

Heterologous gene expression in *Pseudomonas denitrificans* using Cm inducible promoter:  
Case study with KGSADH

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3-Hydroxypropionic acid (3-HP), a commercially important chemical, can be biologically produced from glycerol by two enzymes consisting of glycerol dehydratase and aldehyde dehydrogenase. The alpha-ketoglutaric semialdehyde dehydrogenase (KGSADH) from *Azospirillum brasilense*, an alternate aldehyde dehydrogenase (ALDH) has been selected as the second enzyme in the pathway producing 3-HP from glycerol because of its superior performance. However, the transcriptional level of heterologous *kgsA* gene in *Pseudomonas denitrificans* strain was not up-regulated at expected level To address this limitation, KGSADH expression has been studied at transcription, mRNA stability and translation levels through several approaches. In this study, we focus on manipulation of 3-HP inducible promoter in *P. denitrificans*, Pcm and Pc4 with single or dual promoter strategies, fusion of varying length of N-mmsA to *kgsA*, optimization of the first 10 codons of *kgsA* and 5'-untranslated region (UTR) engineering. The results showed that the KGSADH activity was augmented 20-fold higher in the comparison with the original one.