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RESEARCH PAPER

# *Candida tropicalis* able to produce yeast single cell protein using sugarcane bagasse hemicellulosic hydrolysate as carbon source

**Cristina Emanuela Barbosa Magalhães**[a], **Manoel Santiago Souza-Neto**[b], **Spartaco Astolfi-Filho**[b], **Italo Thiago Silveira Rocha Matos**[b,*]

[a] *Engenharia Química, Universidade Federal do Amazonas, Manaus, AM, Brazil*
[b] *Departamento de Genética, Universidade Federal do Amazonas, Manaus, AM, Brazil*

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**Abstract** The world demand for foods will be the most important bottle-neck for human being maintenance. In this context, the research for alternative proteins sources aiming human and animal feeding is a necessary approach. A wild-type strain of *Candida tropicalis* (KP276650) was identified as potential yeast single cell protein (YSCP) producer using sugarcane bagasse hemicellulosic hydrolysate (SBHH) as substrate. Were evaluated the cell growth and sugar consumption along fermentation assay. Biomass yield ($Y_B$, $g\,g^{-1}$), protein percentage (%), protein yield ($Y_P$, $g\,g^{-1}$) and yeast biomass productivity (YBP, $g\,L^{-1}\,h^{-1}$) were calculated. Fermentation assay reach a total of 96 h, being the total reducing sugar present in the media ($59.94\,g\,L^{-1}$) fully consumed. Final biomass produced was $16.97\,g\,L^{-1}$, and the YBP was $0.1767\,g\,L^{-1}\,h^{-1}$. Percentage of protein in yeast biomass produced was 60.05%, biomass yield ($Y_B$) and protein yield ($Y_P$) were, respectively, 0.28 and $0.17\,g\,g^{-1}$. The $Y_B$ and $Y_P$ obtained are close to the observed for some industrial strains of yeasts. The percentage of protein is greater than the expected for most of yeasts and fungi. These results indicate that the using of *C. tropicalis* (KP276650) for YSCP production may to aggregate high value for sugarcane bagasse.

## Introduction

The world demand for food is currently increasing and will be about 3 billion of tons until 2050 for attending human and animal population (FAO, 2009). Beef, cattle milk, pig meat, fish, poultry meat and eggs are the most significant protein sources for human feeding (Gerber et al., 2013), and all these animal breeding demand pasture area or land using to produce foraging plants. The growth of global population leads to necessity of expanding agricultural land using, resulting in substantial greenhouse gases emissions (Wirsenius, Azar, & Berndes, 2010). In this context, the research for an eco-friendly alternative source of protein usable in animal feeding is a necessary approach.

* Corresponding author.
*E-mail:* italo_matos@ufam.edu.br (I.T. Matos).

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Microbial protein is an option rich in advantages that includes: short time of microorganisms generation, high protein content in the cells, ability to shape amino acids profile according to cultivation conditions or genetic modifications, and possibility of continuous productions by fermentation independent of climatic conditions (Kieliszek et al., 2017). The using of microbes able to grow in low cost and abundant carbon sources makes this alternative moreover interesting.

Sugarcane bagasse is the most abundant agricultural waste around the world, being produced in 2016 about 144.5 millions of ton only in Brazil (BRASIL, 2018a). The use of this waste as carbon source for microbial biomass production with high protein concentration is viable because its abundance and low cost.

A strain of *Candida tropicalis* (KP276650) was isolated from the xylophagous beetle *Veturius transversus* (Matos, Assunção, Carmo, Soares, & Atolfi-Filho, 2017), and identified as high consumer of D-xylose, the most abundant monosaccharide in sugarcane bagasse hemicellulosic hydrolysate (SBHH). As this yeast is widely used for bioprocess using this substrate, the aim of this work was to evaluate the potential of *C. tropicalis* (KP276650) to produce yeast single cell protein (YSCP) in SBHH.

## Material and methods

### Strain reactivation and inoculum preparation

*C. tropicalis*, preserved in sterilized distilled water, was reactivated by culturing a loopful of cell suspension in plates containing Sabouraud Agar (yeast extract 10 g $L^{-1}$, glucose 40 g $L^{-1}$, agar 20 g $L^{-1}$). After 48 h, a loopful was cultured in YPD broth (yeast extract 10 g $L^{-1}$, peptone 20 g $L^{-1}$, glucose 20 g $L^{-1}$) for pre-inoculum preparation (150 rpm, 30 °C, 72 h). The cell suspension was centrifuged (4000 *g*, 40 min, 4 °C) and the sediment was used as inoculum.

### Sugarcane bagasse hemicellulosic hydrolysate preparation

The SBHH was prepared by mixing dry sugarcane bagasse and diluted sulfuric acid (1% v/v), solid:liquid ratio in 1:4. After 24 h, this mix was autoclaved (121 °C, 40 min), its liquid phase was separated and pH was adjusted to 5.0 by $Ca(OH)_2$ addition. After supplementation with yeast extract (15 g $L^{-1}$), total reducing sugar concentration ([TRS]) was of 59.94 g $L^{-1}$.

### Fermentation assay and response variables

Fermentation assay was performed in Erlenmeyer flasks (250 mL) containing 100 mL of SBHH and initial cell concentration about 0.500 g $L^{-1}$ (dry weight). Each 12 h, an aliquot was collected for monitoring the sugar consumption, according [TRS] (g $L^{-1}$), and cell growth. Were evaluated the yeast biomass productivity (YBP, g $L^{-1}$ $h^{-1}$), biomass yield ($Y_B$, g $g^{-1}$), protein yield ($Y_P$, g $g^{-1}$) and protein percentage (PP, %). The flasks were incubated at 30 °C and 150 rpm until total reducing sugar were fully consumed. The assay was performed in triplicate.

### Analytical methods

Initial inoculum and final biomass production were calculated by drying sedimented cells at 70 °C until observed constant weight. Cell growth was monitored evaluating the absorbance of the sample, diluted 10×, in spectrophotometer at 600 nm ($OD_{600}$).

Total reducing sugar was calculated using DNS method, by mixing 200 μL of sample (diluted $10^{-2}$) and 300 μL of 3,5-dinitrosalicylic acid solution. After boiling for 5 min, 1500 μL were added, and absorbance (ABS) was evaluated in spectrophotometer at 540 nm. The total reducing sugar concentration ([TRS], g $L^{-1}$) was calculated according pattern curve, using the formula ''ABS = [TRS] * 0.0153 − 0.0669'' ($R^2$ = 0.9984).

Percentage of total protein (%) was evaluated by Kedhjal method, according to AOAC (2016). Yeast biomass productivity (YBP, g $L^{-1}$ $h^{-1}$) was calculated by ratio ''final biomass concentration (g $L^{-1}$)/time of cultivation (h)''. Biomass yield ($Y_B$) and protein yield ($Y_P$) were determined by ratio ''final biomass or protein/mass of sugar consumed'', expressed in g $g^{-1}$.

## Results

Fermentation assay reach a total of 96 h, being observed exponential growth since 12 h until 84 h, and stationary phase since then until 96 h, when [TRS] was close to undetected. Complete growth curve and [TRS] variation along the assay are presented at Fig. 1.

Final biomass production was 16.97 g $L^{-1}$, and the YBP was 0.1767 g $L^{-1}$ $h^{-1}$. Percentage of protein in yeast biomass produced was 60.05%, meaning a total of 10.19 g $L^{-1}$ of YSCP. By this way, biomass yield ($Y_B$) and protein yield ($Y_P$) were, respectively, 0.28 and 0.17 g $g^{-1}$.

## Discussion

According to García-Garibay, Gómez-Ruiz, Cruz-Guerrero, and Barzana (2014), there are 11 different species of yeasts used for YSCP production around the world. In all these cases, there is no a process that uses SBHH as substrate.

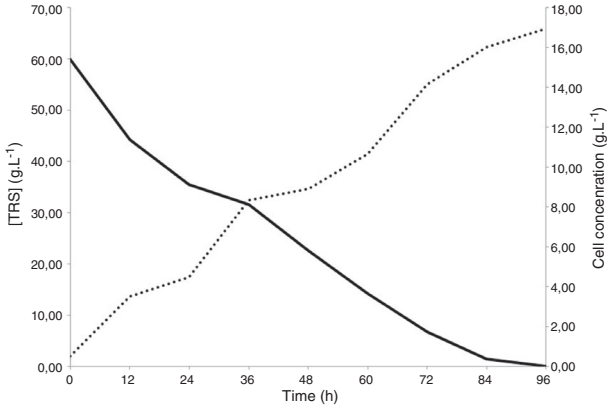


**Figure 1** Total reducing sugar concentration (continuous line, left axis) and cell growth (dashed line, right axis) along fermentation assay.

Considering the percentage of protein greater than 40% in this substrate, this strain of *C. tropicalis* presents a promising potential for a bioprocess aiming protein production.

The final biomass production was 2,6 folds greater than the observed for *Candida utilis* FMJ12 when cultured by Somda et al. (2018) for YSCP production using fruits waste as carbon source. The YBP obtained was close to the observed for *Kluyveromyces marxianus* when cultured in brewery's spent grains hemicellulosic hydrolysate by Duarte, Carvalheiro, Lopes, Neves, and Gírio (2008) and in fresh cheese whey (Yadav et al., 2014). Biomass yield was close to the obtained by Yadav et al. (2014) when culturing *K. marxianus* in cheese whey. Together, these facts indicate that this strain of *C. tropicalis* presents potential close to strains used in industrial process for YSCP production.

According to Nalage et al. (2016), protein percentage in yeasts ranges from 47 to 53%, but *C. tropicalis* (KP276650) presents a total of 60.05%. This high percentage of protein makes by this strain a remarkable protein producer.

Data from Ministry of Agriculture of Brazil, indicates that sugarcane bagasse production for 2017/2018 is estimated about 161.9 millions of tons (BRASIL, 2018b). If the hemicellulose of only 25% of this were used as substrate for YSCP production, it would be about 14.16 millions of tons of substrate available. Considering the protein yield here obtained, it would be produced about 2.41 millions of tons of YSCP. The exploitation of this potential may to aggregate value for sugarcane bagasse, being substrate for producing high amounts of YSCP.

## Conclusion

*C. tropicalis* (KP276650) presents high potential for yeast biomass production in sugarcane bagasse hemicellulosic hydrolysate, presenting potential close to strains used in industrial processes.

The percentage of protein obtained for this strain is remarkable, being about 20% greater than the expected for most of yeasts.

Subsequent efforts will be employed to increase the biomass yield and evaluate the nutritional value of the protein produced.

## Conflicts of interest

The authors declare no conflicts of interest.

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