Oleaginous yeasts as 'cell factories' for food waste valorization

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This PhD research project is aimed at the simultaneous production of multiple products from oleaginous yeasts trough a single sustainable process. Besides single-cell oil (SCO) accumulated into lipid bodies (Fig 1), the natural red pigmentation of some oleaginous strains (as *Rhodosporidium* ssp.) can be exploited to produce carotenoids. To improve economic feasibility and sustainability, oleaginous yeast strains can be cultivated on cheap non-food competing carbon sources, such as food waste. To efficiently use raw materials as a medium where microorganisms can grow an enzymatic pre-treatment has to be set. Instead of the use of expensive commercial enzymes, genetically manipulating the yeast strain it is possible to produce a cocktail of enzymes specifically formulated for the composition of a certain food residue.

State of the art

Oleaginous yeasts are microorganisms able of producing and accumulating lipids up to 70% of their dry weight into vescicles called lipid bodies. Because of this capacity, many of them are already used in bio-fuel industry, but they can find applications also in food and nutraceutical fields. Comparing the fatty acid composition of the oil produced by *Rhodosporidium azoricum* with some classical plant oils (Table 1), we noticed a similarity with olive oil and in particular the high number of unsaturated acids is very promising in terms of quality.

Recent attempts have demonstrated the great potential of microbial carotenoid production employing the microalga *Dunaliella salina* or the filamentous fungus *Blakeslea trispora* (Dufosse et al., 2014). Unfortunately, algal carotenoid production is generally expensive and requires large areas for cultivation, whereas molds are frequently characterized by slow growth that limits their industrial application. This is why worldwide researchers are looking for an alternative production method that considerably elevates the economic profitability of microbial carotenoids processes.

Oils	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
Sunflower oil	6.1	5.3	21.4	66.4	-
Corn oil	10.6	2.0	26.7	59.8	0.9
Canola oil	2.5	1.0	64.4	22.2	8.2
Rapeseed oil	3.5	0.9	19.4	22.3	8.2
Olive oil	14.0	2.0	64.0	16.0	2.0
Rhodosporodium azoricum oil	19.0	5.0	58.0	18.0	1.0

Table 1: Fatty acid composition of common plant oils compared to that produced by *R.azoricum*

In this context, it could be interesting to study the simoultaneous production of carotenoids and lipids in red oleaginous yeasts, as previously done by Saenge et al. (2011) using *R. glutinis*, and evaluate the idea to develop an oil enriched in carotenoids to increase the conservation potential of carotenoids. A similar functional food oil, rich in fatty acids and antioxidants, colored with carotenoids extracted from the microalga *Chlorella vulgaris*, was produced by Gouveia et al., (2007). Also Benakmoum et al., (2008) proposed to enrich refined olive oil and refined sunflower oil with carotenoids and lycopene from tomato peels. A carotenoid enriched oil could be a potential source of bioactive compounds and might have significant antioxidant activity when ingested as part of a dietary regime (Nagao et al., 2013).

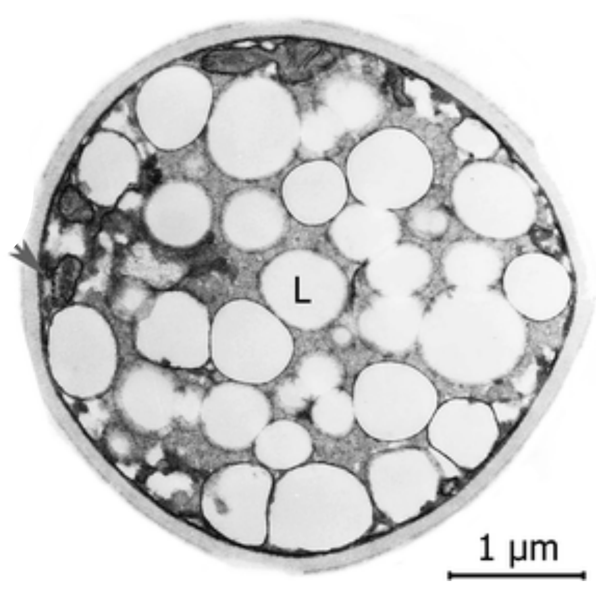

Fig 1: L: lipid body. Image from Manuel Ageitos et al. (2011)

Agro-industrial wastes can provide the carbon and nitrogen sources and other elements necessary to carry out the microbial metabolism, allowing to reduce production costs and, at the same time, avoiding environmental pollution from these agro-industrial wastes. In recent years there has been a growing interest in the use of natural and cheap substrates such as molasses, starch, milk whey, lignocellulose, spent coffee beans and others. In order to make them a base for a medium in which microorganisms can grow, raw matrices must be pre-treated with enzymes as cellulases, hemicellulases, glycosidases and others to release the highest amount of sugars. About this matter, another possible way to greatly increase the economic cost-effectiveness of the process, is to make our yeast able to produce a specific cocktail of enzymes formulated on the composition of each type of food residues. Other genetic interventions can be aimed at improving the overall process, acting for example on carotenogenesis or lipidoenesis, obtaining a hyper-producing strain.

Fig 2: *R.azoricum* process in bioreactor

PhD Thesis Objectives and Milestones

A1) Setting up fast and precise **methods** for
- total carotenoid content by spectrophotometric assay
- relative abundance of each carotenoid by HPLC analyses

A2) Determination and optimization of **enzymatic pre-treatments of different food wastes**. Evaluation of hydrolysate as a medium for yeast growth.

A3) Red yeast wild-type strains (from public and private collections) will be screened for:
- ability to naturally produce lipids
- ability to naturally produce carotenoids
- ability to rapidly grow on hydrolysate obtained from raw substrates

A4) The production process will be **scaled-up in bioreactor** and growth conditions and parameters optimized (Fig 2). A range of different pHs and temperatures will be tested, as well as changing air flow, stirring rates and illumination conditions.

A5) **Genetic interventions**
- Chemical mutagenesis using UV
- Metabolic engineering approach → Possible targets
  - Lipodogenesis
  - Carotenogenesis
  - Enzymes for food waste hydrolisis

A6) Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications

Gantt Diagram

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	*Methods for carotenoid quantification/profile*	■	■	■	■	■	■																		
A2)	*Study of food wastes and pre-treatments*	■	■	■	■	■	■	■	■	■	■	■	■	■	■										
A3)	*Screening for lipid and carotenoid production on food waste*						■	■	■	■	■	■	■	■	■										
A4)	*Scaling-up of the process*															■	■	■	■	■	■	■	■	■	■
A5)	*Genetic intervention*																		■	■	■	■	■	■	■
A6)	*Thesis and Paper Preparation*							■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

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