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

Mauricio Ramírez-Castrillón(1,2), Victoria P. Jaramillo-Garcia(1,3), João A. P. Henriques(3), Caroline Flores(2), Rafaela Hepp(2), Patricia Valente(2)

1Programa de Pós-graduação em Biologia Celular e Molecular, Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Porto Alegre – RS, Brazil; 2Departamento de Microbiologia, Imunologia e Parasitologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil; 3Departamento de Biofísica, Universidade Federal do Rio Grande do Sul, Porto Alegre – RS, Brazil.
E-mail: mauriciogeteg@gmail.com

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.

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