

# **FAPESP BIOENERGY PROGRAM**

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## POPULATION DINAMICS AND INTERACTIONS BETWEEN YEASTS STRAINS IN FERMENTA-TIONS OF MOLASSES AND HYDROLISATES OF SUGARCANE BAGASSE

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Figure 1. Color of the lignocellulose enriched-fractions obtained by using of modified pre-treatments applied to sugarcane as described by Miranda et al. (2015). SCB is a milled sample of sugarcane bagasse in natura

Thermotolerant yeasts are becoming increasingly important in ethanol production due to the continuing changes in the climate and water scarcity responsible for raises on earth temperature. Ethanol production of first and second generation are complex and longterm processes operating in open reactors with cell reuse. As the cells are exposed for months to successive, oscillatory in aggressive environmental conditions all over the harvest season, the use of thermotolerant yeast is essential. Despite the high capacity of starter cells, compete against other yeast cells present in the environment, process conditions can lead to the arising of modified yeast whose effects on the process must be determined. Genetic variants show chromosomal profiles different from the parental yeasts. Inter-delta sequencing or separation of chromosome DNA bands on electrophoresis gels (CHEF) are tools to identify yeast strains. The techniques base on DNA bands are more time consuming and difficult to perform than the inter-delta technique and this can lead to mistakes in identifying strains. The obtaining of strains more resistant to adverse conditions, the development of a dye-based medium for the monitoring of population dynamics and a less aggressive physical-chemical method to pretreat sugarcane bagasse are intentions of the present study.



# SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

A chromogenic medium (Masiero and Laluce, patent n° BR 10 2015 005368-1) was developed to identify genetic variants originated during fermentation as well as wild yeasts (contaminants) using using inter-delta sequencing. In addition, pre-treatments were improved to obtain less recalcitrant cellulose enriched -fractions from the sugarcane bagasse (Miranda et al., 2015). This medium developed to differentiate colonies of yeasts proved very useful for monitoring of the persistence and permanence of yeasts during fermentation. On the other hand, the highest s biomass yields of the cellulose enriched-fractions resulted from the application of assisted microwave pretreatments to the sugarcane bagasse. The cellulosic fraction PT6 resulted from a microwave pre-treatment in sulfuric acid solution, while cellulosic fraction PT7 resulted from a two-step microwave pre-treatment in sulfuric acid solution followed by a pre-treatment in alkaline solution. The highest yields of glucose (enzymatic assay) were obtained from fractions PT6 and PT7, during extraction with a concentrated sulfuric acid solution (30%, w/v). This indicates the two cellulosic fractions went through acidic degradation during extraction. In addition, the liberation of phenols from lignin increased during incubation of the cellulosic fractions in diluted solution of sulfuric acid (4%-6%, w/v) containing Fe<sub>2</sub>Cl<sub>2</sub>. Thus, it seems that the lignin attached to the cellulose fractions can be largely removed by extraction with diluted sulfuric acid solutions in the presence of Fe<sub>3</sub>Cl<sub>3</sub>.

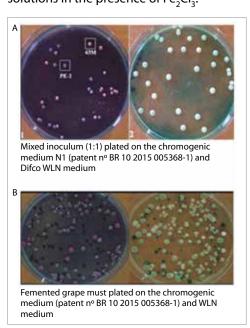


Figure 2. Chromogenic media for monitoring the yeast population dynamics during grape must (B) and control plates (A) showing purple colonies for the industrial strain (A, part 1) and the mutagenized strain 63M (A, part 2) showing colonies of pink colors. Strain 63M obtained as previously described (Souza et al., 2007)

## MAIN PUBLICATIONS

Miranda I, Masiero MO, Zamai TT, Capella M, Laluce C. 2015. Improved pretreatments applied to the sugarcane bagasse and release of lignin and hemicellulose from the cellulose-enriched fractions by sulfuric acid hydrolysis. *J Chem. Technol. Biot.* DOI: 10.1002/jctb.4601, Version online (http://onlinelibrary.wiley.com/doi/10.1002/jctb.4601/abstract).

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Laluce C, Leite GR, Zavitoski BZ, Zamai TT, Ventura R. 2015. Fermentation of sugarcane juice and molasses for ethanol production In "Sugarcane-Based Biofuels and Bio-products". *Wiley-Blackwell*, 1st edition (*in press*).

[PATENT APPLICATION] Cecília Laluce (IQ Unesp), Maria Olívia Campos Masiero (IQ Unesp, Angela Capece (Un. Basilicata), Patrizia Romano (Un. Basilicata. 2015. Composition of a medium the, process of preparation of the medium to identify yeast strains in the mentioned medium (solid culture composition, process for preparation and processor identification of yeast strains in such medium (solid medium composition), process for the preparation thereof and process for identification of yeast strains in such medium. Brazilian filling number BR 10 2015 005368-1; Filling date: 11/03/2015.

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