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A facile and robust non-natural three enzyme biocatalytic cascade based on *Escherichia coli* surface assembly for fatty alcohol production

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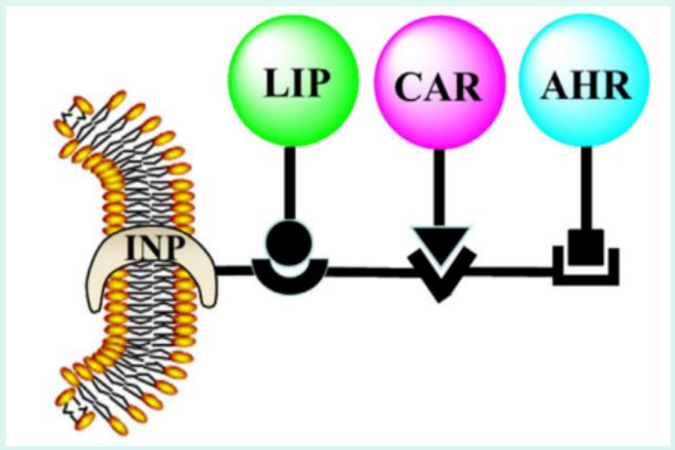
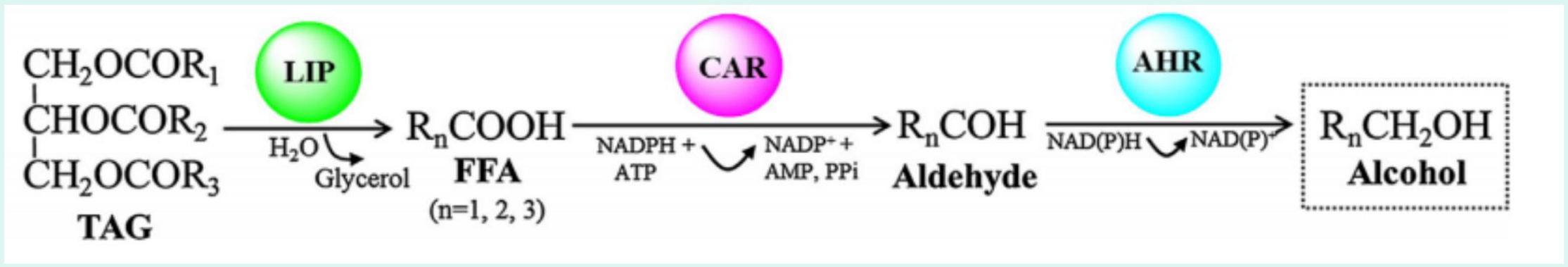
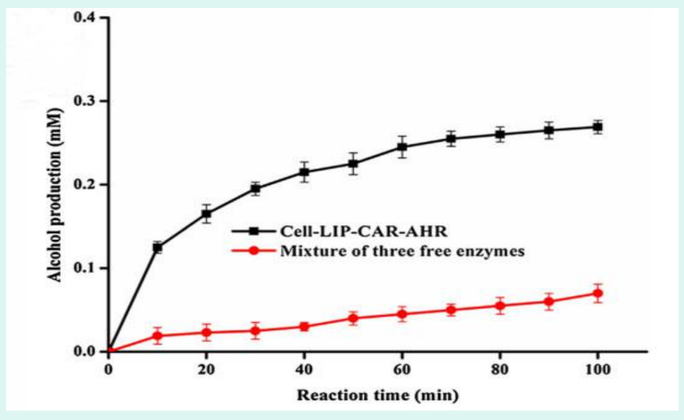
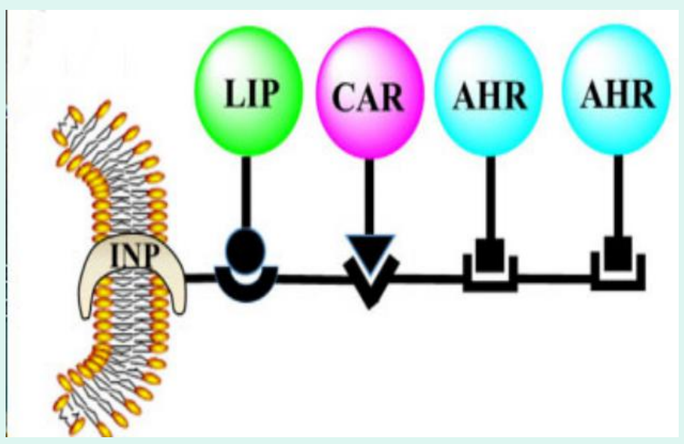




ABSTRACT:Fatty alcohol is considered as a promising clean fuel alternative to fossil fuel. In this study, we developed a facile and robust multiple enzyme complex of lipase (LIP), carboxylic acid reductase (CAR) and aldehyde reductase (AHR) based on cohesin-dockerin specific interaction and E. coli-ice nucleation protein (INP) surface assembly for highly efficient conversion of triacylglycerols (TAGs) to fatty alcohols. The proximity effect, customized ratio and positional arrangement of the three enzymes were readily genetically generated. The optimized cell asso_x0002_ciated three enzyme complex gave 73% of conversion yield, and showed significantly improved stability in higher temperature, acidic/alkaline environments, polar organic solvents, high concentration of substrate, and good recyclability in successive biotransformations. The three enzyme complex provides an efficient non-natural synthetic cascade route for fatty alcohol bioproduction alternative to complex de novo biosynthesis route.





Experiment results:

The proximity effect of the three enzymes in multiple enzyme complex reflected as higher reaction rate compared to three free enzyme mixture

Comparison of the panel of multiple enzyme complex in conversion of TAGs to alcohols showed that the highest conversion yield (56%) was achieved by LIP: CAR: AHR of 1:1:2 Probably, the AHR enzyme responsible for catalyzing the final step of alcohol formation is a rate limiting enzyme in the cascade reaction. Thus increasing AHR enzyme dosage resulted in the highest conversion yield among these enzyme ratio tested.