Lipase overexpression in Yarrowia lipolytica for direct biofuel production

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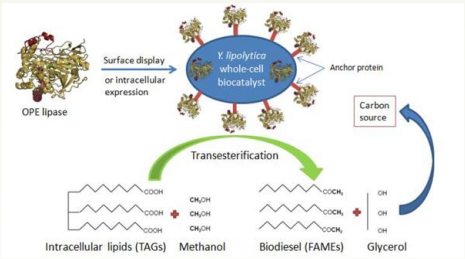
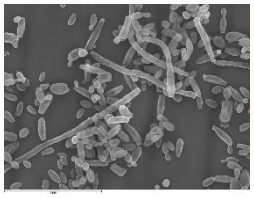
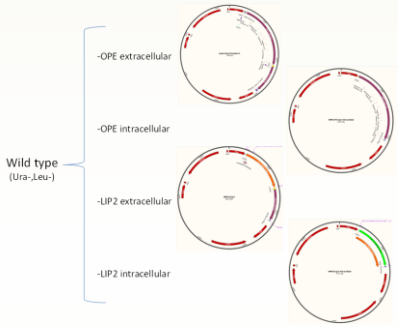
Motivation

The production of biodiesel by the transesterification of microbial lipids is currently an alternative to conventional no-renewable liquid fuels. *Yarrowia lipolytica* is an oleaginous yeast able to accumulate lipids up to the 80% of its total DCW. This yeast also metabolizes a wide variety of substrates generating mainly C16-18 TAGs (Biotechnol Biofuels 8,104). For the accumulation of lipids in *Yarrowia* fermentations are performed starting with an initial cellular growth phase, followed by a N_2 limited stage with a high molar C/N ratio (Biores Technol 82,1). In addition, robust fungal lipases, with high stability to organic solvents, such as those from *Yarrowia* or *Ophiostoma piceae* (OPE) can be used for the enzymatic production of biodiesel.







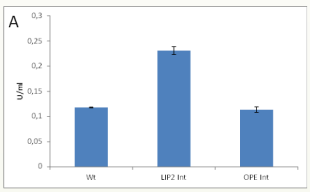
Objectives

The aim was to build a *Y. lipolytica* obese strain expressing a robust lipase for the *in situ* production of biodiesel. The engineered strain will be able to produce TAGs from glycerol and to produce a lipase (either intracellularly or surface-displayed), which after cell lysis with organic solvent and methanol will perform the direct transesterification of TAGs into FAMEs (biodiesel).

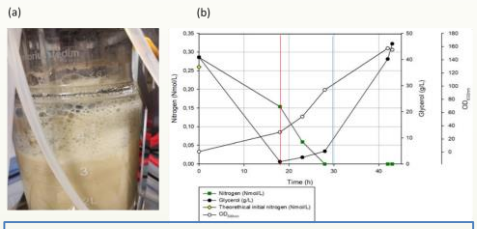
Lipase constructions in the plasmid JMP62 for genomic integration in W29 strain (pTEF promoter, Lip2 SP and Cwp3 for surface display).

Bioreactor batch cultivations were performed using a 1L DASGIP® Parallel Bioreactor System for optimization and a 4L Biostat® B Benchtop glass bioreactors for production. Fermentations were performed using 20 g/L of glycerol as carbon source and $(NH4)2SO4$ as nitrogen source, with 3 L/min aeration. The µmax obtained was of 0.27 h-1.

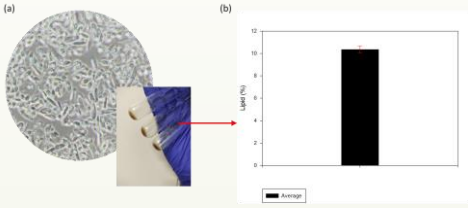
Direct transesterification of triolein with the cell lysates with intracellular Lip2 activity was performed as a poof of concept. Lysates resuspended in petroleum ether and 5 mol of methanol for each mol of lipids were incubated at 30 ºC 24h and 1400 rpm. The products of transesterification were detected after derivatization in a GC/MS Agilent 5975C-7890A



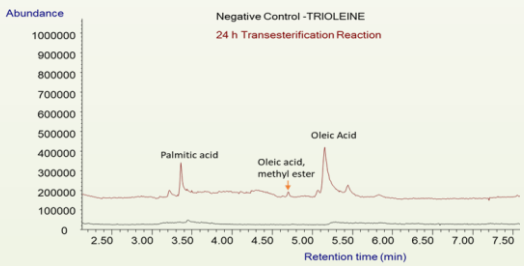
Intracellular esterase activity, normalized to the OD, measured with *p*NPB after 24h culture in YPD.



a) Foam formation during fermentation. (b) Nitrogen, OD600 and glycerol time evolution. Red line indicates the start of the feeding phase and the blue line the change on the pre-programmed exponential substrate feeding rate.



(a) Final microscopic image of yeast cells and total lipid extracted. (b) Lipid percentage of total biomass dry weight achieved at the end of the fermentation.



Transesterification of triolein in red, negative control with no lipase activity in black.

Conclusions

We performed a proof-of-concept producing FAMES from TAGs using *Yarrowia* Lip2 lipase activity. Taking into consideration the obtained results, further work will be mainly related to generate new strain constructions with high lipase activity performance and optimization of the molar C/N relation in fed-batch cultivations to reach a balance between total protein production, protein activity and increased lipid accumulation. In addition, the transesterification reactions will be optimized using different enzyme dosages, methanol molar ratios, etc.

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