



CHARACTERIZATION OF A RESISTANT β -GLUCOSIDASE TO CATABOLIC REPRESSION FROM MUTANT *Cellulomonas flavigena* PR-22

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Introduction. The β -glucosidase is one of the limiting enzymes in the biological hydrolysis of cellulose for producing lignocellulosic bioethanol. Therefore we have sought microorganisms capable of hyper-produce these enzymes and be resistant to catabolic repression [1]. *Cellulomonas flavigena* PR-22 [2], as a result of a mutagenic process, showed a higher β -glucosidase activity than its predecessor PN-120 strain [3]. This work was accomplished to assess if such increased activity was due to a greater amount of expressed β -glucosidase or due to an enzyme with improved catalytic activity.

Methods. The β -glucosidase gene from *C. flavigena* PR-22 and PN-120 was amplified and sequenced according to Sanger (1977). The β -glucosidase enzyme of each strain was purified by ion exchange chromatography followed by gel filtration and later was biochemically characterized. Kinetics of β -glucosidase production were performed in 500 ml bioreactors with sugarcane bagasse as substrate in batch cultures. Catabolic repression kinetics were realized under the same growth conditions using glucose as repressor at concentrations of 30, 40 and 80 mM.

Results. The sequence analysis of *bglA* gene encoding the catalytic subunit of β -glucosidase in *C. flavigena* PR-22, showed no changes in the open reading frame in comparison with its homologous gene from PN-120 strain. The biochemical characterization of β -glucosidase from PR-22 presented no difference in biochemical and kinetic properties of the enzyme regarding to its counterpart in PN-120, so that the increased activity of β -glucosidase in strain PR-22 is unrelated to changes in the structure of this enzyme. The comparative kinetics of β -glucosidase production showed a 40 % increase in specific activity of this enzyme in PR-22 compared to PN-120 (Fig.1). In the catabolic repression studies, it was observed that the highest concentration assayed (80 mM), the PR-22 enzyme still conserved 70 % of its global activity (Fig.2), when it has been reported that glucose

concentrations above 50 mM repressed completely cellulase synthesis in *Cellulomonas* sp. [4].

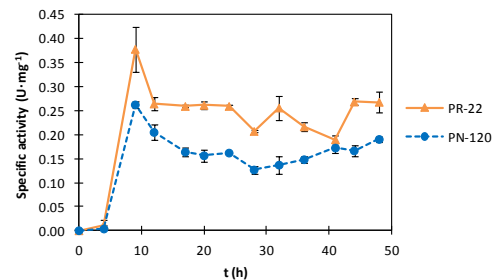


Fig. 1 β -glucosidase production of *C. flavigena* PR-22 and PN-120.

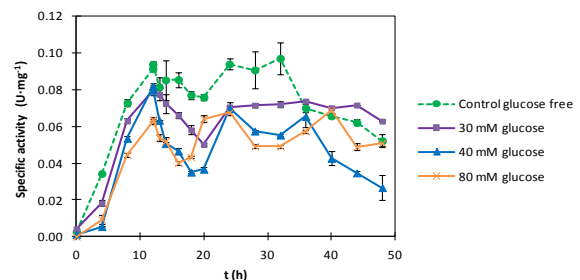


Fig. 2 Effect of glucose on the specific β -glucosidase activity in *C. flavigena* PR-22.

Conclusions. The increased activity of β -glucosidase in *C. flavigena* PR-22 compared to its counterpart in PN-120 could be caused by mutations in the regulatory gene and not in the open reading frame. As a result, PR-22 strain is resistant to catabolic repression and produces a greater amount of enzyme with the same biochemical characteristics as in PN-120.

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