Lipids tracking by a flow cytometer in four oleaginous yeasts

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Oleaginous yeasts are able to metabolize different sources of carbon (e.g., monosaccharides, disaccharides, glycerol) into various kinds of lipids. Oil from yeasts can be used for different applications such as food, pharmaceutical or third generation biofuels, but interest is growing for the production of long-chain polyunsaturated fatty acids as building blocks for the polymer. Yeasts lipids extraction and quantification is a fastidious and time-consuming process involving several steps and the use of non-environmentally friendly solvents such as hexane and methanol/HCl 3M. The classical method involves lyophilization of biomass, trans-methylation in methanolic chloride and extraction in heptane. An alternative method to assess yeasts lipids production during fermentation consists of dyeing intracellular lipids with a fluorochrome. The most popular lipid dye is Nile red, which binds non- specifically neutral lipids. The BODIPY 493/503 dye is more specific to neutral lipid than Nile red. In this study, four oleaginous yeast strains (two strains of *Yarrowia lipolytica* clib 89 and clib 718, one *Lipomyces starkeyi* mucl 27779 and one *Meyerozyma guillermondii* clib 222) were compared for their ability to accumulate lipids during growth. Intracellular lipids accumulation was monitored during yeasts growth, both by a method based on Bligh and Dyer protocol and flow cytometry after cell dyeing with BODIPY 493/503. GC analysis shows that *Yarrowia lipolytica* clib 89 and clib 718 accumulated more lipids in total but *Lipomyces starkeyi* presented the highest production of α-linolenic acid and linoleic acid, a long chain polyunsaturated acid of interest for the chemical industry. Flow cytometry analysis with BODIPY 493/503 dyeing showed a good correlation of fluorescence intensity with both total lipid accumulation and polyunsaturated fatty acid concentration. Yeast marking during fermentation with BODIPY 493/503 associated with flow cytometry is thus a very promising technique to measure and compare the ability of yeasts to synthesize and accumulate lipids, in particular, those containing long-chain polyunsaturated fatty acids.

Biography: Guillaume Delfau-Bonnet is a Ph.D. student who works on fermentation of microalgae residue to produce carboxylic acid for polymers synthesis. He holds a B.Sc. in Biology and Biochemistry and a Master of Biotechnology in Design, Performance of biobased product, University of Reims Champagne-Ardenne. He has done two internships in the valorization of biomass. The first one was linked to the purification of pentose by chromatography in the wheat hydrolysate, and the second one was on *Bacillus subtilis* growth on wheat hydrolysate extracts on growth. He is doing a Ph.D. on the valorization of microalgae by-products with AgroParisTech (France) and University of Mons (Belgium). He uses oleaginous yeast to valorize pretreated microalgae cell wall into unsaturated fatty acids.

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