β-GALACTOSIDASE AND β-FRUCTOFURANOSIDASE ACTIVITIES FROM *CELLULOMONAS FLAVIGENA* GROWING ON DIFFERENT CARBON SOURCES

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Introduction. *Cellumonas flavigena* produces a wide variety of glucanases, which can degrade different polysaccharides from a complex system of intracellular and extracellular enzymes; the main investigations have been focused only to the study of cellulases and xylanases growing on lignocellulosic wastes (1). In this study we demostrate the presence of two hydrolytic enzymes: β-galactosidase and β-fructofuranosidase from *C. flavigena* growing on lactose, fructose, sucrose and raftilose as carbon sources, in extracellular and intracellular fractions as well as comparison of the protein profiles by SDS-PAGE.

Methods. Enzymes were obtained in the exponential phase of growth from 100 mL of culture of *C. flavigena*. After centrifugation, extracellular enzymes in supernatant were concentrated to 2 mL in Ultra-15 Centrifugal Filter Units (Millipore). The cells were disrupted mechanically, and the intracellular proteins were recovered in the supernatant by centrifugation.

The β-galactosidase and β-fructofuranosidase activities (intracellular and extracellular) were analyzed using as substrates 0.2 M of lactose pH 7 and raftilose pH 5.5 in phosphate buffer, respectively. The reaction systems were incubated at 50°C for 20 minutes. The reducing sugars released were quantified by dinitrosalicylic acid method (2) For protein profiles SDS-PAGE were carry out, 10 mg of protein were homogenized with 5 µL of loading buffer. The stacking gel was performed using 5% polyacrylamide and the separator with 12% acrylamide-bisacrylamide (Bio-Rad). The running conditions were: 30 min. 80 V and 120 min to 110 V. A molecular weight marker of 10 to 250 kDa was used. The gel was stained with Coomassie blue.

Results. In extracts from sucrose, lactose and raftilose intracellular hydrolytic β-galactosidase activity were 14.8, 15.4 and 12.3 U/mg, this means that no exist significant differences, in extracts from glucose and fructose the activities were 3.3 and 0.5 U/mg (Table 1), suggesting a catabolic repression. No extracellular activity was presented except for the extract from fructose.

In the same way, analyzing β-fructofuranosidase activity, intracellular enzyme presented higher values than extracellular enzyme 48.6, 37.2 and 28.6 U/mg on sucrose, lactose and raftilose respectively (Table 2) significant differences in the hydrolytic activity were presented on all sugars.

Since in extracellular extracts except glucose, showed hydrolytic activity can be presumed that the microorganism needs to release enzymes that allow the environment to introduce these carbohydrates and then use them to their metabolic requirements. Both intracellular activities were presented on glucose beeing the β-fructofuranosidase almost 4 times higher than β-galactosidase.

Table 1 Hydrolytic activity of the intracellular and extracellular enzymatic extracts of *C. flavigena* using lactose as substrate

	Sucrose	Lactose	Raftilose	Glucose	Fructose
Intracellular	14.8 ± 2^{a}	15.4 ± 2^{a}	$12.35\pm1.^{a}$	3.29 ± 0.3^{b}	0.53 ± 0.1^{b}
Extracellular	0.5 ± 0.1^{b}	0.5 ± 0.2^{b}	1.0 ± 0.25^{b}	0 ± 0^{c}	3.217 ± 1^{a}

Table 2 Hydrolitoc activity of the intracellular and extracellular enzymatic extracts of *C. flavigena* using raftilose as substrate

	Sucrose	Lactose	Raftilose	Glucose	Fructose
Intracellular	48.6 ± 2.5^{a}	37.2 ± 1.3^{b}	28.6 ± 2.6^{c}	13.6 ± 1.0^{d}	0.07 ± 0.08^{e}
Extracellular	1.8 ± 0.13^{a}	1.05 ± 0.56^{b}	1.91 ± 0.1^{a}	0 ± 0^{c}	1.13 ± 0.04^{a}

Data are shown as the average of three replications ± standard deviations are given in units U / mg. Different letters in a row indicate significant differences between treatments ($p < 0.05$)

There were some differences in the protein profiles between the substrate used as inducers, which makes clear that *C. flavigena* has the ability to produce enzymes with different substrates which can be used for their metabolic requirements.

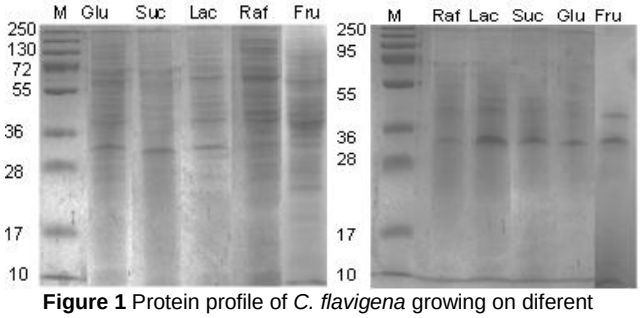

Figure 1 Protein profile of *C. flavigena* growing on diferent carbon sources, A) intracelular B) Extracellular

Conclusions. *C. flavigena* was capable of produce enzymes that hydrolyzed not lignocellulosic carbon sources. We founded hydrolytic enzyme activities for β-galactosidase and β-fructofuranosidase. Furthermore, for each carbon source used, there were differences in protein profiles

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