Poster | 33.Poster

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[P005] Poster

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[P03-353] Deletion of cell wall-related gene enhances D-lactic acid production from methanol in *Komagataella phaffii*

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Methanol is predominantly produced from natural gas but can also be synthesized from carbon dioxide and waste biomass, making it an attractive renewable carbon source for sustainable bioproduction. The methylotrophic yeast *Komagataella phaffii* can utilize methanol as the sole carbon source and has been widely used as a microbial cell factory for producing valuable biochemicals from methanol. In our previous study, we constructed a D-lactic acid-producing *K. phaffii* strain and improved its production capability through UV mutagenesis. Transcriptome analysis of a high-producing mutant revealed that the expression of *PAS_chr4_0305* (*PAS_0305*), which encodes an O-glycosylated cell wall protein involved in cell wall stability, was significantly decreased compared with that of the parental strain. Previous studies also reported that the deletion of the corresponding gene results in a thickened cell wall and the activation of cell wall stress sensors, which enhance methanol assimilation efficiency. Based on these findings, we hypothesized that deletion of *PAS_0305* could improve D-lactic acid production in our engineered strain.
In this study, we deleted *PAS_0305* of D-lactic acid producing strain using a CRISPR/Cas9 genome editing system. The resulting strain was evaluated for D-lactic acid production in shake-flask and fed-batch flask cultivations using methanol as the sole carbon source. In shake-flask cultivation using 50 mL of YPM medium containing 30 g/L methanol, the *PAS_0305* deletion strain showed a 1.20-fold increase in D-lactic acid production compared with the parental strain. To investigate the physiological changes associated with the gene deletion, transcriptome analysis was performed. The results indicated that genes related to methanol metabolism were upregulated in the deletion strain. In addition, genes related to thiamine biosynthesis were also upregulated, whereas genes related to ribosome biogenesis were downregulated. These transcriptional changes suggest that deletion of *PAS_0305* alters cellular resource allocation and metabolic regulation, potentially contributing to improved methanol assimilation and D-lactic acid production.
Furthermore, fed-batch cultivation was conducted in shake flasks using 50 mL of YPM medium buffered with 100 mM potassium phosphate, with methanol feeding (504 µL) and supplementation with 0.5–2.0% (v/v) YP solution (100 g/L yeast extract and 200 g/L peptone) every 24 h. Under these conditions, the *PAS_0305* deletion strain produced 25.7 g/L of D-lactic acid. To the best of our knowledge, this represents the highest D-lactic acid production from methanol. These results demonstrate that engineering of cell wall-related genes can influence methanol metabolism and represents a promising strategy for improving methanol-based bioproduction in methylotrophic yeasts.