Abstract

Lactic acid is a valuable monomer used that utilizes lactate dehydrogenase for its production. Lactate dehydrogenase found in many lactic acid bacteria including *Lactiplantibacillus plantarum.* Utilizing a heterofermentative lactic acid bacteria, such as *L. plantarum*, for lactic acid production is not efficient due to generation of other organic acid byproducts. The yeast *Komagataella phaffii* has emerged as a promising candidate for genetic and metabolic engineering, owing to its well-established engineering tools and strong promoter such as P_{AOX1}. This study explores the feasibility of metabolic engineering in *K. phaffii* by substituting its *Alcohol Dehydrogenase 2* gene (*KpADH2*) with *L. plantarum* lactate dehydrogenase (*LpLDH*) gene using CRISPR/Cas9 technology. Primer pairs for constructing the donor DNA cassette were designed to consist of the *LpLDH* coding sequence which was regulated by the *KpADH2* promoter and terminator. Additionally, sgRNA targeting *KpADH2* was constructed and assembled into a Plasmid BB3cK_pGAP_23*_pLAT1_Cas9 plasmid. An in silico characterization of the *LpLDH* was performed. The gene fragment corresponding to the upstream of *KpADH2*, the *LpLDH* coding sequence, and the downstream of *KpADH2* was successfully isolated, measuring approximately 985, 1019 and 985 base pairs in length. However, construction of these fragments into a donor DNA cassette requires further optimization. The sgRNA was successfully constructed and inserted into the CRIS*Pi* plasmid indicated by colony PCR. Moreover, In silico analysis of the isolated *LpLDH* coding sequence showed 100% similarity with the reference genome, its physicochemical properties suggest the protein to be structurally stable. It showed similar β-sheet and α-helix structures as LDH through protein primary and secondary structure analysis, and its predicted three-dimensional structure conformation. Lastly, the predicted structure showed similar protein motifs and domains. This preliminary research showed the feasibility of the integration of *LpLDH* within the *KpADH2* locus using CRISPR/Cas9 approach.

Keywords: *Komagataella phaffii*, Lactic acid, *Lactiplantibacillus plantarum*, CRISPR/Cas9, Metabolic engineering