

Abstract

Phosphorous (P) is one of the most abundant metallic element found in the earth's crust and is present in soils in both organic and inorganic forms. Though it is present in high concentration, only 0.1% of the total phosphorus is available to plants because of poor solubility and its fixation with other metallic elements in the soil such as calcium, aluminium and iron to form calcium phosphate, aluminium phosphate, and ferrous phosphate and thus becomes unavailable to plants. It is one of the major essential macronutrients for plant biological growth and development. Phosphorous in soil is mainly found as mineral or organic phosphorous, which is insoluble and unavailable to plants. Releasing this phosphorous in soluble form is very important in increasing plant growth and plant product yields. The tremendous application of chemical-based phosphorus fertilizers has a long-term impact on the environment regarding eutrophication, soil fertility depletion, and carbon footprint. This attitude has compelled the scientific community to find a sustainable approach for efficient P availability in agriculture to meet the ever-growing demand for food. Using efficient PSM (phosphate-solubilizing microorganisms) opens up a new horizon for better crop productivity and greater yield performance without affecting soil health. Microorganisms, including bacteria and fungi, play a central role in the natural phosphorus cycle and convert insoluble forms of phosphorus to an accessible form, which is an important trait for the growth and survival of plants.

Considering the importance of Darjeeling tea and the effect it has on the tea industry of India, this region, along with its biotope, became the perfect study area. Though a few studies have been conducted targeting the broad range of microflora of the tea gardens in Darjeeling, a thorough study of this beneficial group of phosphate solubilizers in the region and a detailed investigation of their properties and potential remains unearthed. Subsequently, twenty-four phosphate-solubilizing microorganism consortia were isolated, covering various organic and inorganic tea gardens, which was followed by the isolation of eight phosphate-solubilizing bacteria (PSMR 2.1, PSMR 5.3, PSMR 5.6, PSMR 5.7, PSMR 5.12, PSMR 5.15, PSMR 6.2.9 and PSMR 6.4.3) and were identified based on the DNA sequencing of their 16S rRNA region. PSMR 2.1 was identified as *Brucella pseudogrignonensis*, PSMR 5.3 as *Achromobacter xylooxidans*, PSMR 5.6 as *Staphylococcus hominis*, PSMR 5.7 as *Rothia kristinae*, PSMR 5.12 as *Staphylococcus hominis*, PSMR 5.15 as *Proteus vulgaris*, PSMR 6.2.9 as *Brevundimonas diminuta* and PSMR 6.4.3 as *Bacillus licheniformis*.

Apart from phosphate solubilization, most of the PSMs also possess other plant

growth-promoting abilities such as complex nitrate reduction, ammonia production, auxins such as indole acetic acid (IAA) production, siderophore production for sequestering beneficial elements such as iron and hydrogen cyanide production that is responsible for disease resistance. Each of these, through various mechanisms, helps in the healthy growth and development of plants, increasing the gross yield. Thus, PSMs exhibiting any or all of these properties naturally step up to become a great contender for the application process in fields. When pure bacterial cultures of phosphate solubilizers were investigated for their plant growth promotion activities, all isolates exhibited one or the other property. However, PSMR 5.3, identified as *Achromobacter xylosoxidans*, expressed all the properties except for hydrogen cyanide production in *in vitro* conditions. Pre-treated seeds with the isolated phosphate solubilizing bacteria (PSB) also displayed enhanced germination rate, thus demonstrating the positive effect of these isolates on the growth of plants starting from their seed stage. When tested in *in vivo* conditions, the bacterial isolates had a persistent positive effect on the growth and development of the plants as compared to the untreated control plant with a significant difference.

To further confirm its potential as a plant growth regulator, all eight phosphate solubilizing bacterial isolates were studied for their production of secondary metabolites through GC-MS analysis. The majority of the compounds produced by the isolates were found to have direct or indirect roles in plant growth regulation with a significant percentage of area coverage. The isolate *Rothia kristinae* PSMR 5.7 revealed that all the secondary metabolite compounds produced played direct or indirect role in the plant growth. The metabolites also confirmed their result *in vitro* plant growth promoting experiments, thus establishing their worth as candidates for agronomic use.

In the present era, human intervention and exploitation of resources have led to serious alterations in the natural balance of nature, which is met head-on by the microbial community. Where many fail to cope with the changes and the stress introduced, some successfully modify themselves to cope with their surroundings and emerge as tolerant strains against the posed stress factors. Tea plantations, another face of agricultural practice, meet with a similar fate of unbridled usage of pesticides with toxic organic and inorganic chemicals. Thereupon, when faced with a challenge the microbes of the region modify their genetic makeup through selective adaptation and become tolerant or resistant depending on the rate and extent of contamination. Considering the potential of this ability, their tolerance pattern against high concentrations of pesticides and heavy metals was traced only to reveal extreme levels of tolerance by both the consortia and the pure bacterial cultures of phosphate solubilizers. Isolates like PSMR 5.3 and PSMR 5.7 also demonstrated complete resistance against pesticides like flubendiamide, thiamethoxam, and emamectin benzoate, which had never been reported

before. Even with such high tolerance against the xenobiotics the isolated phosphate solubilizing bacteria were found to be mostly susceptible to the range of clinical antibiotics employed in this study. The enhanced tolerance of the bacterial isolates and consortia may be portrayed as a trump card in situations where reclamation of biodegraded land is in question along with the plant growth regulation property of these phosphate solubilizers. Therefore, high phosphate solubility coupled with extreme tolerance to various stress factors, without the risk of pathogenicity, raises the chances of these bacterial isolates to harness their abilities and probably commercialize them in the near future.