Computer-aided engineering of the Ketoacyl Synthase domain of Yarrowia lipolytica Fatty Acid Synthase for the production of Medium Chain Fatty Acids

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Abstract

Yarrowia lipolytica is a promising organism for the production of lipids of biotechnological interest and particularly for biofuel. In this study, we engineered lipid biosynthesis to modulate fatty acid chain length and produce shorter fatty acids. Based on the hypothesis that the Ketoacyl Synthase (KS), which is the Fatty Acid Synthase (FAS) domain responsible for chain elongation in *Yarrowia lipolytica,* is directly involved in chain length determination, we followed a rational strategy to design mutants of the Ketoacyl Synthase. Molecular modelling of this domain in interaction with a C16-acyl substrate allowed the identification of residue from the fatty acid active site which was targeted by mutagenesis in order to alter KS fatty acid chain length specificity. We applied the TALEN technology for the first time to *Yarrowia lipolytica* and demonstrated the efficiency of the technique for gene silencing and its usefulness to perform site-directed mutagenesis at a specific genomic locus. Among the generated FAS mutants, those having an aromatic amino acid residue in place of the native Isoleucine, led to a significant increase of C14 fatty acid. Particularly, the best mutant gave a 29 fold increase in C14 accumulation (of DWC) and trace amounts of C12 fatty acid. The study provides for the first time, evidences that the KS domain of the fungal FASI system is directly involved in fatty acid chain length specificity.

Keywords: Fatty acid synthesis, enzyme engineering, genome editing, Yarrowia lipolytica

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