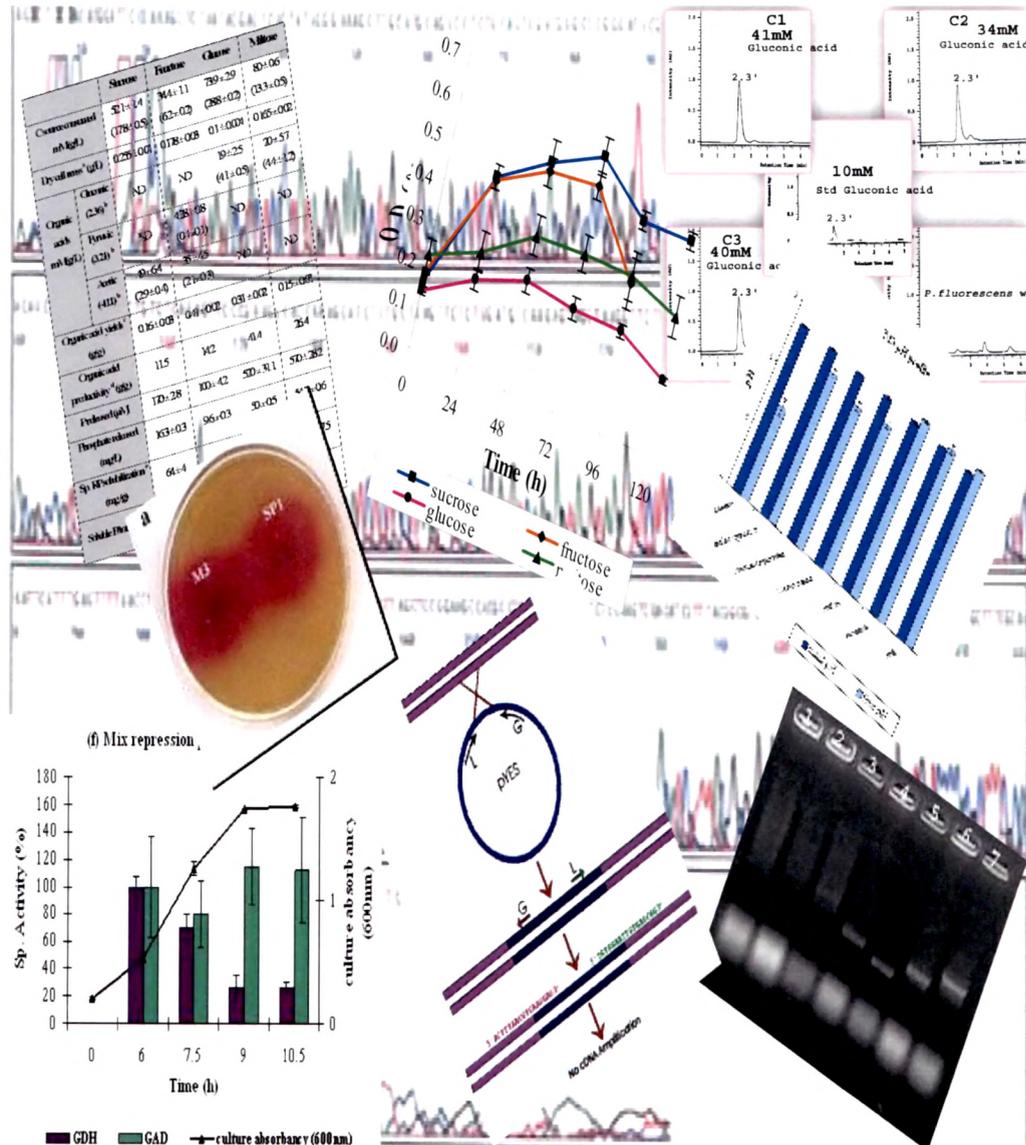


# SUMMARY



Phosphorus (P) deficiency of plants is a problem researched over a century now with no substantial progress towards meeting the ever growing demand of food for the world population. Several factors including depletion of natural rock phosphate (RP) ore reserves, pollution, lack of cost effectiveness along with strong re-fixation ability of free Pi in the soils predominantly limit the use of chemical P fertilizers. Biofertilizers which include phosphate solubilizing microorganisms (PSMs) have been looked upon as an alternative to overcome the disadvantages posed by the synthetic fertilizers. Several microorganisms, including bacteria and fungi, with P solubilizing ability have been isolated from different soil and environmental conditions. However, variations in the laboratory and field performances were observed as a result of the inability of PSMs to be efficient under different soil, plant and environmental conditions. Selection of PSMs by an alternative method using buffered medium could help to isolate better PSMs as the method mimics the alkaline vertisol soil conditions. In addition, there are several factors which need to be addressed in order to develop phosphate biofertilizers effective in the fields as majority of the PSMs have been reported to perform excellently in the laboratory but fail when applied to the soil conditions.

One of the reasons could be the use of glucose as the carbon (C) source for the isolation of PSMs which invariably led to gluconic and 2-ketogluconic acid secretion phenotype. These organic acids produced as a result of direct oxidation of glucose and gluconate via periplasmic enzymes glucose dehydrogenase (GDH) and gluconate dehydrogenase (GAD), respectively, are most effective in P solubilization. However, the plant rhizosphere where the phosphate solubilizing bacteria (PSB) colonize may not contain glucose in sufficient amount to enable them to release P from soils. In contrast the root exudates, which constitute a major source of C and energy for the rhizobacteria, are known to be rich in sugars such as sucrose and fructose along with the presence of low molecular weight organic acids. Thus, it is necessary to isolate PSB with an ability to use alternative C sources which are predominant in the rhizosphere and show P solubilization. Additionally, it is also necessary to study the effect of weak organic acids which are an integral part of root exudates on the mineral phosphate solubilization (mps) phenotype of the PSB. Moreover, it could be advantageous if the microorganisms had additional plant growth promotion abilities along with P solubilization which would benefit the plants in many ways. Hence, the present study was directed towards the use of PGPRs isolated from the plant rhizosphere as PSMs which would also confer

competitive and survival advantages in the soil. Finally, genetic modification of bacteria would be helpful in overcoming some of the limitations faced by the natural soil isolates and in conferring mps phenotype to non-PSB. The present study is a step towards the development of efficient PSB under natural conditions.

A novel PSB, named DHRSS, was isolated from the rhizosphere of sugarcane with the capacity of utilizing sucrose and rock phosphate as the sole carbon and phosphate source, respectively. PCR amplification of the partial 16S rDNA gene and sequence analysis using Ribosomal Database Project (RDP) II, a standard online bioinformatics tool for closest homology search, identified DHRSS as *Citrobacter freundii* with 98.8 % homology, indicating that the isolate belonged to the genus *Citrobacter* of family *Enterobacteriaceae*. The partial 16S rDNA sequence was deposited to the GenBank with the accession No. DQ486057. *Citrobacter* sp. DHRSS exhibited mps phenotype on fructose in addition to sucrose, which are not substrates of the GDH enzyme, along with GDH substrates, viz., glucose, and maltose, as C sources. On sucrose and fructose, *Citrobacter* sp. DHRSS liberated 170  $\mu\text{M}$  and 100  $\mu\text{M}$  free phosphate from rock phosphate and secreted 49 mM (2.94 g/L) and 35mM (2.1 g/ L) acetic acid, respectively. Growth of *Citrobacter* sp. DHRSS on sucrose was mediated by an intracellular inducible neutral invertase. Interestingly, in the presence of GDH substrates like glucose and maltose, *Citrobacter* sp. DHRSS produced approximately 20 mM (4.36 g/L) gluconic acid and phosphate released was 520  $\mu\text{M}$  and 570  $\mu\text{M}$ , respectively. *Citrobacter* sp. DHRSS showed GDH activity when grown on GDH as well as non-GDH substrates mentioned above, indicating that the enzyme was constitutive and could act on a wide range of aldose sugars exhibiting broad substrate specificity. This study demonstrates that the nature of the available carbon source dictates the production of different organic acids and thereby mineral phosphate solubilization by rhizobacteria.

Very efficient P solubilizing bacteria, namely M3 and SP1, were isolated from the rhizosphere of mungbean and sweet potato, respectively. On the basis of presence of fluorescence on pseudomonas agar medium and partial 16S rDNA sequence analysis which showed highest homology with *P. aeruginosa*, the isolates were classified to belong to the broad group of fluorescent *Pseudomonads*. *P. aeruginosa* M3 and SP1

released 793  $\mu\text{M}$  and 661  $\mu\text{M}$  Pi and produced 37 mM and 26 mM gluconic acid, respectively when grown on glucose with RP under 100 mM Tris Cl (pH 8.0) buffered minimal medium conditions. *P. aeruginosa* M3 and SP1 were further studied to determine their ability to solubilize RP in the presence of low molecular weight weak organic acids such as malate and succinate which are present as part of root exudates, in order to mimic their P solubilizing efficacy under field conditions. A strong elimination of the P solubilizing ability of *P. aeruginosa* M3 and SP1 was observed in the presence of glucose along with organic acids. This failure was attributed to the repression of the GDH enzyme which is under the catabolite repression control (CRC) of organic acids in *P. aeruginosa* M3 and SP1. However, another enzyme also implicated in mps phenotype, namely GAD, was not found to be affected by the CRC exhibited by the weak organic acids. Both malate and succinate seemed to affect the P solubilizing ability of *P. aeruginosa* M3 and SP1 to similar extent. The study indicates that the selective preference of the C source could be one of the major reasons for the failure of PSMs in the field conditions.

Screening of *A. thaliana* cDNA library resulted in obtaining organic acid producing transformants of *E. coli* and *P. fluorescens*. However, the *E. coli* transformants were not stable whereas *P. fluorescens* cDNA transformants showed P solubilization on xylose in addition to glucose under Tris Cl (pH 8.0) buffered minimal medium. The mps phenotype was found to be constitutive and the type of organic acid produced varied with the microorganism. *E. coli* cDNA transformants secreted pyruvic acid along with an increase in the acetic acid secretion whereas *P. fluorescens* cDNA transformants exhibited gluconic acid production. The failure of the *E. coli* cDNA transformants to give consistent phenotype could lie in the fact that both, pyruvic acid and acetic acid, are very poor acids as far as P solubilization is concerned and could not confer mps phenotype unless produced in high concentrations. The cDNA responsible for imparting the mps phenotype could not be determined in case of both the bacterial transformants. However, this study demonstrates that organic acid producing ability could be incorporated to the non P solubilizing *E. coli* and *P. fluorescens* by using a random approach of screening the *A. thaliana* cDNA library. These results can be extrapolated to show that mps genes are not limited to PSMs and that overexpression of genes in metabolically distinct organisms could confer mps ability to the host organism.