



Review Article

Phosphate Solubilizing Bio-fertilizers and Their Role in Bio-available P Nutrient: An Overview

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Abstract: Soil is an ecosystem that plays a fundamental role in the availability of plant nutrients and contains a diverse array of beneficial microorganisms which can play a key role in soil fertility enhancement. Soil fertility management strategies for improving plant nutrients and crop productivity include the use of application of composts, vermicomposts and manures, and application of biofertilizer or microbial inoculants. The application of efficient phosphate-solubilizing microbial inoculants in agriculture opens up new insight for future crop productivity besides sustaining soil health. Development in the use of phosphate solublizing bio-inoculants are one of the recently promising options for meeting agricultural challenges imposed by the still growing demand for food. In this regard this review will show that phosphate-solubilizing microbes (PSMs) have tremendous potential as bio-fertilizers. Bio-fertilizer technologies can contribute to efficient utilization of limited resource of phosphorus fertilizers under low-input farming systems and guarantee the environment for livelihood. To strengthen the application of phosphate solublizing bio-inoculants, there is a need to know and understand the methods for their isolation and characterization of phosphate-solubilizing microorganisms and the mechanisms they used to solublize phosphate to make it available for plant nutrition. At the same time there is a need to understand various sources of bio-inoculants used for the primary isolation and characterization of indigenous phosphate solublizers which will be focused in this review. This review will also provide a broad spectrum for the various mechanisms of phosphate solublization and its impact in sustainable agriculture.

Keywords: Phosphate Fertilizer, Phosphate Solubilization, Bio-fertilizers, Vermicomposts

1. Introduction

Sustainable agriculture is the production of sustainable food systems considering the social, ecological and economic dimensions of agricultural practices with less environmental shock that has social profit in the short and long term [1]. Chemical fertilizers have contributed a fundamental role in the agricultural growth productivity in Ethiopia over a period of time. The continuous use of chemical fertilizers may not be considered as sustainable given that soils may decline in the soil biodiversity, topsoil become acidic because of nitrogenous fertilizer application that lowers the pH of the soil

and having unsteady aggregates leading to erosion that direct towards loss of soil fertility and this may leads to yield decline with time in spite of consistent use of chemical fertilizers [2]. Nowadays, a tremendous attention is given to the impact of soil environment due to continuous and misuse of chemical fertilizers and chemical pesticides which have incredible harmful long-term residual effect not only on the soil-health and crop productivity but also they contaminate the ground water level and ultimately they are integrated into the food chain in the ecosystem resulting in human health hazards. Hence, in order to enhance the fertility status of the soil, the natural way of feeding the soil with different types of organic

inputs such as composts, vermicompost, biofertilizers, farmyard manure, etc. has been developed so as to ensure sustained productivity [3].

Keeping substantial soil properties for plant growth and environmental effectiveness necessitates the input of affordable and cheap organic fertilizers and bio-inoculants used as bio-fertilizers for resourceful exploitation of nutrients by plants. This will reduce the environmental impact of chemical fertilizers with an estimated annual consumption of 15 million tons of Urea, worldwide in 2011, which could result in soil acidity, damage to soil bio-diversity and in turn reduction in produce, according to the food and agriculture organization of the United Nations. In these regards sustainable agriculture and soil fertility management is important for successful crop production in all agricultural commodities. Considering the negative effects of chemical P fertilizers, microbial intervention of P-solubilization seems to be an effective way to solve the phosphorus availability in soil. However, the effectiveness of phosphate solubilizers in the soil are dependent on factors such as the soil temperature, moisture, pH, salinity, and source of insoluble P, method of inoculation, the energy sources and the strain of microorganism used [4]. Hence study of phosphate solubilizing microbial (PSM) activity in correlation with these factors has to be done extensively before PSM can be used as bio-inoculants with promising results. Therefore microbial mediated phosphorous management and use of earth worm mediated organic fertilizers is an eco-friendly and cost effective approach and is a realistic alternative in order to lower the environmental risk and enhance the productivity for sustainable development of agricultural crop [5-7]. On the other hand phosphorus exists in the soil forming insoluble metallic complex with iron and Aluminium in acidic soil or with calcium carbonate in alkaline soil; as a result only a small fraction of phosphate is available for the plant growth [8]. This means that the majority of soluble inorganic P is rapidly immobilized by soil fixation and becomes unavailable for plant uptake, leading to insufficient phosphorous acquisition efficiency and potentially limited Phosphorous [9]. Evidence is increasing that phosphate solubilizing bacteria (PSB) and fungi (PSF) play a key role in conversion of insoluble Phosphate to bio-available primary and secondary orthophosphate ions under neutral soil pH [10, 2]. From this one can realize that for living environment of microbes and the availability of phosphorus the neutral pH soil condition is unquestionable otherwise the agricultural system become not well established phosphate solubilizing microbes and phosphorus nutrient deficient. Therefore, the bioavailability of both organic and inorganic phosphate is dependent on the well established phosphate solubilizing microbes in the vicinity of the plant rhizosphere which in turn, the acidity of the soil suppresses the microbial population in the soil [2]. It is the role of phosphate solubilizing microorganisms to convert the insoluble phosphate into soluble forms by acidification, chelation and exchange reaction $\text{H}^+ + \text{H}_2\text{PO}_4^- \rightarrow \text{H}_2\text{O} + \text{HPO}_4^{2-}$ [11]. Due to these phenomena sufficient and efficient bacteria and fungi capable of solubilizing limited amount of phosphate are not always

present in the soil. In such complicated plant rhizosphere in the utilization of chemical fertilizer by the plant root phosphate solubilizers are believed to play a pivotal role facilitating to make P available for plant nutrition. Therefore, the overall objectives of these literature reviews were to assess the current trends of organic fertilizers, P-solubilizing bio-inoculants and mechanisms to make p nutrient available for plant diet.

2. Phosphorus Fertilizers

Next to Nitrogen, Phosphorus (P) is a non-renewable macronutrient, the most important nutrient required by the plants [12]. It is one of the major important element that plays a significant role in biological growth and development such as photosynthesis, respiration, energy storage and transfer in the living plant cells [13] but for plants is also a limiting one due to its low availability of soluble forms in soil [5]. However, most soil phosphorus, approximately 95–99%, is present in the form of insoluble phosphates and hence cannot be utilized by plants [14]. In contrast to nitrogen P is not available in the atmospheric gas and be fixed biologically for plant utilization [15]. Most natural ecosystems in tropical and subtropical areas are predominantly acidic, rich in iron and extremely P deficient [16] due to their strong fixation of P as insoluble phosphates of iron and aluminum resulting P deficiency in the soil. To ease P deficiency in these regions, chemical fertilizers containing soluble forms of P are widely used on large scale to keep soil fertility in crop cultivation. However, only about 25 per cent of the phosphorus applied to the soil is available for the crops. This in turn leads to a need for excessive and repeated application of soluble P fertilizers, which in addition to the economic restriction can pose a serious threat to groundwater [17]. Therefore, the overuse of fertilizers can cause unexpected environmental impacts, damaging human health and polluting the surrounding environment and thus violating the sustainability of ecosystem [18]. In lines with these, the cost of chemical fertilizers and their associated risks on the environmental safety calls for the search of alternative means of plant nutrient management practices such as bio-inoculants, compost, farm yard manure and vermicompost. To achieve maximum benefits in terms of fertilizer savings and better growth, the P-solubilization based inoculation technology should be utilized along with appropriate levels of fertilization.

Due to current trends in phosphate rock scarcity scenario, there has to be a shift towards low reliance on inorganic phosphate and search for locally available agricultural inputs which minimize dependence on inorganic phosphate inputs sustaining agricultural production to feed the world's exponentially growing population [19]. The soil solution is typically in the range of 0.01–3.0 mg per liters representing a small portion of plant needs and remains to be the main source of phosphorus supply to plants. Soil microbe helps in phosphorus (P) release to the plants that absorb only the soluble phosphorus forms like monobasic (H_2PO_4^-) and dibasic ($\text{H}_2\text{PO}_4^{2-}$) forms [20]. Nevertheless a huge percentage

of P exists in unavailable forms and as a result not absorbable by plant roots. Low levels of P reflect the high fixation of phosphate with other soluble components [21], such as aluminum in acid soils ($\text{pH} < 5$) and calcium in alkaline soils ($\text{pH} > 7$) [22]. In addition to soil solution of soluble phosphorus forms, the solid phase such as Rock Phosphate (apatite), hydroxyapatite and oxyapatite could be source of phosphorus through involvement of phosphate solubilizing microbes as well as physical processes.

Moreover, organic forms such as phosphomonoesters, inositol phosphate, phosphotriesters and phosphodiester are major deposits of P in soil, which cannot be absorbed by plants [23].

3. Organic Fertilizer

Although traditional thermophilic compost provides improved availability of nutrients and facilitates plants growth, the presence of heavy metals such as mercury, chromium and lead may pose a threat to human health due to their recycling in the food chain causing carcinogenic effect to human. For this reason technical tests must be carried out to verify its safety [24]. Besides excessive application of manure can generate important reductions of plants growth, extreme levels of nitrogen, ammonia, and salts that could lead to different undesired scenarios for farmers and the soil itself. As a result research strategies have been established to maintain the drawback of manure and using earthworms to avoid problems related to it [25]. Currently, there is increased emphasis in the potential of vermicomposts, as plant growth media and as soil fertility improvement. Vermicompost is finely divided humus-like substance with high porosity, aeration, drainage, water-holding capacity, and formed when organic matter is broken down by the joint action of earthworms and microorganisms [6], which make them excellent soil conditioner. It is different from conventional compost, which is a product formed from the aerobic decomposition of organic waste like animal droppings, crop wastes and even municipal wastes [26]. Vermicomposts and conventional compost have different chemical and physical properties, and hence different effects on plant growth. This is due to differences in how they are produced [6]. Vermicomposts require less production time than conventional composts. In addition, unlike conventional composts which are more abundant in ammonium, vermicomposts contain high amounts of nitrates, which are a more readily available form of nitrogen [26]. It is a sustainable source of macro- and micro-nutrients, which attribute to modifications in soil structure, change in water availability, stimulation of microbial activity and increase of the activities of critical substances as a result of microbial intervention through interactions with earthworms [27]. These properties of vermicompost give it a priority over conventional composts. Vermicomposting is a biological fertilization technique consisting of the use of earthworms' metabolism to produce humus with high nutrient content. It is cost-effective and eco-friendly waste management technology which takes the privilege of both earthworms and the associated microbes and

has many advantages over traditional thermophilic composting [28]. The biochemical process takes place in the earthworm's gut is affected by microbial decomposition of the substrate in the intestines of the earth worms. Most commonly used earthworm species in vermicomposting are *Eisenia foetida* (Red California) and *Eudrilus eugeniae* (African Red) [29]. Vermicompost is taken as excellent agricultural inputs since it is devoid of contaminants and considered to hold available plant nutrients over long time without polluting the environment. Vermicomposting earth worms harbor P solubilizers and decomposers in their intestine and release them in their excreta [30]. Most of these bacteria belong to the genus *Pseudomonas* and *Azotobacter* [31], which can produce plant growth promoters, nitrogen-fixing bacteria, and phosphate solubilizers [32].

A complex interaction between Earthworm's gut and rhizospheric bacteria ingested during vermicomposting and ingested as vermicast influences the dynamics of soil texture which in turn used to heal soil health and increase plant nutrient availability. Vermicasts are full of microorganisms viz., Rhizobium, Pseudomonas, Azospirillum, Bacillus and Azotobacter [25, 33]. Hence, application of vermicasts to agricultural soil are said to be inoculation of bio-inoculants which improves the biological properties of rhizosphere soil.

Even though some harmful spores of Fusarium and Pythium are dispersed along with earth worm casts antagonistic disease-suppressing and other plant growth-promoting beneficial bacteria during vermicomposting outweigh these harmful effects [34]. Hence, vermicomposts with excellent physio-chemical properties and buffering ability, fortified with all nutrients in plant available forms, antagonistic and plant growth-promoting bacteria are fantabulous organic amendments that act as an answer for soil reclamation, enhancement of soil fertility, plant growth, and control of pathogens, pests and nematodes for sustainable agriculture.

Moreover, since the availability of organic matter is the major concern for the establishment of phosphate solubilizing Pseudomonas species in the soil, vermicompost has been suggested having dual purpose as can serve as the best carrier materials for P solubilizers and be used as source of plant growth promoting microbes as well.

4. Bio-fertilizers

The current trends for ecologically and economically feasible fertilizer demands have encouraged the search for a new move towards sustainable agriculture. Sustainable agriculture comprises the most significant approach to work against the environmental quality decline by keeping up the extended ecological balance of environments [35]. Biofertilizer is a breakthrough technology that promises very significant impact on the farmers in terms of increasing farm productivity and income. It is defined as bio-inoculants possessing living organisms which help in nutrient availability and uptake by plant roots in the rhizosphere. Also it can be defined as microbial inoculants which are a promising technology to reduce the use of conventional inorganic

fertilizers [36]. Thus, the term *biofertilizer means the product containing carrier based (solid or liquid (Figure 1)) living microorganisms which are agriculturally useful in terms of keeping the soil environment rich in both micro and macro elements and accelerate production of regulatory hormones and antibiotics in the soil to increase the productivity of the soil and crop.*



Figure 1. Different forms of commercialized biofertilizers. Central & state Govt Approved Organic manure, Phosphorous Rich Organic Manure, Bio-Potash, Bio-enriched Compost, Neem based Organic Manure, Bio-fertilizers, Bio-pesticides, Growth Promoters, Micro-nutrients, CoCo pith, Manufactured and Marketed by: AMRUTH ORGANIC FERTILIZER, malladihalli, Holalkere (T), Chitradurga-577531, Karnataka, India.

Carrier material based bio-inoculants enable long-term storage easy handling and transportation of biofertilizers. They increase and enhance the degree of availability of plant absorbable minerals in the form easily assimilated by plants [38]. Not always soil microbes dwelling in the agricultural soil are as effective as one would expect them to be and hence in vitro characterized and selected bio-inoculants play a very important role in fixation and solubilization effect in the agricultural soil. Microorganisms are the main sources for the preparation of biofertilizer that help crop plant to uptake the nutrients by their interactions in the rhizosphere when applied through seed or soil. They are gaining importance because of eco-friendly, non-hazardous, and nontoxic nature. Several microorganisms such as *Aulosira*, *Tolypothrix*, *Scytonema*, *Nostoc*, *Anabaena*, *Plectonema*, *Azolla*, *Rhizobium*, *Pseudomonas*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Fusarium*, *Sclerotium*, *Aspergillus* and *Penicillium* are commonly used as biofertilizers [39, 40]. The living microorganisms, which promote plant growth by improving the nutrient status of the plant, include rhizospheric fungi, rhizospheric bacteria, symbiotic bacteria and non-symbiotic endophytic bacteria. Rhizospheric fungi, such as arbuscular mycorrhizae [41] and *Penicillium bilaii* (are known to have plant growth-promoting effects by increasing the nutrient status of host plants. The application of bio-fertilizers in agriculture has proven to be eco-friendly, productive and accessible option to continued application of soluble mineral fertilizers [42].

5. Plant Growth-promoting Rhizobacteria and Fungi

The term “plant growth-promoting rhizobacteria (PGPR)” was first described by Kloepper and Schroth in the late 1970s

The management of biofertilizer has evidenced by increased crop productivity, reduction of production costs by reducing the volume of fertilizers applied and a better conservation of environmental resources. Moreover, inoculants are composed of beneficial bacteria and fungi that can help the plant meet its demands for nutrients [37].

[43]. Since then Plant growth promoting microorganisms (PGPM) found in the rhizosphere in association with roots and vermicompost were described by different investigators. These are *Bacillus*, *Enterobacter*, *Azospirillum* and *Pseudomonas* [44], *Azotobacter*, *Chromobacterium*, *Serratia*, *Micrococcus*, *Azotobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Burkholderia*, *Erwinia*, *Caulobacter*, *Flavobacterium*, *Actinobacter*, *Arthrobacter*, *Agrobacterium*, *Hyphomicrobium*, and fungus such as *Trichoderma*, *Aspergillus niger* and *Penicillium* species [45]. They are beneficial microorganisms which can heighten the growth of plants either directly by producing siderophores, phytohormones including indole-acetic acid, cytokinins, gibberellins and inhibitors of ethylene production, and organic acids, N_2 fixation, solubilization of inorganic phosphate for the easy assimilation of plants and for their own use [46] and bio-control of plant diseases [47] or indirectly induction of resistance in host plants and suppression of plant pathogen [5]. Production of organic acid by Phosphate-solubilizing microbes (PSMs) is one of the mechanisms to reduce metal toxicity and to transform metal species to immobile forms or chelate them for mobility [48]. Even if production of HCN by PGPR was originally thought to promote plant growth by suppressing pathogens, this idea has currently been challenged by Rijavec and Lapanje [49], who disagreed that metal chelation due to HCN ultimately amplify P availability. The PSM *Bacillus megaterium* has been commercialized as biofertilizer and can reduce phosphate fertilizer requirements of plantation crops up to 75%. Moreover, strains of P- solubilizing *Pseudomonas striata*, *B. Polymyxa*, and *B. megaterium* have also been commercialized [50]. Their use as ‘plant growth promoting Rhizobacteria (PGPR) is an important contribution to inoculation of agricultural crops.

5.1. Phosphate-solubilizing Bacteria (PSB)

Date back to 1950's PSB were serving as biofertilizer in the agriculture [51]. They are beneficial one capable of solubilizing inorganic phosphorus from insoluble compounds to soluble forms. The ability of P solubilization of rhizospheric bacteria is measured to be one of the most vital characters associated with release of phosphate from complex compound to make it available for plant nutrition. Microorganisms play a central role in the natural phosphorus cycle and among them are strains from genera such as *Pseudomonas*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Erwinia*, *Serratia*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter* and *Flavobacterium* [52, 53]. *Rhizobia*, including *R. leguminosarum*, *R. meliloti*, *M. mediterraneum*, *B. japonicum* and *Bradyrhizobium sp.* are possible P solubilizers [54]. They are reported to have phosphate-solubilizing ability that can convert the insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate, to plant available forms [55].

Among these microorganisms *Bacillus megaterium*, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata* and *Enterobacter* are very effective for increasing the plant available P in soil as well as the growth and yield of crops [53]. Recent report by Pindi et al. [40], was also indicated that *Pseudomonas*, *Bacillus*, *Micrococcus* and *Flavobacterium* are active in the solubilisation process.

Therefore, exploitation of phosphate solubilizing microorganisms through biofertilization has enormous potential for making use of constantly increasing fixed P in the soil, and natural reserves of phosphate rocks. Although several phosphate solubilizing bacteria occur in soil [4], usually their numbers are not high enough to compete with other bacteria commonly established in the rhizosphere. Thus, the amount of phosphate liberated by them is generally not sufficient for a substantial increase in coffee plant growth. Therefore, inoculation of the seed by target bio-inoculants at much elevated population than that normally found in the rhizosphere is essential to take advantage of P availability for the plant growth enhancement.

5.1.1. Mechanisms of Phosphorus Solubilization by Bacteria

Soil microorganisms are said to solubilize inaccessible phosphorous compounds to make it available for plant absorption. They make P available through mechanisms of solubilization to convert inorganic and mineralisation to convert organic into bioavailable form facilitating uptake by plant roots [21]. Phosphate-solubilizing bacteria solubilize inorganic soil phosphates, such as $\text{Ca}_3(\text{PO}_4)_2$, FePO_4 , and AlPO_4 , through the production of organic acids, siderophores, and hydroxyl [56]. The principal mechanism for phosphate solubilization is occurred in two ways.

Release of inorganic phosphate from insoluble chemical form is usually enhanced by the action of acids produced by bacteria to release organic compounds such as citrate, gluconate, oxalate and succinate, and consequently to increase free phosphate in the medium or environment [57]. To determine the amount of acid in liquid culture, filtrates can be

determined by thin layer chromatography or paper chromatography or by high-performance liquid chromatography [58]. Release of mineral phosphate can be mediated by either organic acids as a result of exchange of PO_4^{2-} or by chelation of Aluminium and Iron ions linked to phosphate compounds [59]. Optionally enzymatic acid phosphatase, produced by soil bacteria, can be used to release free P from organic matters by mineralization procedures in the rhizosphere soil [60]. Moreover, enzymes such as phosphatases, phytases and phospholipases which are key drivers can be used in the transformation [61].

5.1.2. Isolation of Phosphate-solubilizing Bacteria

Detection and enumeration of phosphate solubilizing bacteria can be done through the use of plate screening methods [62]. The Pikovskaya's agar culture medium containing tricalcium phosphate (TCP; $\text{Ca}_3(\text{PO}_4)_2$) [63], FePO_4 , and AlPO_4 or rock phosphate (hydroxyapatite), can serve as isolation media. Phosphate solubilizing microbes could be screened by the formation of clear zone around the colonies. *Bacillus* strains when grown in Pikovskaya broth containing rock phosphate as insoluble P-source, individually supplemented with three carbon sources; Sucrose, glucose and maltose, showed maximum P-solubilization after 14 days [64]. An earlier comparative studies on National Botanical Research Institute phosphate growth (NBRIP) medium [65] and Pikovskaya (PVK) medium with eight bacteria such as *Pseudomonas sp. 1*, *Pseudomonas sp. 2*, *P. fluorescens*, *P. aerogenes*, *P. aeruginosa*, *Bacillus polymyxa*, *B. subtilis*, and *Bacillus sp. 1* in a plate assay showed similar results when compared for phosphate solubilization ability. However, in broth assay NBRIP broth showed about 3-fold more efficient compared to PVK broth for all the tested strains [65]. However, Collavino et al. [66] found that there is no correlation between potential phosphate solubilizing bacteria (PSB) isolated on tricalcium phosphate (TCP) and their ability to promote plant growth.

5.1.3. Evaluation of Phosphate Solubilizing Efficacy

Phosphate-solubilizing bacteria (PSB) can not only play key role in liberating P from pools of inorganic phosphate but also can prevent the liberated P from being re-fixed [46]. Solubilization of inorganic P from an insoluble chemical form is usually mediated by the ability of the bacteria to acidify growth medium, to release organic anions such as citrate, oxalate, gluconate and succinate to increase free P in the medium. During qualitative analysis the bacterial characteristic is usually tested in an agar plate medium with precipitated tricalcium phosphate, NBRIP, FePO_4 , and AlPO_4 or rock phosphate (hydroxyapatite) which is clarified by the acid released from the bacterial colony and the efficiency of the solubilizers is measured by measuring the P-Solubilization Index [67]. Moreover, yellow color development was another qualitative measure for p solubilization tendency in the Pikovskaya's solid culture medium containing bromophenol blue when inoculated with the test isolates and incubated at 30°C for 7days. Phosphate solubilization tendency of bacteria can also be quantified and measured in a liquid culture

medium [68]. Such potential activity of bacteria does not always guarantee the efficiency of biofertilizer in the field and hence *in vivo* experiments should be done under field condition to see whether the bacterial inoculum can enhance P availability and behaving as true biofertilizers to improve plant growth.

5.2. Phosphate-solubilizing Fungi

Strains of fungi those contribute to phosphate solubilization includes *Achrothcium*, *Alternaria*, *Myrothecium*, *Oidiodendron*, *Paecilomyces*, *Penicillium*, *Phoma*, *Pichia fermentans*, *Populospora*, *Pythium*, *Rhizoctonia*, *Rhizopus*, *Saccharomyces*, *Arthrobotrys*, *Aspergillus*, *Cephalosporium*, *Cladosporium*, *Curvularia*, *Cunninghamella*, *Chaetomium*, *Fusarium*, *Glomus*, *Helminthosporium*, *Micromonospora*, *Mortierella*, *Schizosaccharomyces*, *Schwanniomyces*, *Sclerotium*, *Torula*, *Trichoderma*, and *Yarrowia* [56, 69, 70] reported that fungi are more efficient and have a greater potential to solubilize insoluble phosphate in release of P from insoluble inorganic compounds than bacteria. Moreover, their ability to withstand biotic and abiotic stress under soil condition makes them the potential candidate for developing bio-inoculant. *Penicillium* and *Aspergillus* spp. are the dominant P-solubilizing filamentous fungi found in rhizosphere [71]. They are said to be the most powerful P solubilizers [53].

Filamentous fungi are highly important in P solubilization. It was reported that *Aspergillus niger* and *Trichoderma harzianum* could be potential candidate for developing bio-inoculants to facilitate P supply to different crops in alkaline and acidic soils with organic and inorganic P content and also possessed plant growth promoting attributes such as auxin and siderophore production [72]. *Aspergillus niger* and some *Penicillium* species have been tested for phosphate solubilization, biocontrol and biodegradation [73]. In addition, Pindi *et al.* [40] were documented the phosphate solubilisation tendency of *Sclerotium*, *Fusarium*, *Aspergillus* and *Penicillium*.

5.2. 1. Mechanisms of Phosphorus Solubilization

Mechanism of phosphate solubilization in fungi is accompanied with and mediated through fungal acid production such as citric, gluconic, fumaric, malic, oxalic, lactic, 2-ketogluconic, malonic acids, succinic, propionic and acetic acid which results in decrease in pH of the medium. Malic acid is said to be more efficient than succinic acid and this may be due to higher number of hydroxyl group in malic acid compared to succinic acid [72].

Phosphate solubilizing fungi are more important to the solubilization of inorganic phosphate in soils than bacteria as they typically produce and secrete more acids than bacteria [56]. Based on the specific attribute of each fungal isolates the amount and nature of organic acid produced may vary under the same production conditions. Hence, quantity and quality of organic acid produced is fully dependent on the type of P-solubilizing microorganisms. Therefore, considerable variation may exist among different fungal isolate for making

available P from the same phosphate source [74]

5.2.2. Isolation of Phosphate-solubilizing Fungi

Phosphate solubilizing fungi (PSF) can be isolated through the use of plate screening methods [62] on Pikovskaya's agar culture medium containing tricalcium phosphate (TCP; $\text{Ca}_3(\text{PO}_4)_2$) [63], NBRIP [75], FePO_4 , and AlPO_4 or rock phosphate (hydroxyapatite). PSF could be identified based on the size of halo zones, and purified by repeated culturing on potato dextrose agar (PDA) incubated at 25°C.

5.2.3. Evaluation of Phosphate Solubilizing Capacity

The release of bioavailable inorganic P from an insoluble chemical compound is usually mediated by the ability of fungal organic acid production such as citric, succinic, propionic, malic and acetic acid in the medium or environment. During qualitative assessment of the solubilization of inorganic phosphate the formation of clear halo zones around the colonies on the National Botanical Research Institute phosphate growth (NBRIP) medium [75] is considered to be indicative of phosphate-solubilizing ability of fungal strain. Moreover, quantitative estimation of the phosphate-solubilizing ability of the fungal isolates in NBRIP broth is determined by liquid fermentation methods [76].

Besides, the Pikovskaya's solid culture media containing tricalcium phosphate (TCP; $\text{Ca}_3(\text{PO}_4)_2$) and broth is another tool used to measure both quantitative and qualitative estimation of inorganic phosphate solubilized [63].

6. Phytobeneficial and Eco-physiological Traits of Phosphate Solubilizing Microbes (PSMS)

To get the maximum benefit of inoculation under different ecological zones, there is a need to develop the bio-inoculants using a microbial strain with multifunctional attributes.

6.1. Phytobeneficial Traits

Plant growth promoting traits such as phosphate solubilization, production of siderophores, indole acetic acid (IAA), Hydrogen cyanide (HCN) and ammonia (NH_3) are important traits in enhancing plant growth and plant disease control. Siderophore, iron-chelating ligands and low-molecular weight, helps a particular microorganism to compete against fungal pathogens for available iron and the role of siderophores in control of diseases has been well documented [77]. The direct promotion of plant growth by PSMs is as a result of the production of plant growth regulators (mainly auxin), enhanced availability of nutrients to the host plant by production of siderophores. Phosphate solubilizing microbes (PSMs) have potential to produce siderophore for improving iron availability to plants [56]. Inoculation of chickpea and soybean seeds with a siderophore-producing *fluorescens pseudomonad* resulted in increasing seed germination, growth, and yield of the plant [78].

Dual role of IAA such as the biocontrol reactions by

inhibiting the germination of spore and development of mycelium of pathogenic fungi [79] and production of high level IAA by isolates has a direct influence on plant growth as they increase plant root length [80] was well documented. Production of high level IAA by isolates has a direct influence on plant growth as they increase plant root length [80]. Spaepen *et al.*, [81] were documented that bacteria in the plant rhizosphere have influenced plant growth due to auxin phytohormones production. The rhizosphere soil isolates which produced high level of IAA have said to show significant increase in the plant height and root length of wheat seedlings along with increase in chlorophyll content when compared with control [82].

Moreover, bacterial IAA facilitates adaptation of host plants in metal-contaminated sites through activation of physiological changes in plant cell metabolism under metal stress so that the growing plants can resist high concentrations of heavy metals [83].

Microbial production of HCN has been reported as an important antifungal trait to control root infecting fungi [84] and it is a potential and environmentally compatible mechanism for biological control of weeds [85].

6.2. Ecophysiological Traits

Stress tolerance towards eco-physiological factors such as high salinity and soil acidity of beneficial soil bacteria may be good attribute to compute with and become a winner strains in alkaline soils. Stress-tolerant bacteria are likely to be found in environments affected by salt and pH stress.

When Various potentially toxic heavy metals, but needed in a trace amount for both animal and plants, are present in elevated concentrations they are known to affect soil microbial populations and their associated activities [86].

On the other hand resistance to antibiotics is a threat phenomenon in medical microbiology. However, in agriculture, it is considered as advantageous for bio inoculants to persist and well established in the soil by resisting agro-chemicals such as pesticides, herbicides and chemical fertilizers. Fast adaptation by antibiotic resistant bacteria is due to spread of R-factors than by natural selection and mutation [87]. Phosphate solubilizing microbes promote the uptake of heavy metals by promoting plant growth through the synthesis of different compounds such as siderophores or due to stimulation of certain metabolic pathways.

7. Conclusion and the Way Forward

Phosphate-solubilizing microbes (PSMs) provide an excellent opportunity to develop environment-friendly phosphorus biofertilizer to be used as supplements or alternatives to chemical fertilizers. They can contribute to efficient utilization of limited resource of phosphorus fertilizers under low-input farming systems and guaranteed the environment for livelihood. PSMs mobilize soil inorganic phosphate to increase its bioavailability for plant use, promote sustainable agriculture, improve the fertility of the soil, and hence increase crop productivity. Therefore, PSMs provide a

wide range of possibilities for the development of conventional agriculture in different localities, economic and cultural backgrounds. Current paper review clearly showed that components of biofertilizer, appropriate culture media for their isolation, mechanisms of their phosphate solubilization and their possession of Phytobeneficial and eco-physiological traits. Moreover, the importance of organic matters in vermicompost technologies that can contribute to increase the inoculums efficiency and the survival rate of bacteria and fungi adherent to the seeds, which are other essential factors for successful inoculation. Indeed biofertilization techniques require less chemical inputs on the soil and facilitate the incorporation of organic and inorganic phosphate with Phosphate solublizers which represents relevant reductions on the environmental impacts associated to agricultural activities. Finally, the search for beneficial bacteria and fungi is important for the development of new and efficient inoculants for agriculture. Thus, the introduction of beneficial bacteria and fungi in the soil tends to be less aggressive and cause less impact to the environment than chemical fertilization, which makes it an affordable agronomic inputs and a mechanism to minimize cost of production to farming system. In future, these are the most feasible candidates who can replace the chemical fertilizers, pesticides and artificial growth regulators showed numerous side-effects to sustainable agriculture. Overall, future research should give more emphasis for the natural field condition applications of phosphate solublizers for the commercialization of bio-inoculums.

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