Title: SCREENING OF YEASTS WITH HIGH CONTENT LIPID ISOLATED FROM RIO GRANDE DO SUL, BRAZIL

Authors: Ramírez-Castrillón, M[1,2], Mendes, S.D.[3], Jaramillo-Garcia, V.P.[1,4], Henriques, J.A.P.[4], Flores, C.[2], Hepp, R.[2], Valente, P.[2]

Institution: [1] Programa de Pós-graduação em Biologia Celular e Molecular, Centro de Biotecnologia, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, prédios 43421/43431 - setor IV - Campus do Vale – caixa postal 15005 - CEP 91501-970, Porto Alegre – RS, Brazil; [2] Department of Microbiology, Immunology and Parasitology, Universidade Federal do Rio Grande do Sul, Rua Sarmento Leite 500, CEP 90050-170 Porto Alegre, RS, Brazil; [3] Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina, Laboratório de Análises de Vinhos e Derivados, Estação Experimental de Videira, Rua João Zardo 1660, Campo Experimental, CEP 89560-000, Videira, SC, Brazil; [4] Departamento de Biofísica, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves, 9500, prédios 43432 - setor IV - Campus do Vale – caixa postal 15005 - CEP 91501-970, Porto Alegre – RS, Brazil.

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. Conventional methods for extraction and gravimetric determination of lipid content of microbial biomasses, are based on solvent extractions, such as methanol and chloroform. There are two major drawbacks of these methods: (1) the results are quite dependent on the cell wall lysis step, hence the process is highly time and labor consuming, making it infeasible for screening; (2) the method uses non-environmentally friendly and strong organic solvents that should be handled with care. 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 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 (triplicate technical and biological of 10^7 cells/mL) of 13 isolates of bromeliads, five isolates of decomposed marshland plants, and strains QU21 (*Yarrowia lipolytica*) and y-024 (*Saccharomyces cerevisiae*, 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 a good 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

Funding: FAPERGS, CNPq