

The impact of metabolic engineering of yeast, designed to produce valuable biomolecules through fermentation, on resulting spent yeast composition

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Introduction

Yeasts are being used as cell factories to sustainably produce valuable molecules for various industries including the pharmaceutical and cosmetics. The metabolic engineered strains developed by Amyris, Inc. (Nasdaq: AMRS) produce high-value biomolecules such as isoprenoids, terpenoids and fragrances among others, through fermentation processes. These processes generate yeast residues which represent up to 15-20% of the total by-products from fermentation. These waste streams are potential new sources of biologically active ingredients. However, these new strains, are not characterized and their compositional profiles are unknown. Efforts should be made to determine the compositional profile of these strains as well as their bioactivities to better define valorization approaches for these streams.

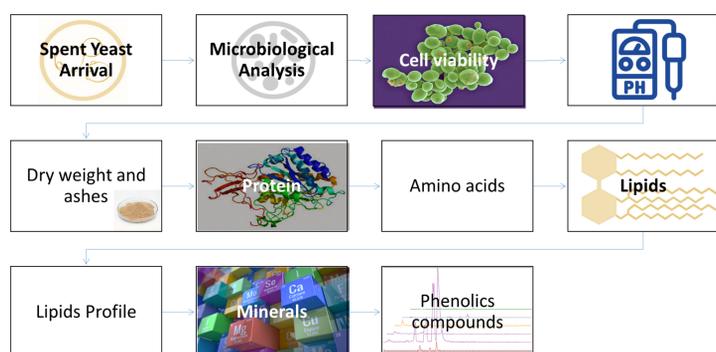
Methods

Yeast strains

6 strains developed by Amyris were analyzed

Strain #	Strains producing different molecules	Sub-strains
1	Wt_F	Wt_F1 Wt_F2
2	Wt_M	Wt_M1 Wt_M3 Wt_M5
3	Wt_P	Wt_P1
4	Wt_R	Wt_R7 Wt_R9
5	Wt_SD	Wt_SD1
6	Wt_S	Wt_S1 Wt_S2 Wt_S3

Experimental methods



Statistical Analysis

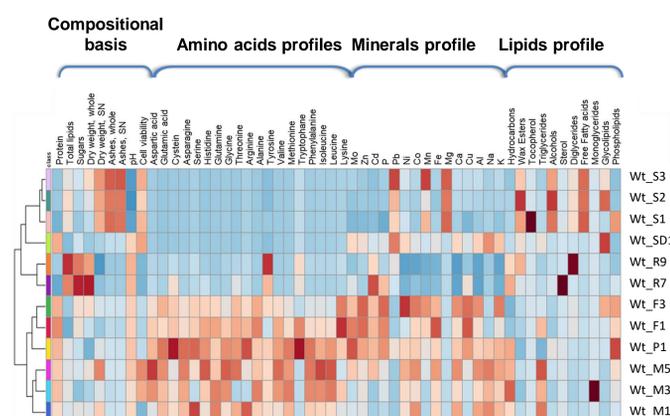
For data visualization a Heatmap with clustering, including all variables and samples was performed. In order to understand if it was possible to distinguish the different strains based on their composition a Partial Least Square Discriminant Analysis (PLS-DA) using all variables and analyzed samples was achieved.

Objectives

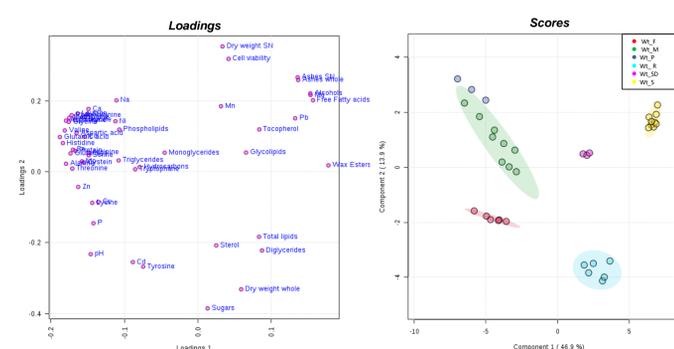
- Characterization of spent yeast resulting from fermentative processes where metabolic engineered yeast are utilized to produce specific molecules.
- Discriminate different spent yeast strains based on their composition.
- Develop knowledge to define valorization strategies for spent yeast resulting from Amyris fermentation processes.

Results and Discussion

Heatmap with clustering of different spent yeasts, based on composition



Partial Least Square Discriminant Analysis (PLS-DA)



The Heatmap shows the extent of the differences in composition among the different strains.

The PLS-DA clearly separates the different strains based on differences in composition.

PLS-DA highlights specific constituents distinctive of each yeast strain:

- Wt_F, Wt_M and Wt_P are highly rich in protein and minerals
- Wt_R has high lipid and sugar contents
- Wt_SD is rich in tocopherol and glycolipids
- Wt_S is rich in magnesium and free fatty acids.

The results show that the final composition of spent yeasts resulting from Amyris fermentation processes is highly dependent on the metabolic changes biotechnologically introduced in each strain.

Conclusions

- The different metabolic engineered yeasts developed for the production of each specific molecule showed significantly different compositional profiles.
- Depending on the molecule produced, the spent yeast strains showed a distinct profile pattern and it was possible, based on their composition, to distinguish the different strains.
- These results clearly show that biotechnologically engineered yeasts significantly differ from each other and should be studied individually to define valorization approaches for these residues of fermentation.

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