Towards a yeast based multivalue technology platform

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ABSTRACT

An important aspect in microbial biotechnology industrialization is economic feasibility. A common approach is to develop a microbial culture to produce a single product. This strategy in many cases fails to be economically feasible. Creating a portfolio of products out of a microbial culture can render the process economically attractive. This work utilized *Rhodosporidium babjevae*, a pink, oleaginous basidiomycete yeast sourced from the Phaff Yeast Culture Collection to test the concept at a lab scale. The yeast was cultured in shake flasks, harvested, and passed through a screw press to recover oil, and high protein yeast meal. The oil was further characterized to contain a triacylglycerol profile ideal for biofuel conversion or high heat cooking since it is rich in monounsaturated fatty acids. Pigments were analyzed using TLC, and could be further processed into high value products. Overall, this work establishes a framework to procure multiple high value products from yeast through an economically feasible, environmentally friendly and sustainable technological platform.

BACKGROUND

One of the main concerns in sourcing bioactives from microorganisms is the economic feasibility of the microbial model. The common approach for production of microbial oil for biodiesel is to grow a specific oleaginous microorganism in a media which induces lipid accumulation, harvest, and recover the oil for biofuel production[1]. The main downside of this approach is the entire process relies solely in the value of the recovered oil. In contrast, a multiproduct model, such as the one within the oilseed industry might result in a more profitable, versatile technological platform. In the oilseed industry a single starting material (e. g. soybeans) is further transformed into a portfolio of high value products, such as edible oil, lecithin, distilled fatty acids (rich in carotenoids and tocopherols), and protein derivatives (e. g. concentrates, isolates, and textured vegetable protein)[2]. The committed step in the oilseed process is the separation of the oil from the protein[3]. It is possible to emulate such model in a microbial system, where from a single culture a bundle of high value products can be obtained. Cellulosic ethanol production is and emerging biorefinery process. Waste lignin is often burned to power boilers. Production of higher value coproducts such as protein, carbohydrates, pigments and vitamins would enable the process to become more economically feasible.

PURPOSE

The present work is a lab scale proof of concept towards establishing a technological platform where oil from a yeast culture is separated via a non solvent process. The major products are a high protein defatted biomass with the potential of being further transformed into high value protein products, and an oil fraction rich in triacylglycerols (TAG), and carotenoid pigments. The oil fraction can be further transformed into high value lipid fractions, in a similar fashion as in the oilseed industry. Several products can thus be sourced from a single culture, increasing the economic feasibility of the model.

MATERIALS AND METHODS

Yeast culture and growth conditions

Yeast strain *Rhodosporidium babjevae* UCDFST 05-775 from the Phaff Yeast Culture Collection was plated in PDA and grown for 3 days. A loopful of cells was then suspended in 3 mL DI sterile water, and 400 microliters were inoculated into either a series of 2.8L Fernbach baffled flask with foam stopper containing 800 mL medium A[4], or a series of 1L Erlenmeyer flasks with foam stoppers, containing 250 mL of media . The molar carbon to nitrogen ratio of the media was 68.2. The culture was incubated for 7 days in room temperature, shaken at 200 RPM. Total intracellular lipid was determined by extracting with Folch reagent[5], evaporating the solvent, and weighing the extracted oil.

Detection of Intracellular lipid using microscopy and Nile red staining

Samples were collected during early exponential phase (12 h) and late stationary phase (6 days), and lipid content was evaluated visually using Nile Red staining[6].

Recovery of Yeast Biomass

Cells were harvested by centrifuging, washed twice, and repelleted. The cells were freeze dried to a moisture content below 5%. Cell biomass yields were calculated gravimetrically.

Non solvent extraction of Oil

Oil was extracted from dried cells using an expeller. Three runs were performed using a Monforts IBG Oekotec CA59G bench screw press, as summarized in table 1.

Characterization of the products

The oil was analyzed for pigments using Thin Layer Chromatography (TLC) with $KMnO_4$ and visualized with UV light. TAGs were identified using Liquid Chromatography-Mass Spectrometry (LC-MS).

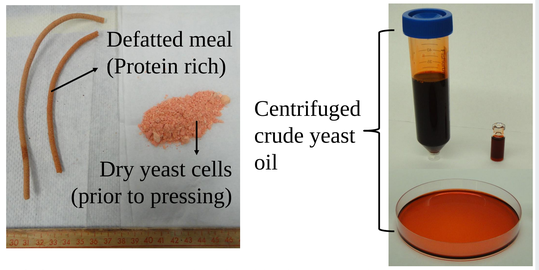

Figure 1. Inputs and outputs involved in the non-solvent extraction step.

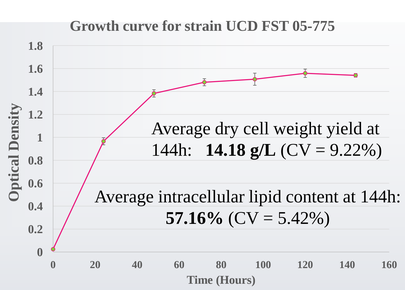

Figure 2. Growth curve of yeast strain UCDFST 05-775. The culture reached stationary phase at 48 hours.

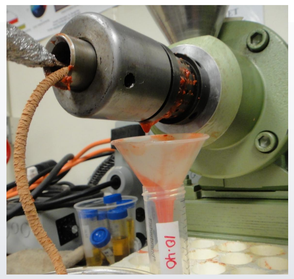

Figure 3. Dry yeast was pressed through a bench top screw press. Oil was collected into the 50 mL tube while defatted yeast cake was expelled out the front

TABLE 1. Processing conditions:

CONDITION	RUN 1	RUN 2	RUN 3
# Passes	3	3	3
Dry Yeast Input (g)	150.1	150.2	51.77
Dry Yeast Input (%)	76.7	76.9	76.6
Moisture content	4.42%		
Rice Hull Input (g)	45.51	45.11	15.78
Rice Hull Input (%)	23.3	23.1	23.4
Temperature	126	118.3	107.7
Flow rate (kg/hour)	1.55	1.13	0.71
Speed (RPM)	71	71	34
Die	5	4	4

Rice hulls were added as a pressing aid. Three runs were performed at different loading rates, temperatures, speeds and die sizes.

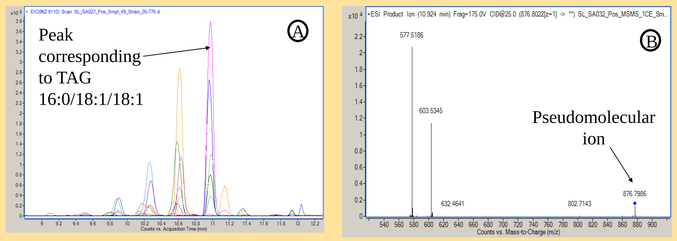

Figure 4. A) Chromatogram depicting the different TAG present in the oil. B) MS/MS spectra depicting the most abundant TAG in the oil, namely 16:0/18:1/18:1. Based on neutral loss of fatty acids.

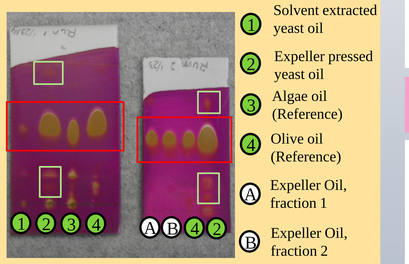

Figure 5. TLC stained with $KMnO_4$, showing TAG bands (red box), and 2 bands associated with carotenoid pigments (pale green box).

RESULTS

TABLE 2. RESULTS FROM MECHANICAL OIL EXTRACTION

RESULTS	RUN 1	RUN 2	RUN 3
Total Recovered oil (g)	44.46	43.27	27.17
Recovery from total extractable oil (%)	51.8	50.4	91.8

- Main TAGS identified: TAG(48:0), TAG(48:1), TAG(48:2)
- The most abundant: TAG 16:0/18:1/18:1.
- Nature of the oil is ideal for biodiesel production, or for high heat cooking purposes, due to high oleic acid content.
- Cells conspicuously changed morphology from smaller, ovoidal forms to bigger spherical shapes with distinctive intracellular lipid droplets.
- 2 Bands identified using UV light, associated with pigments.

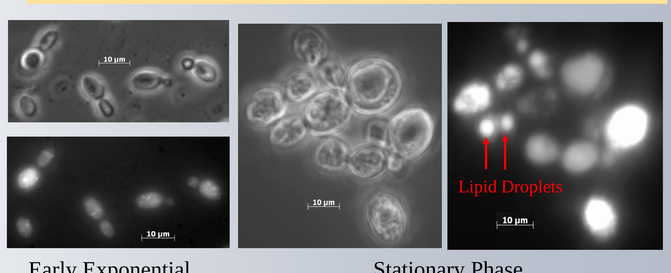

Figure 6. Microscopy images showing the different morphologies of yeast cells during two growth stages: early exponential and stationary phase. The stationary phase cells are significantly larger due in part to oil accumulation.

CONCLUSIONS

- We proved successfully at lab scale that separation of oil from yeast biomass using a solvent-free method is feasible. This is the first step towards a yeast based multi value technology platform.
- We were able to collect other high value yeast fractions that can be processed downstream and transformed into high value products. Work is underway to further demonstrate the feasibility of processing such products.
- A solvent free extraction process constitutes a safe and environmentally friendly process. In addition, it requires less capital investment for scale up since the equipment required does not need to be explosion proof, and additional furnishings, such as safety ditches and underground solvent tanks are not required.
- The advantages of a multiproduct microbial processing technology over the oilseed industry are:
- Year-round production, independence from climatic conditions, faster growth and turnover rates, higher yields of oil per unit biomass, diversification of products and markets, leveraging of sales and profits

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The authors want to acknowledge Erin Cathcart, Enrique Fernandez, Silviana Tjahyono, Ting Lin. This research was funded by Grant Number U01TW008160 from the NIH Fogarty International Center, the NIH Office of Dietary Supplements, the National Science Foundation and the Department of Energy. This project was supported by the USDA Agricultural Food Research Initiative of the National Food and Agriculture, USDA, Grant #35621-04750. The content is solely the responsibility of the authors and does not necessarily represent the official views of the Fogarty International Center or the National Institutes of Health, the Office of Dietary Supplements, the National Science Foundation, the Department of Energy, or the Department of Agriculture. This work was also supported by the Consejo Nacional de Ciencia y Tecnología (CONACYT) grant no. 291795.