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Applications of Carboxylic Acid Reductases in Oleaginous Microbes

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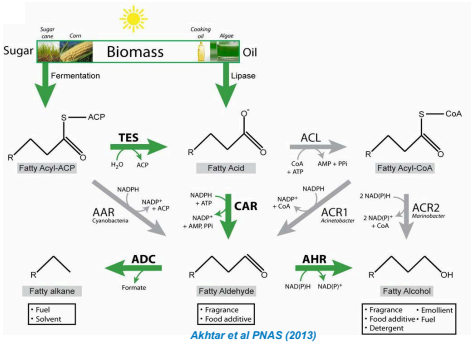
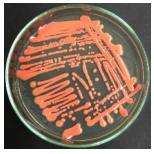
Abstract

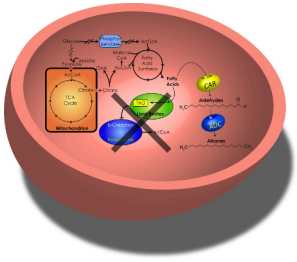
Carboxylic acid reductases (CARs) are recently emerging reductive enzymes for the direct production of aldehydes from biologically-produced carboxylic acids. Recent work has demonstrated that these powerful enzymes are able to reduce a very broad range of volatile- to long-chain fatty acids as well as aromatic acids. Here, we express four CAR enzymes from different fungal origins to test their activity against fatty acids commonly produced in oleaginous microbes. These in vitro results will inform metabolic engineering strategies to conduct mild biological reduction of carboxylic acids in situ, which is conventionally done via hydrotreaing catalysis at high temperatures and hydrogen pressures.



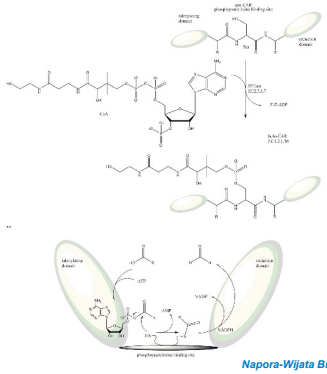
Akhtar et al PNAS (2013)

Engineering Rhodosporidium toruloides for the direct production of Alkanes



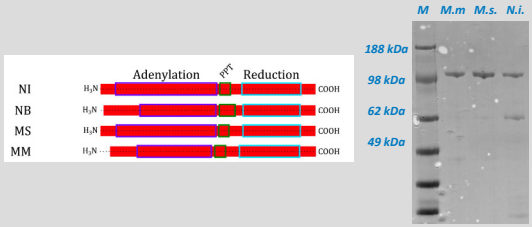


CARs Require Phosphpantaline

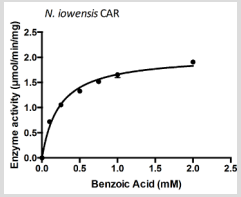


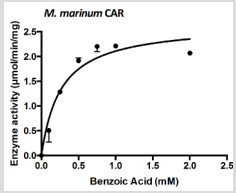
Napora-Wijata Biotech. J. (2014)

Expression and Purification of CARs



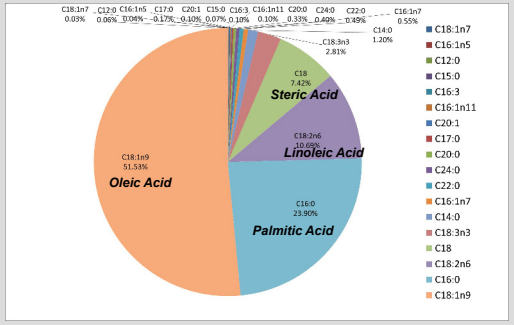
Enzymatic Characterization of CARs



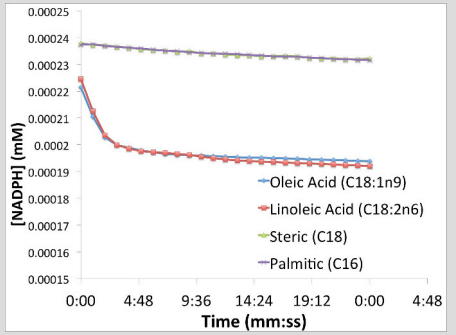


	K_{cat} (min^{-1})	Km (mM)	Vmax (μmol/min/mg)
N. iowensis	265.8	0.2289	2.040
M. marinum	343.2	0.2622	2.661

Composition of Fatty Acids in Yeast



Inhibition of CARs



Conclusions

- *We are able to transform and express genes in R. toruloides*
- *Biophysical characterization of CARs will lead to new insights into improving enzymatic rates*
- *Oleic and palmitic acid account for ~75% of the FA in yeast*
- *Enzymes rates slow and are prone to inhibition ~~NADP, FA or CA, Cofactor limitations, product inhibition~~*

Future Directions

- *Improve expression in R. toruloides*
- *Determine inhibitors*
- *Solve X-tal structure of recombinant enzymes*

This work has been supported by the Department of Energy, Office of Energy Efficiency & Renewable Energy, BioEnergy Technologies Office

SIMB Symposium on Biotechnology for Fuels and Chemicals
Baltimore, MD
April 24-28, 2016
NREL/PO-5100-66304