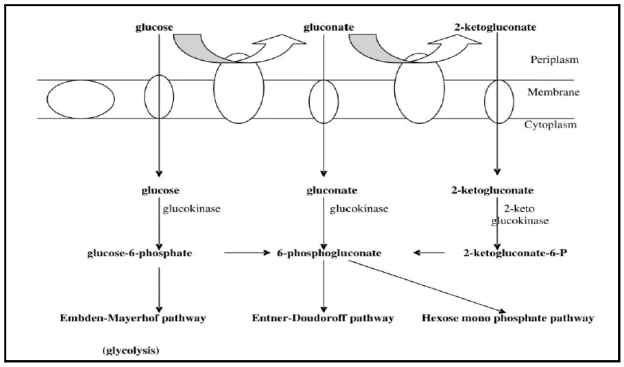

Fig.2. Direct oxidation pathway involved in gluconic and 2-keto gluconic acid production during P solubilizationby PSB (Adopted from Krishnaraj and Dahale, 2014)

key role in this process by catalyzing the oxidation of D-glucose to D- Gluconate. The membrane bound GDH requires pyrroloquinolinequinone (PQQ) as a cofactor. It has binding site for Mg^{2+}, Ca^{2+}, ubiquinone and for the substrate glucose. Many organisms areunable to synthesize PQQ; therefore, GDH occurs as an apoenzyme, but is readily reconstituted by incubation with PQQ and metal ions. The active site of the enzyme is located towards periplasmic space which facilitate release of oxidized product i.e. gluconate to the external environment. Further oxidation into 2-keto-gluconate is catalyzed by another enzyme gluconate dehydrogenase located in the periplasmic region. These two acids are the strongest known organic acids associated with mineral P solubilizing phenotype of Gram negative bacteria.

Other plant growth promoting attributes of Rhizobacteria

Apart from phosphate, nitrogen is another growth limiting macroelement for plants. Although, there is about 78% N_2 in the atmosphere but it is not available to the growing plants (Ahemad and Kibret, 2014). The atmospheric N_2 is converted into plant-utilizable forms by the process known as biological N_2 fixation which converts atmospheric nitrogen to ammonia. Nitrogen fixing microorganisms play the key role in biological nitrogen fixation using a complex enzyme system known as nitrogenase. Symbiotic nitrogen fixing organisms mainly includes the member of the family Rhizobiaceae which are mostly associated with leguminous plants (Ahemad and Khan, 2012) and the actinobacterial genus *Frankia*which are associated with non-leguminous plants. On the other hand a large number of non-symbiotic nitrogen fixing bacteria have been reported that includes *Azospirillum, Azotobacter, Gluconoacetobacter*, *Azocarus, Pseudomonas*, *Stenotrophomonas*etc (Bhattacharyya and Jha, 2012; Ahemad and Khan 2012; Mehnaz *et al*. 2010). Plant growth-promoting rhizobacteria that fix N_2 in non-leguminous plants are also called as diazotrophs which are able to form non-obligate interaction with the host plants. The N_2 fixation process is carried out by a complex enzyme system which is known as the nitrogenase complex.

Production of phytohormonesis another important trait for PGPR organisms. They mainly produce indole acetic acid (IAA) and gibberelic acid (GA_3). About 80% microorganisms isolated from the rhizosphere of different crop plants showed the ability to synthesize IAA as secondary metabolites. As the endogenous IAA pool of the plant may be altered by the interference of the PGPR strains therefore, IAA production by them affects many developmental process of plant (Glick, 2012; Spaepen *et al*., 2007). The precursor amino acid tryptophangreatly alters the quantity of IAA production in different rhizbacterial strains (Zaidi *et al*., 2009b). At least five different pathways have been described for the IAA synthesis in bacteria from the precursor amino acid and most of them showed similarity with plant IAA expect the production of some intermediate compounds (Spaepen and Vanderleyden, 2011).

Iron is another vital nutrient for almost all forms of life. Almost all microorganisms also essentially require iron for their growth. In the aerobic environment, iron occurs principally as $Fe^{3+}$and form insoluble hydroxides and oxyhydroxides, which make it generally unavailable to both plants and microorganisms (Rajkumar *et al*., 2010). Various bacteria can produce some low molecular mass iron chelating compounds which are known as siderophores that have high affinity for Fe^{3+}. Siderophores act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. Numerous studies associated with plant growth promotion by siderophore-mediated iron uptake as a result of siderophore production by rhizobacterial strains have been reported (Rajkumar *et al*.,2010).

Ethylene is an essential metabolite and plant hormone for the normal plant growth and development (Khalid *et al*. 2006). During various stress conditions generated by drought, water logging, salinity, pathogenic attack, heavy metal etc. level of ethylene increases significantlywhich showed negative effects on overall plant growth (Saleem *et al*., 2007). Potential plant growth promoting rhizobacteria produce an enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which facilitate plant growth and development by decreasing this ethylene levels generated during stressed conditions (Nadeem *et al*., 2007; Zahir *et al*., 2008). Currently, bacterial strains exhibiting ACC deaminase activity have been identified in a wide range of genera whichconvert the ethylene precursor ACCinto 2- oxobutanoate and NH_3 and help in plant growth and the negative effect of ethylene is overcomed.

The direct application of microorganisms which are proven non pathogens and non-harmful isconsidered as an environment-friendly approach and is an accepted modelof biological control to prevent plant diseases, (Lugtenberg and Kamilova, 2009). Thus exploitation of this potential of PGPR strains as biological control agent is a major indirect mechanism of plant growth promotion by rhizobacteria (Glick, 2012). They can act as biocontrol organisms by nutritional competition with pathogen, by inducing systemic resistance or by generating dfferent kinds of antimicrobial metabolites (Lugtenberg and Kamilova, 2009). Many rhizobacteria also produce a diverse array of antifungal compounds like, phenazines, HCN, pyrrolnitrin, pyoluteorin, 2,4-diacetyl phloroglucinol, and viscosinamide (Bhattacharyya and Jha, 2012). Interaction of various rhizobacterial strains with the plant roots can also result in plant resistance against some pathogenic bacteria, fungi, and viruses. This phenomenon is known as induced systemic resistance (ISR) (Lugtenberg and Kamilova,2009).

Biofim formation by Plant growth promoting rhizobacteria

Most of the rhizobacteria associated with plant root systems exercise quorum sensing (QS) signals for their cell to cell aggregation, which ultimately improve the rhizosphere colonization through biofilm formation (Loh *et al*., 2002). In Gram-positive bacteria short-chain polypeptide derivative signals help in mediation of this process, while in Gram-negative bacteria an auto-inducer like molecule or QS signal molecule N-acyl homoserine lactone (AHL) is reportedfor the cell to cell communication (Papenfort and Bassler, 2016, Begum *et al*., 2018). Gray and Garey, (2001) reported the LuxR – LuxI type regulatory system is responsible for the quorum sensing mechanism present in some rhizobacteria. The commensal, mutualistic and pathogenic bacteria similar mechanisms with slight variations are observed for effective colonization in the rhizosphere (Danhorn and Fuqua, 2007; Morris and Monier, 2003).

In the genus *Burkholderia* QS system has been extensively studied. Complex of all these species share a conserved QS system known as CepI/R

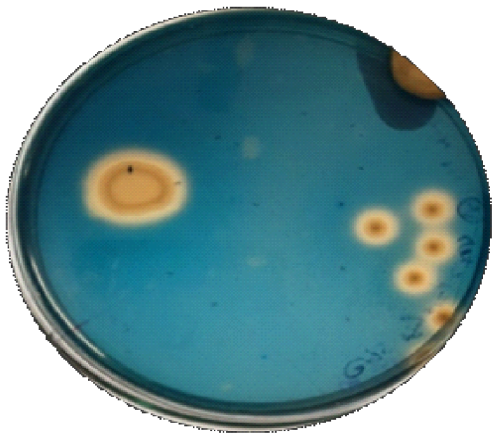

Fig.3. An efficient biofilm forming strain of *Burkholderia* sp. producing high amount of siderphore on CAS agar plate by orange colored halo zones

(Gotschlich *et al*., 2001) that produces and responds to *N*-octanoylhomoserine lactone (C8-AHL). CepI/R Qs system regulates virulence and several important phenotypes like biofilm formation and siderophore production (Fig.3) (Eberl, 2006; Huber *et al*., 2001; Venturi *et al*., 2004). It consists of CepI which synthesizes mainly C8-AHL and the CepR, C8-AHL sensor response regulator. On the other hand Suárez-Moreno *et al*. (2008) reported BraI/R quorum sensing system which produces and respond mainly to C12-3-oxo-AHL and stringently regulated by a repressor RsaL. Later on they have also reported that BraI/R QS system is related to EPS production and biofilm formation by some plant associated *Burkholderia* spp. (Suárez-Morenoet. al., 2010).

The PGPR organisms which have efficient root colonizing ability through biofilm development is beneficial than those strains which do not have the same phenotype (Velmourougan-eetal.,2017).Thus, the QS-based formation of biofilms may function as an important phenotype for the PGPR isolates to excel their highest benefits to the plants. A number of pathogenic prokaryotes are well characterized for their QS-based biofilm formation which includes *Agrobacterium tumefaciens*,*Burkholderia glu-mae*,*Erwinia carotovora*,*Ralstonia solanacearum*, *Xanthomonas* spp. and *Xylella fastidiosa* as reviewed by Ansari and Ahmad (2018), Sibanda *et al*.(2018).On the contrary little is known on communication signal for biofilm formation for *Ensifer* spp., *Rhizobium* spp.,*Sinorhizobium meliloti, Azotobacter vinelandi* and *Azospirillum lipoferum.*

Yasmeen *et al*.(2020) has shown that two halotolerant PGPR strains viz., *Bacillus licheniformis* AP6 and *Pseudomonas plecoglossicida*PB5 with could help in growth of the sunflower in saline soil. They have also observed that this ability of the strains is achieved through biofilm formation. Both the strains were found very effective in generating antioxidant functions through upregulation of different enzyme functions like catalase superoxide dismutase and guaiacol peroxidase. Their ability to solubilize insoluble phosphate, produce phytohormones and ACC deaminase activity were found positively correlated with biofilm formation.

A biofilm can be defined as an assemblage of surface associated microbial cells embedded in an extracellular organic polymeric matrix of microbial origin. For bacteria, there are several advantages of biofilm formations. One of the important features of most of the biofilm forming bacteria is the production of extracellular polymeric substances (EPS) which consist larger of polysaccharide and smaller amount of protein and DNA. The EPS molecules are considered as the major factors which influence biofilm formation by microorganisms (Czaczyk and Myszka, 2007). The extensive production of EPS occurs during the specific adhesion stage of biofilm formation. EPS are also responsible for the architecture and morphology of the biofilm matrix (Mattos- Guaraldia *et al*., 2000; Langille *et al*., 2000).The concentration of the QS-signalingmolecule increasesalongside the bacterial population density and, when it reaches a significant level, bacteria respond and modulate target gene expression.

During solubilization of insoluble P, biofilm formation by the bacterial isolates probably create a close environment that help better solubilization of phosphate granules by organic acids secreted by the isolates. Mukhopadhyay *et al*. (2010) also observed biofilm formation by *B. cenocepacia* C11 on JRP granules during solubilization. Comparison between the extents of biofilm formation on different rock phosphate granules reveled maximum biofilm formation by all the isolates on MRP granules. It has been reported earlier that phosphate content in MRP was lowest in comparison to other rock phosphates (Pal, 2006). By assuming the role of available P on the extent of biofilm formation in *Burkholderia* spp., quantitative approaches were taken where biofilm formation by the isolates were quantified using crystal violet staining technique in the presence of different concentrations of soluble P (K_2HPO_4) (Ghosh *et al*., 2016, Ghosh *et al*., 2019). Measurement of optical densities of crystal violet solutions indicated maximum biofilm formation by isolates of *Burkholderia* spp. in P limitation conditions. These outcomes suggested that the P solubilizing bacterial isolates attached to the surfaces of insoluble P granules and release more soluble P to overcome the stress generated by P-limitation (Ghosh *et al*., 2019). It was also found that phosphorous limiting condition enhances the biofilm formation of plant pathogenic *Agrobacterium tumefaciens* (Danhorn *et al*., 2004). Increase of planktonic cell densities along with increased concentrations of K_2HPO_4 also supported the positive correlation between P

limitation and increase of bacterial biofilm.The biosynthesis of EPS serves many functions like promotion of bacterial attachments to solid surfaces and formation as well as maintenance of microcolony and mature biofilm structure (Czaczyk and Myszka, 2007). Increase of EPS along with biofilm production by bacterial isolates strongly indicated their direct involvement in biofilm formation during P limitation condition. Maximum EPS production in the presence of MRP was observed by Ghosh *et al.* (2016) in *Burkholderia*. This probably directs the bacterial organisms to produce more condensed biofilm structures to release enough quantity of soluble P from MRP. Presence of higher concentration of available P from the rock phosphates may create less stress, thus reduce EPS production and subsequently biofilm formation. This leads to again reduction of solubilization and a homoeostasis is maintained for phosphate solubilization. Highest degrees of EPS production in the presence of lowest concentration of K_2HPO_4 also suggested its strong correlation with available phosphate. Involvement of EPS production in biofilm formation have been reported by several workers for various bacterial species (Czaczyk and Myszka, 2007). Yi *et al*. (2008) found four bacterial strains under the genus *Enterobacter*, *Arthobacter*and *Azotobcter* which were able to produce significant amount of EPS and showed strong abilities for TCP solubilization.

AHL dependent quorum sensing system plays significant role in biofilm formation in various Gram negative bacterial organisms. Ghosh *et al*.(2019) reported in their study that in *B. tropica* P4 and *B. unamae* P9 presence of BraI/R QS system was detected. As the primer sets were effective to amplify the *braR*and *rsaL*gene from 20 species of *Burkholderia* (Suarez and Moreno *et al*., 2008) therefore they were used to detect the presence of BraI/R QS system in isolated bacterial strains. Suarez-Moreno *et al*. (2010) also reported that biofilm formation and EPS productions were regulated by BraI/R QS system in some plant associated *Burkholderia* spp. On the other hand no amplification was detected for *cep*loci of *B. cepacia*10 using the above mentioned primers. Ghosh *et al.* (2019) have also pointed out that biofilm formation and EPS production by *B. tropica* P4 as well as *B. unamae* P9 was highly correlated with available P in the medium. So there may be some positive relation of this QS system which regulates the biofilm formation or EPS production during P solubilization by the isolates.

The biofilm helps in protecting microbial communities inside and maintain a homeostasis from the available antibiotic like substances in the rhizosphere environment. This film can prevent the influx of the undesired agents and restrict their diffusion inside. The exopolysaccharide produced by the efficient biofilm forming PGPR has tremendous power to sequester toxic metal ions and thus provide protective function to themselves as well as the plants.

The mature biofilm in many cases contain functional spaces and water channels which provide an increased surface area for the exchange of mineral nutrion. As the water channels are interconnected and travel deep inside the film, it ensures nutrition and even the trace elements required for the plant's growth in a symbiotic rhizobacterial system. (Stoodley *et al*., 1998). The complex biofilm architecture also creates an opportunity to sharing of metabolic requirements among the symbionts at various environments (Davey and Ó Toole, 2000).Biofilms in PGPR also help in overcoming salinity stress in sunflower plants through antioxidative pathways (Yasmeen *et al*., 2020).Harrison and Buckling (2009) had also observed that there is a strong correlation between siderophore formation and biofilm development in human pathogenic *Pseudomonas aeruginosa*. It is evident from their study that in the clones defective in their genes for iron scavenging through mutation leads to stoppage of siderophore formation and the clones were deficient in biofilm development. This has also been found in many PGPR where siderophore production is crucial in their functioning to help the crop plants and efficient biofilm forming strains are found to be good siderophore producers. A very good correlation is observed between biofilm and siderophore formation by Kalam *et al*., (2020). Biofilms with the help of EPS made many apparently impossible things into possible. The best example is cited in nature by many aerobic nitrogen fixers and under experimental conditions in a strain of *Pseudomonas stutzeri*A1501 which induces biofilm formation during depletion of provided nitrogen and could fix atmospheric nitrogen very efficiently (Wang *et al*., 2017).

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