ISOLATION AND PURIFICATION OF PHOSPHATE SOLUBILIZERS

Introduction

Phosphorus (P) is one of the major essential macronutrients for biological growth and development. It is present at levels of 400–1200 mg·kg^{-1} of soil. Its cycle in the biosphere can be described as 'open' or 'sedimentary', because there is no interchange with the atmosphere. Mineral forms of phosphorus are represented in soil by primary minerals, such as apatite, hydroxyapatite, and oxyapatite. They constitute the biggest reservoirs of this element in soil because, under appropriate conditions, they can be solubilized and become available for plants and microorganisms. Mineral phosphate can be also found associated with the surface of hydrated oxides of Fe, Al, and Mn, which are poorly soluble and assimilable. Microorganisms play a central role in the natural phosphorus cycle. This cycle occurs by means of the cyclic oxidation and reduction of phosphorus compounds (Rodriguez and Fraga, 1999), where electron transfer reactions between oxidation stages range from phosphine (–3) to phosphate (+5). The concentration of soluble P in soil is usually very low, normally at levels of 1 ppm or less (10 M $H_2PO_4^{-1}$). The cell might take up several P forms but the greatest part is absorbed in the forms of HPO_4^{2-} or $H_2PO_4^-$. Most agricultural soils contain large reserves of phosphorus, a considerable part of which has accumulated as a consequence of regular applications of P fertilizers. However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants. The phenomena of fixation and precipitation of P in soil is generally highly dependent on pH and soil type. Thus, in acid soils, phosphorus is fixed by free oxides and hydroxides of aluminium and iron, while in alkaline soils, it is fixed by calcium, causing a low efficiency of soluble P fertilizers, such as super calcium.

Chemical fertilizers, e.g., manufactured water-soluble phosphatic (WSP) fertilizers (superphosphates) have played a significant role in the green revolution and are commonly recommended to correct phosphorus deficiencies. After nitrogen, phosphorus is an essential plant nutrient whose deficiency restricts crop yields severely. Tropical and subtropical soils are predominantly acidic, and often extremely phosphorus-deficient with high phosphorus fixation capacities. On average, most mineral nutrients in soil solution are present in millimolar amounts but phosphorus is present only in micromolar or lesser quantities. The low levels of phosphorus are due to high reactivity of soluble phosphate with other elements. For instance, in acidic soils phosphorus is associated with aluminium and iron compounds, whereas calcium phosphate is the predominant form of inorganic phosphate in calcareous soils. Organic phosphate may also make up a large fraction of soluble phosphate, as much as 50% in soils with high organic matter content. New approach to farming, often referred to as sustainable agriculture, requires agricultural practices that are friendlier to the environment and that maintain the long-term ecological balance of the soil ecosystem. In this context, use of microbial inoculants (biofertilizers) including PSM in agriculture represents an environmentally friendly alternative to further applications of mineral fertilizers. A continued exploration of the natural biodiversity of soil microorganisms and the optimization/manipulation of microbial interactions in the rhizosphere represents a prerequisite step to developing more efficient microbial inoculants with phosphorus-solubilizing ability (Khan et al., 2006).

Microbial solubilization of phosphate

The beneficial role of microorganisms in the solubilization of insoluble phosphates in soil became increasingly important for biotechnology related to agriculture during the past decade. Phosphate utilization efficiency in soils is very low because applied phosphorus is mostly fixed to aluminum and iron in acidic soils and to calcium in alkaline soils. Several bacteria and fungi were isolated from the soil and their mineral phosphate-solubilizing activity was evaluated using various P sources such as $Ca_3(P0_4)_2$, $FePO_4$ and $AlP0_4$ (Gadagi and Sa, 2002)

Phosphate-dissolving microorganisms can be vital in promoting the bioavailability of phosphorus in soil characterized by high total P. Their additional property of phyto-hormone production can be advantageous for improving crop growth. *Pseudomonas striata* evaluated for phytate mineralization and solubilization of tricalcium, rock, ferric, and aluminum phosphate showed high potential as phosphobacteria. Chromatographic analysis of cell-free culture filtrate showed the presence of tartaric acid, malic acid, citric acid, succinic, and gluconic acid. Tartaric acid was effective in solubilization of tricalcium phosphate. The test strain also produced extracellular phytase (43.05 EU ml−1) in phytase-specific broth medium. The level of indole acetic acid (15.59 μg ml−1) recorded was higher in the absence of tryptophan than in its presence.

Inoculation of phosphobacteria occupy prime position among biofertilizers because of their vital role in solubilization of insoluble phosphates. In soil. Several genera of phosphate solubilizing microorganisms (PSM) have been released, but very little is known about their establishment in soil. This was in fact due to difficulties faced by studying interaction of microbes and plant roots *in situ*. The true potential of microbial inoculants can only be realised when we have complete knowledge of their persistence and saprophytic competence (Maurya and Jauhri, 2002). Several methods have been suggested to detect and enumerate indigenous and released microorganisms in natural system, but they have limitations.

Phosphate-solubilizing microorganisms

They are ubiquitous, whose numbers vary from soil to soil. In soil, phosphate-solubilizing bacteria constitute 1–50% and fungi 0.5%–0.1% of the total respective population. Generally, the phosphate-solubilizing bacteria outnumber phosphate-solubilizing fungi by 2–150 times. The high proportion of PSM is concentrated in the rhizospheres and is known to be more metabolically active than those isolated from sources other than the rhizosphere. Conversely, the salt-, pH- and temperature-tolerant phosphate-solubilizing bacteria have been reported to be maximum in the rhizoplane followed by the rhizosphere and root-free soil in alkaline soils. The role of rhizospheric organisms in mineral phosphate solubilization was known as early as 1903. Since then, there have been extensive studies on mineral phosphate solubilization by naturally abundant rhizospheric microorganisms. Important genera of mineral phosphate solubilizers include *Bacillus* and *Pseudomonas* (Khan *et al.,* 2007).

Isolation of phosphate solubilizer

Phosphate-solubilizing microorganisms can be isolated by serial dilutions or enrichment culture techniques on/in Pikovskaya medium (Pikovskaya, 1948) from non-rhizosphere and rhizosphere soils, the rhizoplane, and also from other environments, such as rock phosphate deposit area soil and marine environments. Upon incubation of the organisms on the solid plates containing insoluble phosphate, PSM are detected by the formation of clear halos around their colonies. Recently, a few other methods for the isolation and selection of PSM have been suggested. Once the efficient phosphate-solubilizing organisms are selected, they are tested for their ability to solubilize insoluble phosphate under liquid culture medium. Finally, the selected efficient phosphate-solubilizing cultures are used for making the inoculants and their performance under pot/field conditions is tested against various crops.

Efficient PSM cultures are mass-produced for supply to the farmers as microphos. The production of microphos, i.e., a preparation containing microorganisms with phosphate-solubilizing activity, includes three phases: the first concerns selection and testing of phosphate-solubilizing strains; secondly, inoculant preparation, including selection and processing of the material carrier and mass culture of PSM; and thirdly, quality control procedures and distribution. For microphos production, peat, farmyard manure (FYM), soil and cow dung cake powder have been suggested as suitable carriers. Finally, the cultures are packed in polybags and can safely be stored for about three months at 30 ± 2 °C. In India, a microbial preparation termed Indian Agricultural Research Institute (IARI) microphos culture (Gaur, 1990) was developed that contained two efficient phosphate-solubilizing bacteria (*Pseudomonas striata* and *Bacillus polymyxa*) and three phosphate-solubilizing fungi (*Aspergillus awamori*, *A. niger* and *Penicillium digitatum*).

Mechanism of phosphate solubilization

Many researchers have quantitatively investigated the ability of PSM to solubilize insoluble phosphate in pure liquid culture medium. The microbial solubilization of soil phosphorus in liquid medium has often been due to the excretion of organic acids. For instance, oxalic acid, citric acid, lactic acid, etc. in liquid culture filtrates were determined by paper chromatography or thin layer chromatography or by high-performance liquid chromatography and certain enzymatic methods to allow more accurate identification of unknown organic acids. *Pseudomonas striata* produced malic, glyoxalic, succinic, fumaric, tartaric and α-ketobutyric acid (Gaur, 1990). Such organic acids can either directly dissolve the mineral phosphate as a result of anion exchange of PO_4^{2-} by acid anion or can chelate both iron and aluminium ions associated with phosphate. However, no definite correlation between the acids produced by PSM and amounts of phosphate solubilized are reported. The role of organic acids produced by PSM in solubilizing insoluble phosphate may be due to the lowering of pH, chelation of cations and by competing with phosphate for adsorption sites in soil.

Conclusion

Since most soils are deficient in plant-available phosphorus (Khan *et al.*, 2007), and chemical fertilizers are not cost-effective, there is interest in using rhizosphere competent bacteria (RCB) or soil microorganisms endowed with phosphate-solubilizing ability as inoculants to mobilize phosphate from poorly available sources in soil.

References

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