Transcriptional and translational regulation of enzymes involved in 3-hydroxypropionic acid production using synthetic cassette architecture

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Commercial production of 3-hydroxypropionic acid (3-HP) from glycerol encounters several challenges such as 3-hydroxypropionaldehyde (3-HPA) accumulation, cofactor availability and 3-HP toxicity. Among them, 3-HPA accumulation is considered as a serious issue as the 3-HPA inactivate glycerol dehydratase (DhaB) and aldehyde dehydrogenase (KGSADH), the key enzymes for 3-HP production, and reduce the cell growth. Therefore, balancing the activity of DhaB and KGSADH is very important. To this end, we tried to control the expression levels of DhaB and KGSADH by range of transcription levels of dhaB with higher transcription level of KGSADH. The translation efficiency was normalized for both the genes. We used different strength synthetic constitutive promoters to alter the transcription levels and a bicistronic ribosome binding site that nullify the effect of mRNA secondary structures on translation efficiency. dhaB and kgsadh were cloned under the control of low and medium strength promoters, respectively and expressed in E. coli W. The controlled expression of DhaB and KGSADH were validated in both transcriptional and translational level, using RT-PCR and SDS-PAGE analysis, respectively. The recombinant E. coli did not accumulate even small amount of 3-HPA throughout the production and produced ~52 mM 3-HP which is ~20% higher than the reference strain (~41 mM). The activity of DhaB and KGSADH were also present till the end of fermentation. Further tuning of this system will increase the productivity and titer of 3-HP production.