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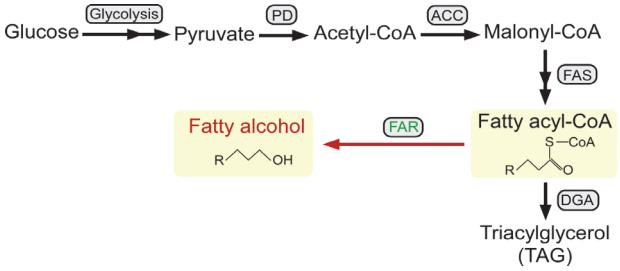
Metabolic engineering of oleaginous yeasts for fatty alcohol production

Wei Wang, Hui Wei, Eric Knoshaug, Stefanie Van Wychen, Qi Xu, Michael E. Himmel, Min Zhang
National Renewable Energy Laboratory, Golden, CO 80401 * *Wei.Wang@nrel.gov*

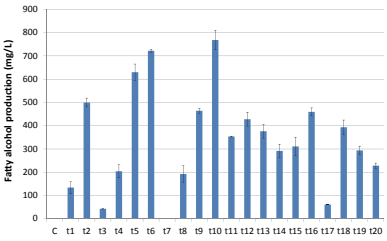
Abstract

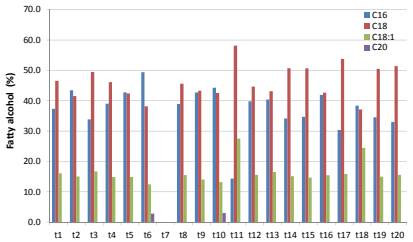
To develop pathways for advanced biological upgrading of sugars to hydrocarbons, we are seeking biological approaches to produce high carbon efficiency intermediates amenable to separations and catalytic upgrading to hydrocarbon fuels. In this study, we successfully demonstrated fatty alcohols production by the oleaginous yeasts, *Yarrowia lipolytica and Lipomyces starkeyi*, by expressing the bacterial fatty acyl-CoA reductase (FAR) gene, resulting in the production of 167 and 770 mg/L of fatty alcohols in shaking flasks, respectively. Moreover, we find much higher extracellular distribution of fatty alcohols produced by FAR-expressing *L. starkeyi* compared to *Y. lipolytica*. In both yeasts, long chain length saturated fatty alcohols, i.e., hexadecanol and octadecanol were predominant, accounting for more than 85% of total fatty alcohols produced. Our work demonstrates that, in addition to *Y. lipolytica*, *L. starkeyi* can also serve as a platform organism for production of fatty acid-derived biofuels and bioproducts following metabolic engineering.

Engineered pathway for fatty alcohols production in oleaginous yeast



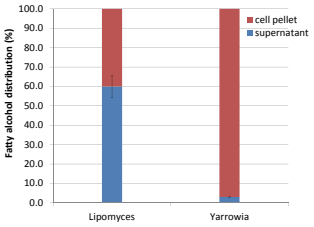
Production of fatty alcohol in Lipomyces Ls[FAR]





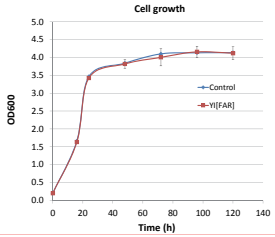
- 70 transformants were confirmed to produce fatty alcohols with varied levels, among which # 10 produced 770 mg/L. This production level is about 5 fold more than in *Y. lipolytica*, which could be related to the robust fatty acid synthesis in *L. starkey* as indicated by high lipid content in wild type cells;
- The patterns of fatty alcohol composition in *L. starkeyi* FAR transformants were similar to that of *Y. lipolytica*. i.e. long chain fatty alcohols were the major constituents.

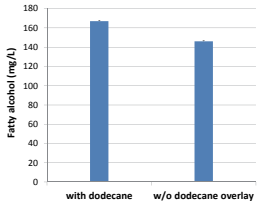
Partition of fatty alcohols in FAR transformants



- The distributions of the fatty alcohols produced by *Y. lipolytica* and *L. starkeyi* were quite different. In *L. starkeyi*, more fatty alcohols, at least 50% of the total, were secreted out of the cells into the medium. This difference could be related to the permeability of cell membranes.

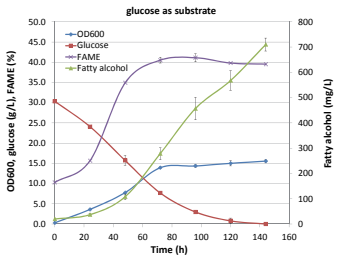
Production of fatty alcohols in Yarrowia YI[FAR]

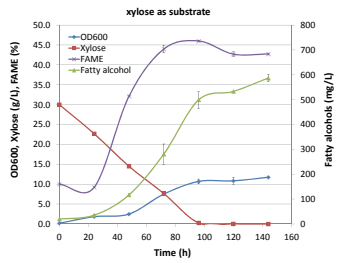




- we did not find that our YI[FAR] transformant exhibited slower growth than the parent strain; Expressing the FAR gene in *Y. lipolytica* resulted in production of fatty alcohol up to 167 mg/L in mineral medium

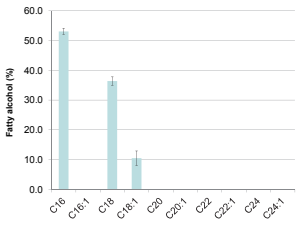
Fatty alcohol production with glucose and xylose as substrate





- *L. starkeyi* can utilize both glucose and xylose for fatty alcohol productions;
- The profile of fatty alcohol production on xylose is similar to that on glucose, except cell mass and the fatty alcohol titer were slightly lower than those on glucose.

Fatty alcohols composition in YI[FAR]



- Over-expressing the FAR gene in *Y. lipolytica* led to a decrease in lipid content, indicating the carbon flux originally to TAG synthesis was partially redirected towards fatty alcohol production;
- The fatty alcohols with long chain length, i.e. hexadecanol (C16), octadecanols (C18), and (C18:1) accounted for 53.1%, 36.4%, and 10.5% respectively of total alcohols. Saturated fatty alcohols were predominant in all produced fatty alcohols which could be beneficial for downstream hydrotreating to hydrocarbons

Conclusions and challenges

- This study demonstrates fatty alcohol production by oleaginous yeasts *Y. lipolytica* and *L. starkeyi*. Our work indicates that, in addition to *Y. lipolytica*, *L. starkeyi* could also serve as a platform organism for production of fatty acid-derived biofuels and bioproducts via metabolic engineering.

- To achieve this goal, however, more efficient molecular biology tools have to be developed for further genetic manipulation of *L. starkeyi*; in addition, more genomic information has to be acquired.

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