Conversion of Glycerol to 3-Hydroxypropanoic Acid by Genetically Engineered Bacillus subtilis

Authors : Aida Kalantari, Boyang Ji, Tao Chen, Ivan Mijakovic

Abstract : 3-hydroxypropanoic acid (3-HP) is one of the most important biomass-derivable platform chemicals that can be converted into a number of industrially important compounds. There have been several attempts at production of 3-HP from renewable sources in cell factories, focusing mainly on Escherichia coli, Klebsiella pneumoniae, and Saccharomyces cerevisiae. Despite the significant progress made in this field, commercially exploitable large-scale production of 3-HP in microbial strains has still not been achieved. In this study, we investigated the potential of Bacillus subtilis to be used as a microbial platform for bioconversion of glycerol into 3-HP. Our recombinant B. subtilis strains overexpress the two-step heterologous pathway containing glycerol dehydrates and aldehyde dehydrogenase from various backgrounds. The recombinant strains harboring the codon-optimized synthetic pathway from K. pneumoniae produced low levels of 3-HP. Since the enzymes in the heterologous pathway are sensitive to oxygen, we had to perform our experiments in micro-aerobic conditions. Under these conditions, the cell produces lactate in order to regenerate NAD+, and we found the lactate production to be in competition with the production of 3-HP. Therefore, based on the in silico predictions, we knocked out the glycerol kinase (glpk), which in combination with growth on glucose, resulted in improving the 3-HP titer to 1 g/L and the removal of lactate. Cultivation of the same strain in an enriched medium improved the 3-HP titer up to 7.6 g/L. Our findings provide the first report of successful introduction of the biosynthetic pathway for conversion of glycerol into 3-HP in B. subtilis.

Keywords : bacillus subtilis, glycerol, 3-hydroxypropanoic acid, metabolic engineering

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