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Comprehensive Review on the Role of Microbial Phosphate Solubilization in Citrus crops

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Abstract

A detail analysis of the microbial diversity in citrus crops is presented in this article, the main growth limiting nutrient is phosphorus, and dissimilar nitrogenand it cannot be made naturally available from a large atmospheric source. The characteristics linked to phosphorus nutrition include root development, stalk growth and steam strength enhancements, flower & seed development, crop ripeness and production fixation in legumes, crop quality, and showing the resistance to plant diseases. Rhizobacteria from genera including *Alcaligenes, Acinetobacter, Arthrobacter, Azospirillum, Bacillus, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Paenibacillus, Pseudomonas, Rhizobium,* and *Serratia* are among the strains that stimulate plant growth in the crop field. They are employed as control agents or biofertilizers to enhance agriculture, and many researchers are working to save the agricultural environment. Extensive biogeographic sampling the physical and purposeful makeup of the citrus rhizosphere microbiome has been better understood thanks to the shotgun metagenomic technique. The suggestions to enhance fertilization, disease and pest management programs will be made easier with an understanding of the extent to which beneficial bacteria react to grow the citrus crop using the significance of PSM. The quest for biopesticides and active biofertilizers that agrochemical businesses may develop into new green technologies will be focused by these research activities.

Keywords: Phosphate Solubilization, Citrus Crop, Soil Fertility and PSM)

Introduction

The phosphate is one of the important macronutrients required for plant growth and development is phosphate. According to T. Karpagan and P.K. Nagalakshmi (2014), a large number of microorganisms in the rhizosphere are known for solubilization and make the insoluble phosphorus available to the plants in the form that they need. Phosphorus (P) is one of the important growth-limiting macronutrients required for proper plant growth, particularly in tropical areas, due to its low availability in the soil (Santana E. B., 2016). It accounts for between 0.2 and 0.8% of the dry weight of plants and it is contained within nucleic acids, enzymes, coenzymes, nucleotides, and phospholipids.

Phosphorus is essential in every aspect of plant growth and development, from the molecular level to many physiological and biochemical plant activities including photosynthesis (development of roots, strengthening the stalks and stems, formation of flowers and seeds, crop maturity and quality of crop, energy production, storage and transfer reactions, root growth, cell division and enlargement, N fixation in legumes, resistance to plant diseases (Sharma S. B 2013, Walpola B. C. 2013), transformation of sugar to starch, and transporting of the genetic traits. The molecular level to numerous physiological and biochemical plant processes, such as photosynthesis (root development, stem and stalk strengthening, flower and seed formation, crop maturity and quality), energy production, storage and transfer reactions, root growth, cell division and enlargement, N fixation in legumes, resistance to plant diseases, conversion of sugar to starch, and transport of genetic traits, phosphorus is necessary for all aspects of plant growth and development.

Obtainability of P' in the soil

For the primordia of plant reproductive organs to be laid down in the early stages of plant development, enough P obtainability is also necessary. It is essential for the proper operation of critical enzymes that control the metabolic pathways. About 98% of Indian soils are expected to have insufficient amounts of available phosphorus, which is necessary for determining plant growth.

Only a small portion of the phosphorus that is actually provided as phosphate fertilizer is absorbed by the plant. Widespread phosphate deficiencies need the use of phosphorus fertilizers in order to sustain crop productivity. Only a little amount of it is used by plants when it is applied to the soil as phosphate fertilizers (Padmavathi Tallapragada, 2010). A significant number of bacterial species have the capacity to positively impact plant growth. The term "rhizobacteria" refers to the fact that they are primarily found in the rhizosphere of plants. Phosphorus is the mineral nutrient that most frequently restricts crop growth, second only to nitrogen.

The synthesis of various organic acids is the main mechanism for the solubilization of mineral phosphate, and acid phosphatases are important for the mineralization of organic phosphorus in soil. The action of organic acids produced by soil microbes is widely acknowledged to be the primary mechanism of mineral phosphate solubilization. When organic acids are produced, the microbial cell and its environment become more acidic. Generally the bacteria that solubilize phosphate can produce organic acids.

Many chelating substances and inorganic acids such as sulphideric acid, carbonic acid and nitric acid are considered as other chemical mechanisms for phosphate solubilization. Nevertheless the efficiency and their role to phosphate molecules release in soils seem to be less than organic acid production.

Various screening techniques have made it feasible to identify bacteria and estimate their capacity to solubilize phosphate. Clearing zones are created all over the place in the form of microbial colonies in media by phosphate solubilizers. The media contain phosphates in insoluble mineral form such tri-calcium phosphate molecules.

In addition, the bromo-phenol blue approach, which creates yellow halos after a PH drop by releasing organic acids, is more correlated and reproducible than the straightforward halo method. Nonetheless, the clearing zones on agar plate technique are frequently employed. Phosphate solubilizers selection can be done generally using Pikovskays' medium. Nonetheless, the clearing zone on agar plate technique is frequently employed. Phosphate solubilizer selection can be done generally using Pikovskays' medium.

Diversity of PSM in citrus crop rhizosphere

Increased plant growth and harvest are typically the outcomes of PSM inoculation, especially in glasshouse settings. More importantly, studies employing maize and wheat plants at the field level have demonstrated that PSMs could greatly reduce the use of organic or chemical enrichers.

Rock phosphate Tri-calcium phosphate, di-calcium phosphate and, hydroxyapatite, are the examples of insoluble inorganic phosphate compounds that have been shown in numerous examples to be soluble by various bacterial species (Goldstein, 1986). Two fungal isolates that have been shown to have the capacity to solubilize phosphate are *Aspergiluus and Penicillium.* According to T. Karpagam and P.K. Nagalaxmi (2014), *Psudomonas Azospirillum, Bacillus, Ranzobiumburkholderia, Orthobacter, Alcaligenes, Serratia, Enterobactor, Acinetobacter Flavobacterium, and Erwinia* are among the bacterial genera that possess this ability. The bacteriome exhibited more taxonomic richness than the mycobiome, and the majority of citrus trees' microbial biodiversity was found in the plant rhizosphere (Blaustein *et al*. 2017; Ginnan *et al*, 2020).

Beta diversity plots showed that the microbial composition of those groups differed significantly between the above and below ground compartments, despite the fact that Ascomycota fungi and Proteobacteria dominated the colonization of trees across all compartments (Trivedi *et al*., 2010; Passera *et al*., 2018 Xu *et. al*, 2018 Bai *et al*., 2019).

The flush and flower have little microbial diversity, with *Ascomycota* and *Proteobacteria* accounting for 80% of the relative microbial abundance in those tissues. By contrast, the microbial assemblage below ground was more diverse, particularly for bacteria, and comprised a variety of taxonomic groupings from various bacterial phyla.

A few of species (3.4%) were able to colonize both above-ground and below-ground habitats, according to the findings. The core taxa of the citrus holobiont were characterized as genera that were present in at least 50% of our samples, had a relative abundance of at least 1%, and had ASVs that could colonize at least one bio compartment above and below ground. The fungi *Alternaria spp., Cladosporium spp., Fusarium spp. (syn. Fusicolla, Gibberella, Neocosmospora), Mycosphaerella spp., Sigarispora spp., and Symmetrospora, as well as the bacteria Actinopianes spp., Bacillus spp., Burkholderia spp, Firmicutes spp., Massilia, Mesorhizobium spp., Pseudomonas spp., Sphingomonas, and Streptomyces spp.,* were identified as core members of the citrus holobiont based on these criteria. That list might be reduced, though, by more sampling from orchards situated in various citriculture regions. Many of these bacterial species are known to enhance plant development, operate as biocontrol agents, and give the host an advantage in terms of fitness (Lemanceau *et al*., 2017; Xu *et al*., 2018).

But the function of fungal taxa is still unclear. Our dataset included representatives of several genera (*Fusarium, Giberella, Fusicolla, and Neocosmospora*) that belong to the *Fusarium* species complex. These organisms have a variety of behaviors, such as parasitism, mutualism, and commensalism (Crous*et al*., 2021).

Although *Fusarium solani*, for instance, is a recognized citrus pathogen that causes wood dry rot (Sandoval-Denis *et al*., 2018), it is unknown how other species in the Fusarium complex live. Other citrus holobiont fungal taxa, including *Alternaria alternate spp., A. arborescens spp., and Mycosphaereilacitri spp*, have also been seen to cause fruit blemishes (Mondal *et al*., 2003, Wang *et al*., 2021). *Sigarispora* and *Symmetrospora*, on the other hand, have been discovered in a variety of environments but have no recognized roles in citrus. It has been demonstrated that only *Cladosporium cladosporioides* inhibits *Liberibacter crescens*, a cultural surrogate of CLas, and may offer some advantages to the host (Blacutt *et. al*., 2020).

Identifying the fungus species linked to citrus will be made easier with deeper amplicon-based sequencing, which could also reveal some details about their lifestyle.

The geographic distribution and functional characteristics of the key fungal taxa within the citrus holobiont will be clarified by large-scale sampling in conjunction with omics technologies; however, the availability of reference genomes will still limit this strategy (Xu *et al*., 2018).

Citrus crop in central India

Orange production in Maharashtra state is concentrated in the districts of Nagpur and Amravati in the Vidarbha region, which is known as the California of Maharashtra state. At 15,205 hectares, Nagpur district has the largest area, production, and productivity, earning it the moniker "orange city." The district produces 1,35,613 tons of oranges annually.

In addition to its significant position in the domestic market, oranges are also found in the global market (Nighot *et al*. 1984; Sapate 1993). With broader principles, greater potential, and comprehensive effort required to boost orange production in the nation with significant export potential, it has taken on a definite significance (Bhende, 1965; Gangawar and Singh, 1998; Gangawar *et al*., 2005; Gupta and George, 1974; Ingley, 1983).

According to Ropan Bante *et al*. (2015), several agencies acknowledged the pressing need to increase Nagpur orange output due to the growing demand for the oranges for both domestic and foreign markets. Manufacturing of Inoculants to create inoculants, microorganisms that have the capacity to stimulate plant development have been employed. When producing inoculants, potential components that can promote healthy bacterial growth and survival are required. Various coals, bentonite, maize oil, mineral soils, peat, peat moss, vermiculite, and perlite are among the many materials that have been assessed.

A typical substance for inoculant carriers is peat. Conventional inoculant production is the most typical usage of finely ground peat. However, peat is not always accessible because some types of peat can prevent certain strains of Rhizobium from growing. The challenge of acquiring autoclaved or gamma-irradiated peat as carriers has been reported. Toxic chemicals for bacteria may be produced by the high temperature during steam sterilization or the high dosage required for irradiation.

Microbes play a crucial role in the phosphorus cycle that occurs naturally. Phosphate solubilizing microorganisms (PSMs) have long been the focus of research on their potential as biofertilizers to improve agriculture. The purpose of this paper is to give a brief overview of soil P availability and PSM variety, P solubilization mechanisms, PSM's ability to promote plant growth, and their potential use as biofertilizer in crop production.

Material and Methods

Search the Literature. Using a web-based search engine, we searched Pubmed, Google and Google Scholar. Web of Science, and Scopus for systematic review articles that compared advances data. We used the search terms microbial diversity, PSM, sustainable agriculture, Biofertilizers and bio pesticides. We manually searched the references to the mentioned publications for more relevant research. To include unpublished papers, keywords were used to scan abstracts from 2000 to 2024.

Examine the literature. A web-based search engine was used to look up Pubmed and Google Scholar. Scopus and Web of Science for English-language systematic review publications that contrasted data on advancements. We searched using the following terms: sustainable agriculture, PSM, microbiological diversity, biofertilizers, and biopesticides. We looked for further pertinent research by hand through the cited articles' references. In order to incorporate unpublished publications, abstracts upto 2024 were scanned using keywords.

Study range

All studies assessing the development and biodiversity of PSM around the citrus plant rhizosphere as well as the other antagonistic activities. Studies were considered included if they reported full-text articles, meta-analyses, and systematic review papers. The study was excluded if the abstract and title did not match the inclusion requirements. All studies evaluating the growth and richness of PSM surrounding the rhizosphere of citrus plants, along with other antagonistic actions, were chosen. Studies were deemed included if they reported systematic review papers, meta-analyses, and full-text articles. If a study's abstract and title did not meet the inclusion criteria, it was eliminated.

Data Extraction and Analysis: A review manager was employed to perform every meta-analysis. An extensive literature assessment and analysis were conducted, following to the selected reporting items for Systematic Review Articles and Meta-analysis (PRISMA) guidelines. Analysis and Data Extraction: Each meta-analysis was carried out by a review manager. The Preferred Reporting Items for Systematic Review Articles and Meta-analysis (PRISMA) criteria were followed in conducting a thorough literature review and meta-analysis.

Results

160 studies that met the keyword requirements were found in the literature search. Following the elimination of duplicates, 145 study titles and abstracts were examined. After removing studies that had no relevance to our search, 40 studies were left for full text evaluation.15 articles containing data unrelated to our meta-analysis were found during the full-text review. As a result of reviewing the references to the included research, 4 additional papers were added to the meta-analysis. Data were taken out of 64 researches.

The literature search turned up 160 studies that fit the keyword criteria. 145 study titles and abstracts were reviewed after duplicates were removed. Forty studies remained for full text examination after studies that were irrelevant to our search were eliminated. During the full-text review, 15 publications with data unrelated to our meta-analysis were discovered. Four further publications were included to the meta-analysis after the references to the included study were reviewed. 64 studies' worth of data were extracted.

Discussion

Phosphorus is not present in its elemental form because it is a reactive element. According to Walpola (2012), there are two forms of phosphorus in the soil, insoluble organic phosphorus and insoluble inorganic phosphorus. It's biosphere cycle can be characterized as "alluvial," as no significant air source can be rendered biologically accessible (Bai Y. 2019). Phosphate deficiency therefore significantly limits crop development and output. The soil contains around 0.05% phosphorus (Agusti M. 2022). Generally speaking, soil test results are substantially higher, although the majority of them—roughly 95–99%—are insoluble phosphates (Bodenhausen N 2013). The amount of soluble phosphorus in soil suspension is often quite low, ranging from parts per billion in extremely poor quality soils to 1 mg/L in soils that have received a lot of fertilizer (Augusti M. 2022, Alvarez-Perez S, 2013, Bai Y. 2019 Bodenhausen 2013).

Phosphorus fertilizer application is the primary source of inorganic P in agricultural soil. Cation fixes and converts inorganic P to around 70 to 90 percent of phosphorus fertilizers that are applied to soils (Bai Y 2019).According to Aleklett K (2014), P is immobilized by cations like Ca++ in calcareous or normal soils to create a complex calcium phosphate ($Ca_3 (PO_4)_2$), and with Al+++ and Fe+++ in acidic soils to generate ferrous phosphate (FePO) and aluminum phosphate (ALPO).

Since these forms are insoluble, they are not accessible. With the help of PSM, these phosphates that have accumulated in agricultural soils might be mobilized and transformed into soluble P forms, which would sustain maximum crop output globally for approximately a century (Bai Y. 2019). Therefore, there has been more worry about finding a different method that uses inexpensive technologies to provide plants with enough phosphorus. Both organic and inorganic phosphorus molecules can be hydrolyzed from insoluble substances by a class of helpful microbes known as phosphate solubilizing microorganisms (PSMs). The bacterial (Bacillus, pseudomonas, and Rhizobium) and fungal (Aspergillus and Penicillium) strains, as well as the actinomycetes and arbuscularmycorrhizal (AM) strains, are important among these PSMs. Many PGPR species, such as *Pseudomonas, Bacillus, Enterobacter, Klebsiella, Azotobacter, Variovorax, Azosprillium,* and *Serratia,* have been researched for decades, and some of them have even been marketed (Bulgarelli D. 2012).

On the other hand, only a small portion of agricultural practices worldwide use plant growth promoting rhizobacteria. This is because the inoculated plant growth promoting rhizobacteria uneven qualities may have an impact on crop yield. The longevity of plant growth promoting rhizobacteria in soil, compatibility with the crop it is inoculated on, ability to interact with native soil microorganisms, and environmental conditions are all necessary for its effective use (Burgess E. C. 2022).

The fact that plant growth promoting rhizobacteria has a variety of modes of action and that different rhizobacteria have different mechanisms presents another difficulty (Callahan B. J. 2016). These drawbacks restrict the use of plant growth promoting rhizobacteria. As a result, the worldwide agricultural output required feed the large population, which is expected to reach 8 billion by 2025 and 9 billion by 2050.

The rhizospheres are colonized by bacteria, fungus, actinomycetes, protozoa, and algae. The most prevalent microorganism in the rhizosphere, however, is bacteria (CDFA 2021). Applying their microbial populations to plants has been shown to improve their growth (Nighot 1986, Ozanne P. G. 1980).

In order to facilitate further research on these helpful microbes, Kloepper and Schroth proposed the name "plant growth promoting rhizobacteria." Conferring to Chen M. (2018) and Chiyaka (2012), plant growth promoting rhizobacteria especially PSm are linked to the root and have a good impact on both plant development and the management of phytopathogenic bacteria. Plant growth promoting rhizobacteria is hence one of the active elements in the composition of biofertilizer.

Several studies have shown that bacteria which are solubilize inorganic or organic phosphorus from soil that can promote plant growth when they are injected into soil or plant seeds (Kloepper *et al*., 1988, Gaur and Ostwal, *et al*., 1972; Subbarao *et al*., 1982; Gerretsen *et al*., 1948; Cooper et al., 1959). The production of tomato, potato, ion, banana, coffee, and other crops was found to be enhanced by a strain of Burkholderiacepacian that is commercially utilized in cuba as a biofertilizer. This strain exhibits high amount of mineral phosphate solubilization and reasonable phosphatase activity (Chabot *et al*., 1993).

Together with a strain of *Azospirillium brasilense*, co-inoculation of *Pseudomonas spp.* and *Bacillus polymyxa strains* showing phosphate solubilizing action led to remarkable increase in grain and dry matter yields, as well as a corresponding rise in nitrogen and phosphorus uptake (Alagawadi *et al*., 1992). Phosphate solubilizing bacillus relates with vesicular arbuscular mycorrhizae by liberating phosphate ions in the soil, conferring to a number of studies. This synergistic interaction allows improved manipulation of phosphorous sources that are faintly soluble (Ray *et al*., 1981).

As per Ozanne *et al.* (1980), phosphorus is a mechanical element of numerous coenzymes, phosphor-proteins, and phospholipids. These are contributes to the genetic memory, or "genetic material," of every live thing. It includes the storage and transfer of energy needed for development and reproduction. According to Borch *et al*. (1999) and Williamson et al. (2001), phosphorus is essential for photosynthesis, carbon metabolism, and membrane development. It is also vital for the elongation process of root propagation, and an absence of it can have an impact on root design.

The rhizosphere of plants has a stable population of phosphate-solubilizing bacteria, which include both aerobic and anaerobic strains. Aerobic strains are more conjoint in underwater soils. The rhizosphere of the citrus crop often has a much advanced concentration of phosphate solubilizing bacteria than non-rhizosphere soil (Alexander M *et al.,* 1977, Katznelson H, Peterson E.A., Rovatt J.W. *et al*., 1962), Raghu K, Mac Rac IC *et al.,* 1966).

Although there are a number of PSB in soil, they are typically insufficient in quantity to compete with other microbes that are often found in the rhizosphere. By way of a result, their Phosphorous liberation is typically insufficient to significantly boost in situ plant growth. Therefore, in order to benefit from the chemical nature of phosphate solubilization for the enhancement of plant yield, a target microorganism must inoculate the plant at a concentration that is far greater than normal for soil. The growing promotion of lettuce crop and maize crop by several microorganisms capable of solubilize different minerals, such as phosphates is one of them. In India, citrus is one of the most important fruit crops.

The citrus crop includes lemons like Assam and Baramasi Lemons, sweet oranges like Mosambi, and mandarins like Nagpur, Kinnow, Darjeeling, and Coorg mandarins. According to the Ministry of Agriculture and Farmer's Welfare's Horticulture Statistics Division, 12.04 million tons of citrus were produced in 0.94 million hectares in India during 2016 and 2017.

The Vidharbha region harvests the majority of the fruit to provide a boost to the citrus industry in Central India, which has the ideal growing conditions for citrus. Oranges can be eaten raw or in the form of syrup, jam, juice, or squash. Citric acid and cosmetics with global market value are mostly derived from it. One of the most significant fruit crops in India is citrus, which is grown on over 0.62 million hectares of land and yields 4.72 million tons annually. Mandarin orange (citrus reticulate), sweet orange (*citrus sinensis*), and acid lime (*Citrus aurantifolia*) are the most prevalent citrus fruits in India, accounting for roughly 41%, 23%, and 23% of the country's total citrus fruit production, respectively. About 40% of India's total citrus-growing land is used for orange farming. The orange (citrus reticulate), known locally as Santra, is a member of the Rutaceae family. In terms of acreage and orange production, Maharashtra leads India, followed by Tamil Nadu, Andhra Pradesh, West Bengal, Assam, Punjab, and Karnataka. In Maharashtra state, the yield of mandarin oranges is 9.2 million tons per hectare, or 1,21,495, ha, 7,21,217 million tons.

Conclusion

PSM offers an economically and naturally viable solution to the phosphorous scarcity and citrus plant adoption of it in citrus production areas. Effective PSM utilization creates new opportunities for increased yield performance and crop productivity without compromising sod health. The factors that are crucial to determining the process of citrus crops' uptake of phosphate at different parameters are revealed by the study. Molecular techniques and diversity analysis reveal several new levels that could be useful in increasing citrus crop yields. Enhancing the phosphate uptake will be possible through bioprospecting of effective strains.

This work offers fresh insights on the composition of citrus microbial communities. Our findings from a single grove provide evidence of the citrus microinome is made up by the primary soil-derived taxonomic groupings that have the ability to colonize trees systemically. A bacteriological niche distribution with specific taxa (Group) that can colonize both the more or less ground bio compartments is seen.

Our results confirm the abundance of transient taxa during blooming and tree thinning, when thier colonization patterns are in coordination with the host phenology. *Burkholderia, Sphingomeras, and Streptomyces* are among the possible plant growth-promoting bacteria that we found embedded in all biocompartments and that could be used to commercialize by products to improve the plant health. We also find tissue-specific microorganisms (such as *Acinetobacter and Epiconcion*) that may colonize the citrus flowers and expand tree or plant productivity.

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