DETERMINATION OF HYDROLYTIC ACTIVITY OF CELLULOSOMES PRODUCING STRAINS ISOLATED FROM RUMEN AND TERMITE

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Introduction. Certain bacterium and yeasts have different types of hydrolytic enzymes which act synergistically on a specific substrate (1). Recent research has shown that these enzymes can be grouped to form cellulosomes, which have catalytic domains similar to free hydrolytic enzymes (2). It has been reported the presence of cellulosomes on rumen fluid and termites digestive extract, although enzymatic hydrolysis is a slow and sometimes incomplete process, it has been documented that in a relatively short time (48 h) these consortium from rumen strains hydrolyze cellulose to 60-65%, meanwhile certain termite strains have achieved the 90% of assimilation of wood cellulose (3).
The aim of this study was to evaluate the extracellular and membrane hydrolytic activity of four cellulosomes producing strains of cellulosomes.

Methods. Experimental tests were carried out with four strains: three of this were ruminal (*Candida tropicalis, Klebsiella pneumoniae y Acinetobacter baumannii)* and the last one from termite (T-7). Cell growth was measured by optical density, free reducing sugars and total protein content were determined by DNS (Miller, 1959) and Lowry (Lowry, 1951) methods respectively. The hydrolytic activity was determined as established by IUPAC and expressed at international units (IU = enzyme amount hat liberates 1 µmol of glucose / min * mg of protein). Carboxymethyl cellulose and cellobiose were used as substrates (4).

Results. Significant differences ($p<0.05$) between extracellular and membrane hydrolytic activity of each strain were obtained. The hydrolytic activity of beta-endoglucanase and beta-glucosidase extracellular and membrane as well as the growth curves of each strain are shown in figure 1. Increase of membrane enzymes activity in both yeasts was observed, with maximal hydrolytic activity for T-7 and *Candida tropicalis* as described below: for beta-glucosidase 58UI and 30UI, respectively and for beta-endoglucanase 28UI and 15UI respectively. These peaks of activity were observed at 6 hours of growth which corresponds to the beginning of the exponential phase. Furthermore, yeasts growth was similar in both substrates, with maximal growth at 16 hours.

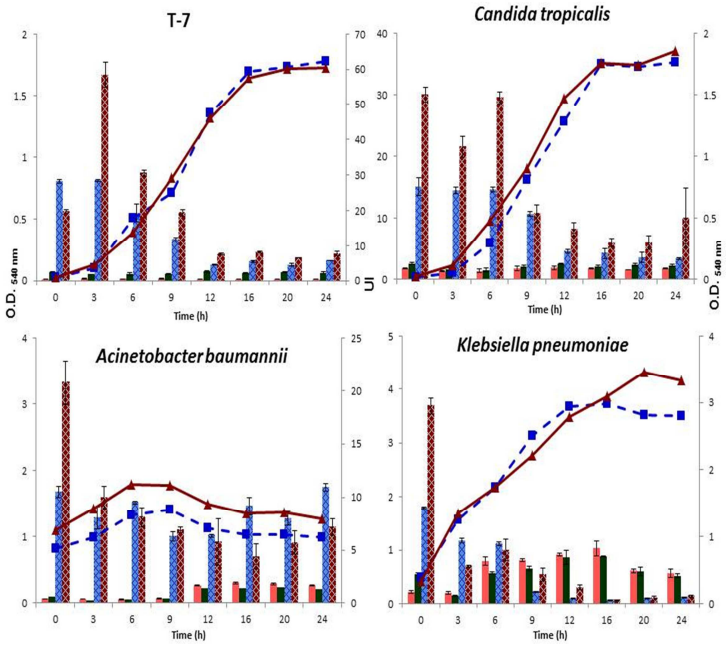

Fig.1 Solid bars refers to extracellular activity, =Beta-endoglucanase, =Beta-glucosidase. Gridded bars refers to membrane activity, =Beta-endoglucanase, =Beta-glucosidase. =Growth in CMC, =Growth in cellobiose.

Conclusions. According to statistical analysis, membrane hydrolytic activities values were considerly higher in yeasts, obtaining the highest hydrolytic activity for T-7 followed by *Candida tropicalis, Acinetobacter baumannii* and *Klebsiella pneumoniae*.

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