

Extended Abstract

Incorporation of Vitreoscilla hemoglobin gene mitigates biofilm formation in *Bacillus Subtilis* DK1042

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Abstract

Biofilm formation is often considered as a stress combating strategy adopted by bacteria in response to a variety of cellular and environmental signals. Impaired respiration triggers biofilm formation in *B. subtilis*. Vitreoscilla hemoglobin (VHb) is known to supply oxygen to respiratory chain and hence improves aerobic growth and bioproduct synthesis of a variety of bacteria including *Bacillus* spp. Although VHb improves respiration, very little efforts have been made in elucidating its effect on biofilm formation. *B. subtilis* DK1042 was genetically modified to develop two integrants NRM1113 and NRM1114 containing vgb-gfp operon under 2 and 5 copies of P43 promoters, respectively, at an amyE locus by double-crossover events. Effect of VHb on biofilm formation by integrants and wild-type (WT) was assessed on both solid and pellicle biofilm in lysogeny broth (LB) and LB supplemented with 1% glycerol and 0.1mM manganese (LBGM). Here, we report that genomic integration of vgb gene in *B. subtilis* DK1042 mitigates biofilm formation and associated sporulation under different conditions. It also decreases the sporulation associated brown pigment production in minimal medium in shake flask cultures. These findings suggest that VHb mediated prolonged vegetative state may augment the production of desired bioproducts by host *Bacillus* spp. Reduced biofilm forming phenotype in LBGM medium and hyperosmotic conditions indicates that VHb has a profound impact on entire regulatory network governing biofilm formation. Use of VHb harboring *Bacillus* biofertilizers will have a tremendous advantage during their sessile lifestyle in rhizosphere that may enhance their performance as Plant Growth Promoting Rhizobacteria (PGPR) and Rhizoremedial agents.

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A chimeric construct consisting of the double CaMV35S promoter fused to the Myc-vhb gene encoding Vitreoscilla (bacterial) hemoglobin (VHb) was introduced into *Arabidopsis thaliana* and transgenic plants were cultured under various growth and stress conditions with the aim of studying the generality phenomena. Molecular analysis indicated that the exogenous vhb was stably integrated into the *Arabidopsis* genome and successfully expressed.

Constitutive vhb expression improved the germination rate of transgenic seeds. Under submergence conditions, in vitro and in vivo bioassays revealed that the vhb expression protected transgenic plants against prolonged waterlogging. Leaf discs from the transgenic plants maintained in standard growth conditions reveal increased tolerance to nitrosative stress induced by sodium nitroprusside (SNP) treatment. Significant differences in chlorophyll a, b, total protein content and conductivity were observed among the transgenic, wild type (WT) and blank transformation (CK) plants.

No significant differences were observed between the tested VHb, WT and CK lines in the presence of oxidative stresses by paraquat treatment. VHb expression also affected the levels of some *Arabidopsis* endogenesis genes that are involved in oxygen metabolism and antioxidants biosynthesis; up-regulating their expression at transcriptional level.