

## SEARCHING FOR OLEAGINOUS YEASTS IN RIO GRANDE DO SUL: FLUORESCENCE AS HIGH THROUGHPUT SCREENING METHOD

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### **Abstract:**

Second-generation biodiesel production utilizing oleaginous microorganisms is a very promising alternative to overcome the critical bottlenecks of 1st generation biodiesel production. Yeasts are a promising source of microbial oil, since some strains can accumulate up to 70% of their dry weight in lipids. It is important to assess and select oleaginous yeast strains to establish their suitability for biodiesel production. Therefore, there is a need for a rapid, robust and highly efficient method for quantifying lipid contents in microbial biomasses. Consequently, we proposed a high throughput screening (HTS) for comprehensive evaluation of the lipid-accumulating ability of yeast strains, isolated of bromeliads in Itapuã Park and decomposed plants of “Lagoa dos Patos” marshland.

A yeast culture collection of “Mycology Lab at ICBS/UFRGS” was assessed comprising approximately 200 yeasts isolates of Rio Grande do Sul. We established two-steps screening in order to select one promising oleaginous yeast: (1) exponential growth at 72 hours (max), and (2) content lipids higher than our positive control (QU21). Therefore, we measured fluorescence intensity with Red Nile (technical and biological triplicate of  $10^7$  cells/mL) of 13 isolates of bromeliads, five isolates of decomposed marshland plants, strains QU21 (*Yarrowia lipolytica* as positive control) and y-024 (*Saccharomyces cerevisiae* as negative control). We used fluorescence microscopy with the same dye to visualize lipid drops. Eight isolates showed higher average fluorescence intensity (AFI) than QU21, and four isolates lower AFI than y-024, so we discarded these isolates as oleaginous yeasts, and selected the isolate BI281 (*Cryptococcus flavescens*) as candidate of oleaginous yeast because their fluorescence intensity was two times higher than QU21 with lower standard deviation. This species has not been reported before as oleaginous yeast.

**Keywords:** oleaginous yeast, screening, high content lipid, fluorescence intensity

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