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# GROWTH OF LACTIC ACID BACTERIA IN THE PRESENCE OF 2G ETHANOL FERMENTATION SUGARS

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#### 1. INTRODUCTION

Second generation ethanol (2G) is produced through the use of lignocellulosic biomass that is previously treated to release the sugars fermentable substances to obtain ethanol (AMORIM et al. 2011). The production of biofuels from lignocellulosic waste could supply the energy matrix in the near future. The present study proposes the evaluation of the physiology of lactic acid bacteria (LAB), with heterofermentative and homofermentative metabolism, contaminants of the ethanol production process in the presence of the most abundant sugars predicted in the 2G ethanol fermentation. Evaluate the performance of these contaminating bacteria against the sugars is extremely important to assess whether additional care for the control of these microorganisms should be taken in the production processes of 2G ethanol.

#### 2. METHODS

The growth of LAB was conducted in 96-well microplates, with 200  $\mu$ L of culture medium: yeast extract (5 g.L<sup>-1</sup>), peptone (5 g.L<sup>-1</sup>), K<sub>2</sub>HPO<sub>4</sub> (2 g.L<sup>-1</sup>), MgSO<sub>4</sub> (0.2 g.L<sup>-1</sup>), and MnSO<sub>4</sub> (0.01 g.L<sup>-1</sup>) (BASSO et al. 2014). The pH was adjusted to 6 and temperature conditions were controlled at 37°C for 24 h. For each treatment was added 20 g.L<sup>-1</sup> of the following sugars: sucrose, xylose, arabinose, fructose, glucose and combination of glucose and fructose. The LAB used were *Lactobacillus plantarum* CECT 221, *Lactobacillus fermentum* DSM 20391, *Lactobacillus plantarum* E4 and *Lactobacillus fermentum* E3. The cultures were monitored by the Tecan Infinite Pro 200 plate reader, which performed the OD<sub>600</sub> reading at 20-minute intervals, during 24h of cultivation without agitation. The maximum specific growth rate ( $\mu$ ) h<sup>-1</sup> was obtained by plotting the natural logarithm of the culture absorbance against time. The slope of the linear regression line represents the  $\mu$ 

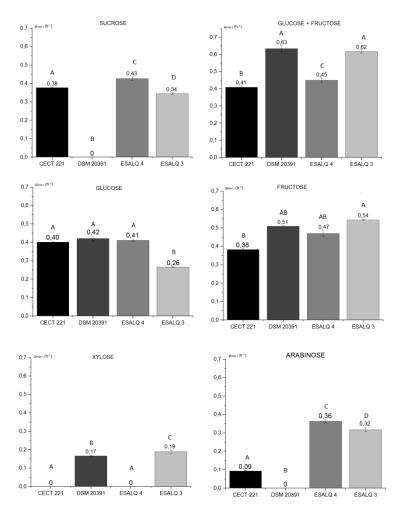
#### 3. RESULTS

Hexoses were metabolized by LAB through the EMP pathway, when homofermentative, they generate only lactic acid and when heterofermentative, they generate equimolar amounts of lactic acid and ethanol/acetic acid (KANDLER, 1983). DSM 20391 strain did not show growth in sucrose, may be due to it being isolated from an environment where sucrose does not represent a viable carbon source. Therefore, in the strain DSM20391, genes linked to sucrose assimilation are absent or unexpressed, reinforcing the dependence of heterofermentative bacteria on induction by the substrate for the expression of sucrose transport genes as FOLLADOR and GANZLE (2012) reported. Evaluating the behavior of lactic acid bacteria in the presence of different sugars from the pretreatment step, it was observed that only the LAB heterofermentatives have the ability to metabolize xylose (the main component derived from hemicelluloses). Thus, it is possible that in a 2G fermentation, heterofermentative bacteria will be in greater quantity, since they consume both C6 and C5 sugars.



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**Figure 1**. Comparative graphs of the maximum specific growth rate in for: L. plantarum CECT221, L. fermentum DSM 20391, L. plantarum E4 and L. fermentum E3. Growth was carried out at 37°C in culture medium with 20g.L<sup>-1</sup> of sugar: sucrose, xylose, arabinose, fructose, glucose and combination of glucose and fructose. Tukey test with 95% confidence where means that do not share a letter are significantly different for each chart.

### 4. REFERENCES

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