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Functional metagenomics of phosphate solubilising microorganisms in sustainable wheat production –A review

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ABSTRACT

Phosphorus being one of the major nutrients required for the plant growth and development is available in very little proportions to the plant. In order to overcome the deficiency of phosphorus, it is added externally to the soil in the form of chemical phosphorus fertilizers. To overcome the potential problems caused by the use of chemical fertilizers, phosphate solubilizing microorganisms can be an alternative for sustainable agriculture. Wheat is an important staple in our dietary requirements. Therefore, the production of wheat through sustainable agriculture techniques must be undertaken. Very little information is available about the phosphate solubilizing microorganisms having potential in increasing wheat production. This is possibly due to the lack of knowledge about their cultural characteristics and also only few percentages of them are cultivable in the laboratory. To find out the potent phosphate solubilizing microorganisms from the wheat rhizosphere functional metagenomics can be a useful tool. With this technique we can directly isolate the environmental DNA and then clone it in suitable vector, introduce it into appropriate host and then study its phosphate solubilizing capacities. Through this technique, one can easily identify phosphate solubilizing microorganisms directly from their environment without using traditional isolation procedures. Functional metagenomics can be a useful tool to reveal the potent phosphate solubilizing microorganisms found in the wheat rhizosphere which are still unknown. Thus, metagenomics is a very helpful technology that can help to understand the microbial ecology and the functions associated with them can be studied by functional metagenomics.

Keywords: Phosphate solubilizing microorganisms, Wheat, rhizosphere, Functional metagenomics, sustainable agriculture.

INTRODUCTION

Phosphorus being one of the major nutrients available to the plant have very little mobility in the soil. Therefore, it is available in limited proportions (Khan *et al.*, 2007b). In order to overcome the problem of phosphorus deficiency and uptake, the use of phosphate solubilizing microorganisms can

prevail to be an efficient way for sustainable agriculture. Phosphorus accounts for about 0.2-0.8% of the plants dry weight (Sharma *et al*., 2013). In matter of world's production, wheat amongst the cereals ranks third after rice and maize, give about 35% of the total food production. Wheat has a very high nutritional value and hence it is considered to be one of the important dietary constituents. The second largest wheat producing country is India with 11.9% production out of 12% of total area under cultivation. Phosphorus is nutritionally important to plants for the development of root, improving the strength of stalk and stem, proper development of flower and seed, N-fixation in legumes, improving quality of crops and providing resistance to the plant from phytopathogens ((Mohammadi, 2012), Agrifacts., 2013, (Sharma *et al*., 2013)). In wheat, phosphate solubilizing microorganisms plays a very important role in increasing straw strength and fruit production, better tilling in wheat, early maturation of plant and seed (Sial et al., 2017). Deficiency of phosphorus causes reduction in plant sizes and growth (Sharma *et al*., 2013). Bacterial populations of wheat rhizosphere are mainly studied by cultural techniques. Only small proportions of these microbes are cultivable in laboratory. Thus, molecular methods can prove to be a useful tool to gain insight regarding the actual populations of bacteria that lies in the environment (Velázquez *et al*., 2012). Inoculation of soil or crop with phosphate solubilising/mineralizing microorganisms is therefore a promising strategy for the improvement of absorption of phosphorus by the plants, which ultimately will lead to a reduction of chemical fertilizers who have led to a negative impact on the environment (Alori *et al*., 2017).

URGENT NEED FOR PSM:

Rapid increase in human population around the world has laid an emphasis for an increase in food production by more than 50 % in the next 20 years. This cannot be achieved through conventional agricultural practices because the use of chemical fertilizers has reached to its maximum peak (Khan *et al*., 2007a). Moreover, the production of chemical Phosphate fertilizers is a very energy conserving and cost-effective procedure. A second green revolution in the world is the need of the day in order for the food production to increase in next 20 years to sustain the increased population (Gyaneshwar *et al*., 2002).Therefore, there is an urgent need to replace the chemical fertilizers by using phosphate solubilising microorganisms to solubilise the available phosphorus in the soil. Microbial population can drain ample amount of nutrients from the natural repository making it available to the soil. Crop microbial ecosystem can thus be efficiently used for employing sustainable agriculture (Khan *et al*., 2007a). Wheat farmers face a huge problem in wheat cultivation for increasing the yield of the crop. One of the major advantages of applying phosphate solubilizing microorganisms to the wheat crop is that it can better colonize and establish in the rhizosphere and makes phosphorus available to the plants(Khan *et al*., 2017). To comply with phosphorus requirement of the plants, phosphorus is added in the form of chemical fertilizer. Out of this added phosphorus fertilizer; most of the phosphorus is precipitated by metal-cation complexes and becomes fixed in soils (Sharma *et al*., 2013). Microbial inoculants can thus be an alternative to chemical fertilizers because they can act as phytostimulants, biofertilizer or microbial biocontrol agents. They have the efficiency to facilitate plant growth and minimize the effect of loss top-soil, soil infertility, poor plant growth, low yield index and insufficient diversity of indigenous microbes (Babalola & Glick, 2012).

BIODIVERSITY OF PHOSPHORUS SOLUBILIZERS:

A significant number of microbial species exhibit phosphorus solubilisation capability, which includes bacteria, fungi, actinomycetes and even algae (Sharma *et al*., 2013) as shown in table 1.1 Bacteria have more efficiency to solubilise phosphorus as compared to fungi. High concentrations of phosphate solubilizing microorganisms are concentrated in the rhizosphere and they are metabolically more active than from other sources. Usually, one gram of fertile soil contains 10^1 to 10^{10} bacteria, and their live weight may exceed 2,000kg /ha (Sharma *et al*., 2017).

MECHANISM OF PSM:

Phosphate solubilizer promotes wheat growth through solubilisation of insoluble phosphorus. The soluble phosphorus is then taken up as a nutrient element by plants. Besides making soluble phosphorus available to plants, the phosphate solubilizing microorganisms also secretes some important active biomolecules that directly or indirectly enhance the growth and productivity of crop plants. Phosphorus solubilization mechanisms employed by soil microorganisms include:

Release of mineral dissolving compounds:
Organic acid (Mohammadi, 2012), siderophores, protons hydroxyl ions, CO_2 (Sharma *et al*., 2013), Indole Acetic Acid, and cyanogenic compounds plays an important role in phosphate solubilisation (Khan *et al*., 2017). Organic acid production is considered as the chief mechanism for mineral phosphate solubilisation in bacteria. This was presumed by cloning of two genes involved in gluconic acid production: pyrroloquinoline quinone (PQQ) synthase and gabY gene. Gluconic acid is the main organic acid produced by *Pseudomonas spp.*, *Erwinia herbicola*, *Pseudomonas cepacia* and *Burkholderia cepacia. Rhizobium leguminosarum, Rhizobium meliloti* and *Bacillus firmus* produce noticeable amounts of 2-ketogluconic acid. Other organic acids, such as lactic, isovaleric, isobutyric, acetic, glycolic, oxalic, malonic and succinic acids are also generated by different phosphate-solubilising bacteria (Igual *et al*., 2001).

Liberation of extracellular enzymes such as 1-Aminocylclopropane-1-carboxylate (ACC) deaminase :
It is an important growth regulator of plants that induces metabolic changes and hence increases the growth of plants directly by hindering or reducing ethylene secretion (Khan *et al*., 2017).

Release of phosphorus substrate degradation:
Microorganisms plays an important role in soil phosphorus cycle by dissolution-precipitation, sorption-desorption, mineralization-immobilization(Sharma *et al*., 2013).

Organic P Solubilization:
It is also called mineralization. It occurs with the help of enzymes.
Nonspecific Acid Phosphatases: It dephosphorylates phosphoester or phosphoanhydride bonds of organic matter.

Phytases: It causes release of phosphorus by phytate degradation.

Phosphonotase and C-P lyases: Cleaves C-P bond of organo phosphonates (Sharma *et al*., 2013).

Inorganic P Solubilization:
Many saprophytic bacteria and fungi acts on sparingly soluble soil phosphates, mainly by chelation method of phosphate solubilization. Organic and inorganic acids such as gluconic and ketogluconic acid secreted by phosphate solubilizing bacteria in which hydroxyl and carboxyl groups of acids chelate the cations (Al, Fe, Ca) and decrease the pH in basic soils (Sharma *et al*., 2017).

Table 1.1 Phosphate Solubilizing Microorganisms

Microorganism		REFERENCE
BACTERIA	*Pseudomonas spp., Agrobacterium spp., Bacillus circulans*	Babalola et al., 2012
	Azotobacter	Kumar *et al*., 2014
	Burkholderia	Zhao *et al*., 2014
	Bacillus subtilis, Serratia marcescens	Mohamed *et al*., 2018
	Enterobacter, Erwinia	Chakraborty *et al*., 2009
	Kushneria	Zhu *et al*., 2011
	Rhizobium spp., Bacillus megaterium, Bacillus circulans, Bacillus subtilis, Bacillus polymyxa, Bacillus sircalmous, Pseudomonas straita	Mohammadi., 2012 Egamberdiyeva *et al*., 2004
FUNGI	*Achrothcium, Alternaria, Arthrobotrys, Aspergillus, Cephalosporium, Cladosporium, Curvularia, Cunninghamella, Chaetomium, Fusarium, Glomus, Helminthosporium, Micromonospora, Mortierella, Myrothecium, Oidiodendron, Paecilomyces, Penicillium, Phoma, Pichia fermentans, Populospora, Pythium, Rhizoctonia, Rhizopus, Saccharomyces, Schizosaccharomyces, Schwanniomyces, Sclerotium Torula, Trichoderma, and Yarrowia*	Srinivasanetal.,2012; Sharma *et al*.,2013, Mohammadi, 2012, Pradhan *et al*., 2005
ACTINOMYCETES	*Micromonospora and Streptomyces.*	Sharma *et al*.,2013
ALGAE	*Cyanobacteria*	Sharma *et al*.,2013

EFFECT OF PSM ON CROP WHEAT CROP PRODUCTION:

Use of phosphate solubilizing microorganisms can increase crop yields up to 70%. Significant increase in growth traits and yields of wheat were observed by using *Pseudomonas fluorescens* (Zia-ul-hassan *et al*., 2015). *Pseudomonas spp*.Isolated from wheat has significantly enhanced seedling growth in the terms of shoot and root length, shoot and root dry weight and nutrient contents (%N,%P,%K) as compared to the control (Sarker *et al*., 2014). Effects of phosphorus-solubilising bacteria like *Bacillus megaterium var. phosphaticum* [M13] and nitrogen-fixing bacteria like *Stenotrophomonas maltophilia* and *Ralstonia pickettii* were observed on the quality of wheat yield against the chemical fertilizer treatments used as control. The yield of wheat and phosphorus content in plants is significantly improved by the use of *Penicillium oxalicum* (Singh *et al*., 2011). Seed inoculation of wheat varieties with phosphate solubilising and phytohormone producing *Azotobacter chroococum* showed a higher increase in yield traits of grain and straw (Narula N, Kumar V, 2005). Combined0 use of phosphate solubilising microorganism *Pseudomonas fluorescens* BAM-4 and *Burkholderia cepacia* BAM-12 and arbuscular mycorrhizal fungus, *Glomus etunicatum* significantly increased growth, yield and nutrient uptake in wheat plant (Minaxi SJ, Chandra S, 2013). Combined inoculation of arbuscular mycorrhiza and phosphate solubilizing bacteria give better uptake of both native phosphorus from the soil and phosphorus coming from the Phosphatic rock (Mohammadi, 2012). The combined effect of bioorganic phosphate with phosphorus solubilising bacterial strain *Bacillus* MWT-14 on the growth and productivity of wheat was tested. The results significantly showed increase in growth yield as compared to control (Tahir *et al*., 2018).Mutant strains of *Azotobacter chroococcum* showed higher increase in grain (12.6%) and straw (11.4%) yield over control and their survival (12–14%) in the rhizosphere as compared to their parent soil isolate (P4) (Kumar *et al*., 2001).

Functional metagenomics in mineral phosphate solubilization:

There is a large gap between the current average global wheat yields and that achievable through best agronomic management and crop genetics. It is expected that this gap can be reduced by manipulating soil processes especially those that involve microbial ecology. Development of prognostic understanding of the link between soil biology and agronomy and crop performance would be a first step towards improving crop productivity of intensive cereals and would have a major impact of global food production (Donn *et al*., 2015). Culture dependent procedures have been over looked, having a vast majority of naturally occurring bacterial metabolites for obtaining high yield of wheat crop. Even though most bacteria are not readily cultivable in the laboratory, it is possible to extract microbial DNA directly from the naturally occurring consortia of bacteria present in environment samples. The bacterial populations that inhabit the rhizosphere of wheat plants have mainly been studied by culture methods. However, only a small proportion of these populations can be grown In laboratory conditions.

Molecular methods can give a better indication of the actual population of bacteria that lies in the environment (Velázquez *et al*., 2012). In many studies, the genes involved in mineral phosphate solubilisation (MPS) activity from bacterial isolates were identified by cloning and complementation of mineral phosphate solubilisation traits in bacterial host system such as *Escherichia coli,* which can be a useful host for studying microbial phosphate solubilisation. This is because *Escherichia coli* is mineral phosphate solubilisation (MPS) negative. It does not synthesize holo-Glucose dehydrogenase and is therefore incapable of initiating direct oxidation pathway and therefore cannot produce gluconic acid necessary for phosphate solubilisation (Babu-Khan *et al*., 1995). Also in a study conducted , it was found that *Escherichia coli* lacks the necessary genes required for P solubilisation activity such as pyroquinoline quinone (PQQ) synthesis (Chhabra *et al*., 2013). *Escherichia coli* is capable of synthesizing the Apo-glucose dehydrogenase enzyme (GDH) but not the cofactor pyrroloquinoline quinone (PQQ), which is essential for formation of the holoenzyme.

Therefore, in the absence of exogenous pyrroloquinoline quinone (PQQ), *Escherichia coli* does not produce gluconic acid (Liu *et al*., 1992).The exact genetic mechanism for mineral phosphate solubilisation (MPS) activity is not yet completely known (Rodriguez and Fraga 1999). However it is known that gluconic acid (GA) and 2-ketogluconic acid (2-KGA) biosynthesis in the periplasm of bacteria (direct oxidation pathway) can be an important basis for the mineral phosphate solubilisation (MPS) activity in many gram-negative

bacteria. Gluconic acid (GA) biosynthesis is commonly carried out by the enzyme glucose dehydrogenase in the presence of the co-factor pyrroloquinoline quinone (PQQ). Alternatively, genes involved in gluconic acid production have also been identified for example: a gabY gene cloned from *Pseudomonas cepacia* in *Escherichia coli* was shown to be involved in mineral phosphate solubilization (MPS) activity. The deduced amino acid sequence of this gene was shown to have no similarity to the commonly known gluconic acid biosynthesis genes pyrroloquinoline quinone (PQQ) OR glucose dehydrogenase (GHD) but showed homology to histidine permease membrane bound components (Babu-Khan *et al*., 1995). Also a DNA fragment from *Serretia marcescens* that induce GA synthesis in *Escherichia coli* was identified which showed no homology to pyrroloquinoline quinone (PQQ) or glucose dehydrogenase (GHD) genes (Chhabra *et al*., 2013; Krishnaraj, P.U. and Goldstein, 2001).

CLONING AND ISOLATION OF PHOSPHATE SOLUBILISING MICROORGANISMS DIRECTLY FROM SOIL SAMPLE:

The environmental DNA needs to be first isolated from the soil of Rhizospheric root region soil of wheat crop. The isolation of environmental DNA can be carried out by using Brady S F 2007 protocol (Brady, 2007).. In this protocol DNA is extracted directly from environmental samples and screening these clones for the phosphate solubilising potential. DNA is extracted directly from environmental samples by heating in the presence of a strong detergent and the free high molecular weight environmental DNA is then gel purified. This purified high molecular weight environmental DNA is blunt ended, ligated into a vector, packaged into a lambda phage and transformed into *Escherichia coli* (Brady, 2007). The cloned bacteria capable of persistent and high phosphate solubilisation can be identified by phenotypic characterization or genotypic characterization. Phenotypic characterization relies mainly on numerous biochemical tests on solid medium such as Pikovaskaya's solid medium to analyse phosphate solubilisation qualitatively and quantitative analysis of phosphate solubilisation can be done in liquid medium such as NBRIP (National Botanical Research Institute's growth medium) and then testing it colorimetrically(Igual *et al*., 2001) .

CLONING OF GENES INVOLVED IN MINERAL PHOSPHATE SOLUBILISATION:

In a study conducted by Goldstein *et al* 1987 cloning of a gene involved in mineral phosphate solubilization from the Gram negative bacteria *Erwinia herbicola* was done in *Escherichia coli* HB101. Observation showed that the expression of the gene allowed production of gluconic acid in Escherichia coli and was found to solubilize hydroxyapatite(Goldstein a h, 1987).

In a study it was demonstrated that 1.8 kb locus of *Erwinia herbicola* encodes a protein similar to the gene III product of a pyrroloquinoline quinone (pqq) synthesis genes complex from *Acinetobacter calcoaacetium* and pqqE (Coenzyme PQQ synthesis protein E) of *Klebesiella pneumoniae* (Liu *et al*., 1992).

Study was done in *Rahnella aquatilis* where a 7.0 kb fragment was transcomplemented to *Escherichia coli* HB101 and *Escherichia coli* DH5-alpha. The results conferred the ability to solubilize hydroxylapatite and the production of gluconic acid to *Escherichia coli*, which is unable to produce gluconic acid on its own. Further nucleotide sequence analysis revealed two complex open reading frames (ORF1 and ORF2) and a partial ORF. ORF1 and ORF2 encoded proteins of molecular mass 10 kDA and 44 kDA respectively. The 44 kDA protein showed extensive similarity to pqqE (Coenzyme PQQ synthesis protein E) of *Erwinia herbicola* and *Acinetobacter calcoacetium* (Kim *et al*., 1998).

With the help of restriction base cloning, it was found that a DNA fragment from *Serretia marcescens* induces gluconic acid synthesis but showed no homology to pyrroloquinoline quinone (pqq) or glucose dehydrogenase (GHD) gene. They suggested that this gene acted by regulating gluconic acid production under cell signal effect(Krishnaraj *et al*., 2001).In one study, it was showed that genes responsible for conferring mineral phosphate solubilization were cloned from *Synechococcus* PCC 7942, a unicellular cyanobacterium, which itself does not show mineral phosphate solubilization activity. In Gram negative bacterium, genes encoding pyrroloquinoline quinone (PQQ) biosynthetic protein and glucose dehydrogenase gluconic acid are mainly responsible for mineral phosphate solubilization activity. However, in some cases other genes which either influence the synthesis of pyrroloquinoline

quinone (PQQ) cofactor or complement the glucose dehydrogenase directly are responsible for mineral phosphate solubilization phenotype (Gyaneshwar *et al*., 1998).

FUTURE ASPECTS:

Despite their ecological habitats and their phosphate solubilising capabilities, still a lot needs to be explored in case of PSM. Current developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious applications, are likely to facilitate their use as reliable components in the management of sustainable agricultural systems. Although significant studies related to PSM and their role in sustainable agriculture have been done over the last few decades, the required technique still remains in its infancy. Nevertheless, with an awareness of the limitations of existing methods, a reassessment can be expected, so that the use of PSM as potential biofertilisers in different soil conditions becomes a reality. Efforts should be made to use microorganisms such as PSM to reduce the applications of pesticides. Biotechnological and molecular biological tools must be imposed to develop a single efficient strain of PSM that can perform dual function of phosphate solubilisation as well as plant growth and its protection. To meet the increasing food demands new and advanced methods must be used for the enhancement in the phosphate solubilising activity by the microorganisms.

CONCLUSION:

Phosphate solubilising microorganisms opens up a new horizon in sustainable agriculture. This can be efficiently achieved by using approaches such as functional metagenomics. Through this we can explore novel isolates capable of solubilising phosphate, thereby increasing phosphate uptake of the plant. New metabolic pathways can be explored with the help of the metagenomics study. This could definitely reach us towards better crop cultivation and decreasing the use of chemical fertilizer.

Competing interests

Authors have declared that no competing interests exist.

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