Abstract

Plant growth promoting rhizobacteria (PGPR) are known to promote plant growth through variety of mechanisms such as nutrients mobilization, production of plant growth hormones, inducing abiotic stress tolerance, biocontrol of plant pathogens. However, efficacy of plant growth promotion depends on the abundance and functional status in the rhizosphere. *Bacillus* has been one of the dominant plant growth promoting rhizobacteria (PGPR) with efficient biocontrol properties involving secretion of small antimicrobial compounds and enzymes. Since the *Bacillus* spp. possesses good biocontrol abilities and mycorrhizal helper ability, *Bacillus* spp. with phosphate and potassium solubilizing properties could be a very effective PGPR. High amount of oxalic acid secretion is reported in fungi, including *Aspergillus niger*, *A. fumigatus*, *Botrytis cinerea*, *Fomitopsis palustris* and *Penicillium* spp. etc. In fungus oxalic acid synthesis is governed by a cytoplasmic enzyme oxaloacetate acetyl hydrolase (OAH) which breaks down oxaloacetate in oxalic and acetic acid. On the other hand, high amount of oxalate secretion in fungi is mediated by efficient oxalate transporter, which could explain the high amount of oxalate secreted. *Formitopsis palustris* is a wood rotting fungus and degradation of wood is mediated by oxalic acid secretion with the help of an oxalate transporter encoded by *FpOAR* gene. *Bacillus* strains secreting oxalic acid in sufficient amount will be very effective in mineralizing the inorganic phosphate and inorganic potassium.

In addition to this, *Bacillus subtilis* has been considered a model organism to study formation of complex multicellular structures called biofilm. Impaired respiration is one of the environmental signals triggering biofilm formation in *B. subtilis*. Low oxygen concentration impairs aerobic respiration in *B. subtilis* which is sensed by KinA and KinB. KinB - a membrane embedded histidine kinase senses impaired electron transport through respiratory chain via its transmembrane segment 2 and KinA senses decrease in NAD^+ levels through its PAS A domain. Further, transduction of this signal leads to matrix production and consequent colony wrinkling by *B. subtilis*. Similar colony wrinkling is triggered by a combination of glycerol and manganese (GM) in lysogeny broth (LB) medium and biofilm production due to glycerol and manganese is sensed by the extracellular CACHE domain of KinD. Colony wrinkling is an outcome of decreased oxygen concentration that increases the surface to volume ratio facilitating greater access

to oxygen. *Vitreoscilla* hemoglobin (VHb) improves aerobic growth and bioproduct synthesis by supplying oxygen to respiratory chain. However, its effect on multicellularity is not clear in *Bacillus* spp. Here, we report that genomic integration of *vgb* in *B. subtilis* DK1042 mitigates complexity of biofilm and associated sporulation under different conditions mainly by improved respiration.

Our broad aim was to incorporate traits which can enhance the biofertilizer potential of *B. subtilis* DK1042. With this approach, we could incorporate oxalic acid production and secretion ability in *B. subtilis* DK1042 but the yield of oxalic acid was not sufficient enough to impart mineral phosphate and potassium solubilization ability. Oxalic acid in the range of 5 – 10 mM is required to solubilize mineral complexes in the rhizosphere. Oxaloacetate occurs at the very important place of anaplerotic node and it is used to replenish the citric acid cycle and for the synthesis of several amino acids so, it is possible that the activity of the enzymes other than OAH that use oxaloacetate as a substrate could be higher than OAH because of which we could not achieve higher production and secretion of oxalic acid in *B. subtilis* DK1042. On the other hand, incorporation of VHb in *B. subtilis* DK1042 enhanced its biofilm formation ability, and biofilm formation is positively correlated with the colonization and biocontrol activity of *Bacillus* spp. Therefore, these findings also emphasize on the potential use of genetically modified *Bacillus* species containing VHb as biofertilizers/biocontrol agents for sustainable crop production in future.