TITLE: SCREENING OF YEASTS ABLE TO PRODUCE CELLULASES ASSOCIATED TO TERMITES

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ABSTRACT:

Cellulose is the most abundant component of plant biomass, with a promising prospect as a substrate in the biofuels industry. Specifically for the pre-treatment of cellulose, must be employed cellulases, enzymes that cleave the glycosidic bonds dissociating glucose for the alcoholic fermentation. Yeasts associated with termites play a key role in the degradation of polysaccharides present in the diet of these insects, since they do not secrete all the enzymes necessary for the cellulose hydrolysis. Considering that most of these are produced by microbial symbionts of the digestive tract, the aim of this work was to isolate cellulolytic yeasts associated to termites *Nasutitermes macrocephalus*, and the enzymatic index (EI) was verified. Ninety specimens of *N. macrocephalus* were collected from fragments of arboreal nests in Central Amazonia (3º05'59.7 "S 59º58'26.8" W). Each specimen had the gut dissected and inoculated into YPD medium (Yeast Extract 10 g/L, Peptone 20 g/L and Dextrose 20 g/L), 3 units for each 5 ml of medium. After 72 h, 100 µL were cultured in plates containing Sabouraud Agar (Yeast Extract 10 g/L, Dextrose 40 g/L, Agar 20 g/L) and plates containing sugarcane bagasse hemicellulosic hydrolyzate (Agar: 20 g/L , YNB: 6.7 g/L and total reducing sugar 10 g/L), incubated at 28 °C for up to 72 h. The isolates were submitted to cellulase secretion test using the replica-plating technique on plates containing Manchini-CMC Agar, composed of Manchini's solution (KH_2PO_4, 2 g/L; $(NH_4)_2SO_4$ 1 g/L; $MgSO_4$, 0.1 g/L; $Na_2HPO_4.2H_2O$, 0.9 g/L; yeast extract, 1 g/L), Agar (20 g/L) and Carboxy-Methyl Cellulose (10 g/L). The plates were incubated at 28 °C for up to 24 h and stained with 0.1% Congo red solution. Sixteen yeast colonies were isolated, of which six presented cellulolytic activity, viz. Lev01, Lev02, Lev04, Lev09, Lev12 and Lev14. The halos measured on average 10.1 mm, varying between 7 and 13 mm. The observed enzymatic indices indicated promising activity (EI ≥ 2.0) for the Lev04 (EI = 2,0), Lev09 (EI = 2,3) and Lev12 (EI = 2,0) isolates. For the other isolates, EI values were 1.8, 1.25 and 1.2 for Lev01, Lev02 and Lev14, respectively. These results indicate that the isolates Lev04, Lev09 and Lev12 present potential for simultaneous saccharification and fermentation assays. Subsequent efforts will be concentrated on the taxonomic identification of these.

Keywords: Cellulases, Yeast, Ethanol, *Nasutitermes macrocephalus*, Cellulose.

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