PRODUCTION OF MEDIUM CHAIN FATTY ACID BY *YARROWIA LIPOLYTICA:* COMBINING MOLECULAR DESIGN AND TALEN TO ENGINEER THE FATTY ACID SYNTHASE.

Coraline Rigouin, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France
Marc Gueroult, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France
Christian Croux, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France
Gwendoline Dubois, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France
Vinciane Borsenberger, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France
Sophie Barbe, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France
Alain Marty, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France
Fayza Daboussi, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France
Isabelle André, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France
Florence Bordes, LISBP, Université de Toulouse, CNRS, INRA, INSA, Toulouse, France

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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 through rational engineering of the giant multifunctional Fatty Acid Synthase (FAS) enzyme to modulate fatty acid chain length and produce shorter fatty acids. Based on the hypothesis that the Ketoacyl Synthase (KS) domain, responsible for chain elongation in Yarrowia lipolytica, is directly involved in chain length specificity, a computer-based strategy was undertaken to re-design mutants of the Ketoacyl Synthase.

Molecular modelling of this domain in interaction with a C16-acyl substrate enabled identification of a key residue from the fatty acid binding site. This site was then targeted by mutagenesis in order to modify KS fatty acid chain length specificity. To introduce point mutations in this essential gene, we applied, for the first time, the TALEN technology to Yarrowia lipolytica and demonstrated the efficiency of the technique to perform site-directed mutagenesis at a specific genomic locus. Some mutants led to a significant increase of C14 fatty acid. Thanks to the use of an elegant combination of genome editing technology and molecular modelling, this 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.