Chapter 1

Introduction

Phosphorus, being a macronutrient just after nitrogen, is a very crucial element that aids in the growth and development of plants. It is an indispensable component of any living system due to its absolute role in cell division and proliferation, a major component of enzyme activity and energy currency of a cell. In molecular level, it is the part of the backbone of nucleic acids such as the DNA and the RNA. Though a large amount of phosphorus is naturally present in the soil, only a minute amount of this element is actually available for the plants to uptake. This is mostly due to the fact that the naturally occurring phosphorus is highly reactive in nature and forms complexes with various metals like calcium, aluminium and iron for example. These complexes are generally unfit for the plant to uptake since plants take up nutrients in ionic forms. Thus, in the absence of bioavailable forms of phosphorus plants often show phosphorus deficiency syndromes. This compels the farmers to resort to chemical fertilizers that are marketed in complex forms as well. Hence, the soil is only added with more insoluble phosphate salts that eventually fail to come to any use by the plants. To top this off, the process of addition of these inorganic phosphate fertilizers also increases the cost of production without an increase in the yield.

The insoluble phosphates present in the soil can only be made available for the plants by breaking the complex salts to their respective anionic and cationic forms. This is achieved by the tiny yet very instrumental inhabitants of the soil which is a part of the beneficial microflora, the phosphate solubilizing microorganisms (PSMs). These group of microorganisms includes a wide range of microbes such as bacteria (*Pseudomonas spp., Agrobacterium spp., Bacillus spp.,* etc.), fungi (*Alteraria spp., Cephalosporium spp., Cladosporium spp., Fusarium spp., Glomus spp.*, etc.), actinomycetes, algae and arbuscular mycorrhizal (AM) fungi (Kalayu 2019). Soil fungi can travel long distances in the soil more easily than bacteria and can better solubilize insoluble phosphates as they have the ability to secrete more organic acids responsible for the solubilization of inorganic phosphates. Generally, these group of microbes comprises of a significant part of the entire microbial community ranging from 1-50% differing from soil to soil. The PSMs isolated from the rhizospheric soils of various plants, are found to be metabolically more active and forms a healthy plant-microbe interaction.

The PSMs work through different mechanisms to make the phosphates available for absorption by the plant roots. The organic acids released by these microbes enhance in the breakdown of the complexes by lowering the pH of the soil. The solubility of the insoluble phosphates increases with decrease in soil pH. Acidity is also created through the release of carbon dioxide which is generally observed in calcium phosphate solubilization. This decrease in pH due to the secretion of organic and inorganic acids results in phosphate solubilization (Son et al., 2006). These organic acids are the metabolites produced by the phosphate solubilizers, and are basically products of oxidative respiration or fermentation by utilizing glucose as the carbon source (Alam et al., 2002). The types of organic acids produced and their quantity of secretion differ with every organism. The extent of phosphate solubilization is largely dependent on the kind of acid and its strength. Tri- and dicarboxylic acids have been proved to be more effective in phosphate solubilization than monobasic acids. Similarly, aliphatic and aromatic acids too are better candidates for solubilizing phosphates than phenolic, citric, and fumaric acids (Walpola and Yoon, 2012; Mahdi et al., 2011). Some widely reported organic acids that are responsible to solubilize phosphates are primarily citric, lactic, gluconic, 2- ketogluconic, oxalic, glyconic, acetic, malic, fumaric, succinic, tartaric, glutaric, propionic, butyric, glyoxalic, etc. (Ahmed and Shahab, 2009). Among these organic acids, gluconic acid and 2-ketogluconic acids are the most common agents of mineral phosphate solubilization.

Other than this, it also helps in the chelation of the cations by competing with the phosphates for adsorption sites in the soil. The functional groups like hydroxyl (OH) and carboxyl (COOH) groups of the acids chelate the cations such as aluminium and iron bound to the phosphate, thereby releasing the anionic part that is taken up by the plants. These acids complete for binding sites of Al and Fe insoluble oxides and reacts with them, stabilize them, thus forming "chelates". 2- ketogluconic acid is known to be a powerful chelator of calcium. The PSMs producing siderophores such as hydroxamates also aid in the chelation of iron from the soil, thus leaving the anionic phosphates that can be utilized by the plants.

The organic remains of plants and animals account for a huge source of phosphorus in the forms of nucleic acids, phospholipids, sugar phosphates, phytic acid, polyphosphates, and phosphonates. PSMs also mineralize soil organic phosphorus through the production of enzymes like phosphatases (Aseri *et al*., 2009) that hydrolyze organic phosphate compounds, and releases inorganic phosphorus (Pi). This Pi is then immobilized by the plants. Enzymes like alkaline and acid phosphatases use organic phosphate as their substrate and convert it into an inorganic form. Phosphoesterases, phytases, and phospholipases are some other extracellular enzymes that aid in the mineralization of organic phosphates in the soil.

Among the soil microorganisms, arbuscular mycorrhizal fungi (AMF) form a basis of strong plant-microbe interaction. They function as scavengers of available phosphate through their hyphae that provides large surface area and their high affinity towards mechanisms involving phosphate uptake (Hayman 1983). Among the mycorrhizal fungi, ectomycorrhizal fungi possess phosphate-solubilizing activity (Lapeyrie *et al*., 1991) and are capable of utilizing phosphate from inositol phosphates. They also possess phosphatase activity, that releases the phosphates from the soil organic matter (Koide and Schreiner,1992). In addition, arbuscular mycorrhizal fungi can exert a selective influence on soil microbial communities through multiplication of -ketoglutarate catabolizing microorganisms (Duponnois *et al*., 2005). In addition, arbuscular mycorrhizal fungi can also make iron phosphates available to developing crops.

With its high efficiency phosphate solubilizing microorganisms have often been employed in agricultural fields as biofertilizers to increase gross yield. Many of the isolates have been previously reported to increase the availability of phosphorus by considerable quantities. Walpola and Yoon, 2012 reported increased phosphate uptake leading to better grain yield in wheat when inoculated with phosphate solubilizing *Pseudomonas* and *Bacillus* species. More importantly, the biochemical composition of the soil remains undisturbed during the phosphate solubilization process by the PSM. This is of great importance where chemical phosphatic fertilizers cannot be used. Another advantage of PSM lies in the fact that it is not host specific and can be utilized for various crops. Since phosphorus also play a vital role in disease tolerance and resistance in plants, application of PSMs have also been reported to improve the quality of various crops. Proper seed formation and early crop maturation is highly dependent on adequate supply of phosphate (Sharma *et al*., 2013). It helps in root penetration as well as root branching in young plants and causes early ripening of fruits (Mehrvarz *et al*., 2008). They also help in increasing the total dry matter weight of plants which is a significant contribution to plant health. The commercially available phosphate inoculum Jump Start, was developed with a strain of *Penicillium bilaii* (Satyaprakash *et al*., 2017). The influence of PSM on cane yield and its juice quality is well established, and the role of phosphate application has become an essential part of a sugarcane fertilizer programme (Sundara *et al*., 2002). PSMs have been proven time and again as effective biofertilizers and biocontrolling agents. Some of them that have proved to have promising output as effective biofertilizers are *Bacillus megaterium, Bacillus subtilis,* and *Pseudomonas striata (*Satyaprakash *et al*., 2017).

Tea, that has made its way as the first cup of beverage of the day among the major population worldwide find its history back in India in the nineteenth century. Introduced by the Britishers then, the Indian tea eventually got popular due to its undeniable quality, flavour and aroma. Though Indian tea industry offers a range of tea varieties, Darjeeling tea secured a special place in the taste buds of the tea connoisseurs worldwide. Due to its unmatchable aroma and flavour, Darjeeling tea also holds the much coveted GI tag. Hence, Darjeeling along with its world famous tea is an important sector for the tea industry of India as it brings in a lot of revenue for the country. Moreover, a huge population is dependent on the industry for its livelihood. Thereupon, working for the betterment of this industry and understanding its ecology, especially the microflora of the region, through a scientific approach might hold a promising outcome. With this view in mind, this study area was chosen mainly focusing on the phosphate solubilizers among the diverse microflora of the region.

The tea plantations of Darjeeling hills harbours both organic and inorganic tea gardens. Hence, needless to say, there remains a fair chance of the development of tolerance by the microflora of the region against the xenobiotics in use. Therefore, tracing a tolerance pattern of the consortia and the pure culture bacterial isolates of phosphate solubilizers is an important step for a better understanding of their potential. A considerable level of tolerance against various xenobiotics such as pesticides, heavy metals, etc. can also be advantageous in regions with extreme contamination levels lacking beneficial microflora due to the prevailing stress factors. The tolerant phosphate solubilizers can, therefore, aid in nutrient cycling and develop the plant-microbe interaction, slowly but surely reclaiming the land for agricultural use. Since soil microbes are also very well established to possess the ability to form beneficial plant-microbe interaction, it is crucial to look into this phase of the phosphate solubilizers as a positive result can bring forth means for application of these in agricultural uses and hopefully commercialization of them in the near future.