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

Riddhi Vyas

The Maharaja Sayaji Rao University of Baroda, India

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.

rvvyas.biotech@gmail.com

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