Cell engineering for Ricinoleic Acid production in oleaginous yeast Yarrowia lipolytica

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Abstract

Ricinoleic acid (RA) is an hydroxylated fatty acid of industrial interest currently extracted from castor oil. A microbiological alternative strategy to product RA was developed in order to overcome castorbean farming linked issues and to meet market demands.
An engineered strain of the oleaginous yeast *Yarrowia lipolytica* was chosen for the RA production. This strain – OleoX combines 10 deletions which maximize the pool of available oleic acid which is the substrate used for bioconversion to RA. As a result, the fatty acid composition in the chassis OleoX strain contains mainly oleic acid ($> 80\%$).

Two hydroxylases originated from castorbean (RcFAH12) or fungi (CpFAH12) were expressed in OleoX. The strain expressing CpFAH12 was able to product RA (52%), with a coproduction of linoleic acid. Using promoter 4UAS-pTEF, stronger than pTEF, led to a strain producing RA up to 65% of its total lipid content.
With the objective to optimise this chassis strain for library construction, a docking platform was introduced in OleoX strain to guide the integration of expression cassettes at a specific locus. The objectives are to improve both transformation efficiency and expression level reproducibility. This strain will be used to construct a library of mutants of FAH12.

Keywords: Lipids, Yarrowia lipolytica, Ricinoleic acid, Membrane, bound Desaturases

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