Genetic engineering of lipid metabolism and substrate utilization for improvement of biolipid production by oleaginous yeast

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TEAM DESCRIPTION

The BIMLIP team has pioneered the development of genetic and metabolic engineering in the yeast *Yarrowia lipolytica*, with a main focus on lipid metabolism. Both modification of targeted candidate genes and OMIC approaches (genomic, comparative genomics, transcriptomic, proteomic and metabolomic) has been carried out on pathways involved in lipid metabolism (synthesis, accumulation, storage, remobilization, degradation of lipids) and on the compartments involved in these metabolisms such as peroxisomes and lipid bodies.

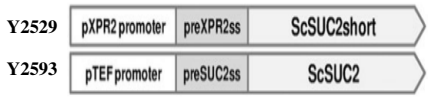
PROJECT DESCRIPTION

The yeast *Yarrowia lipolytica* is currently the subject of numerous studies due to its ability to produce molecules of interest such as citric acid, erythritol and biofuels. The production of alternative fuels by the mean of microorganisms from non food resource has become an important challenge to face the increasing demand and pricing, for this reasons, *Y. lipolytica* appears to be a promising microbial biofuel producer. The scientific purpose of the project is to discover and to understand the mechanisms of lipid biosynthesis and accumulation in *Y. lipolytica*. In addition, carbon source utilization remains an essential step for these processes, therefore hexose transport into the cell occurring in this yeast is an additional purpose of this project. In the yeast *Saccharomyces cerevisiae* glucose and fructose are uptaken by the same family of transporters (HXT1-17). Therefore, to identify the presence of similar transporters in *Y. lipolytica* or to determine specific ones for glucose and fructose uptake will get insight about this phenomenon. It will be equally essential to examine the mechanisms controlling gene expression as well as the factors that influence the rate of transport.

MATERIALS AND METHODS

Microorganism

Clones of *Yarrowia lipolytica* overexpressing SUC2 gene from *S. cerevisiae* in two different constructions were analyzed.



CA and invertase biosynthesis conditions

Production medium in 1L of tap water contained: sucrose (100 g), NH_4Cl (1.5 g), KH_2PO_4 (0.7 g), $MgSO_4 \times 7H_2O$ (1 g), YE (0.3 g), thiamine 300 µg. The CA biosynthesis as batch fermentation (BC) was conducted in stirred 2L bioreactor "Biostat B Plus" during 72 hours, at 30°C with agitation rate of 800 rpm and aeration of 0.36 vvm. Culture acidity was automatically controlled at pH 6,8 (40% NaOH). Inoculum medium contained in 1L: carbon source (50 g), NH_4Cl (1.5 g), YE (1,0 g), peptone (0.1%).

Analysis

CA, sucrose, glucose and fructose were determined by HPLC separation on Animex HPX-87H column (flow rate of 0.6 mL/h, elution with 0.01 N H_2SO_4, UV detection at 210 nm for acids and refraction index-RI- for sugars).

qPCR and overexpression of putative hexose transporters in *S. cerevisiae*

Fold of genes upregulation in fructose based medium compared to their expression in glucose based medium was checked by qRT-PCR with SYBR Green (34 genes).

Genes encoding putative hexose transporters were amplified by PCR (21 genes). Constructs for their overexpression were prepared with 2µ plasmid pRS426ADH1 (gift from Dr. M. Künzler). Transformation of *S. cerevisiae* (hxt null mutant, gift from Prof. E. Boles) were performed by lithium acetate method. Transporter activity was analyzed with glucose, fructose, galactose and mannose as a carbon source.

RESULTS

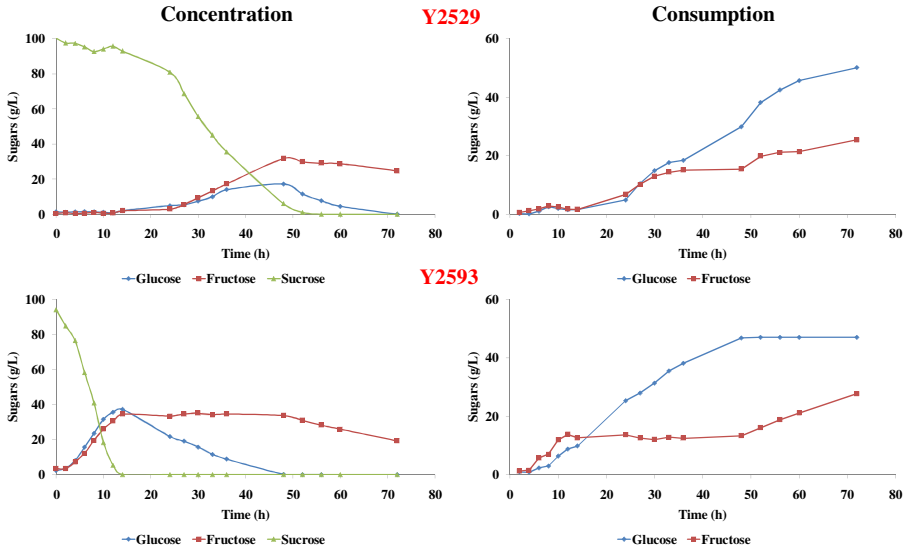


Fig. 1. Sugar concentration and consumption during *Y. lipolytica* SUC+ transformants culture in sucrose based medium

Tab. 1. Putative transporter activity analysis by its overexpression in *S. cerevisiae* hxt null mutant

Gene	Glu			Fru			Man			Gal		
	%											
	0.1	1.0	2.0	0.1	1.0	2.0	0.1	1.0	2.0	0.1	1.0	2.0
YALI0C06424	++	++	++	++	++	++	++	++	++	++	++	++
YALI0C16522		+									±	
YALI0C08943				++	+							
YALI0B01342	++	++	++				++	++	++	++	++	++
YALI0B06391							+	+			±	±

Tab. 2. Fold of genes upregulation in fructose based medium (normalized to actin)

Gene	Fold of upregulation	SD
YALI0D01111	21.02	1.64
YALI0F25597	2.70	0.01
YALI0F18084	9.03	3.03
YALI0B00396	2.51	0.46
YALI0E34903	5.17	0.92
YALI0C00825	72.17	12.5

SUMMARY

1. Expression of SUC2 gene under TEF promoter and with its native signal sequence resulted in increased invertase activity, what allowed for higher rate of sucrose hydrolysis.
2. Glucose as a carbon source was preferentially consumed over fructose.
3. Assimilation of fructose was inhibited by high concentration of glucose in the medium.
4. Among 21 genes, 5 of them had transporter activity towards different sugars.
5. Among 34 genes, 6 appeared to be fructose inducible.