Biochemical Characterization of a Glucose-stimulated β-D-Glucosidase Produced by *Humicola grisea* var. *thermoidea* Grown in Sugarcane Bagasse

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The effect of several carbon sources on the production of β-glucosidase by *Humicola grisea var. thermoidea* in submerged fermentation was investigated. Maximum production occurred when cellulose was present in the culture medium, but higher specific activities were achieved with cellobiose or sugarcane bagasse. Xylose or glucose (1%) in the reaction medium stimulated 2-fold β-glucosidase activity in crude extracts from mycelium grown in sugarcane bagasse. The enzyme was purified by ammonium sulfate precipitation followed by Sephadex G-200 and DEAE-cellulose chromatography, showing a single band in PAGE and SDS-PAGE. The β-glucosidase exhibited a carbohydrate content of 43% and apparent molecular masses of 57 and 60 kDa, estimated by SDS-PAGE and gel filtration, respectively. Optima of pH and temperature were 6.0 and 50ºC, respectively. The purified enzyme was thermostable up to 60 min, in water, at 55°C, and exhibited half-lives of 7 and 14 min at 60ºC, when incubated in the absence or presence of 50 mM glucose, respectively. The enzyme hydrolyzed *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-fucopyranoside, *p*-nitrophenyl-β-D-xylopyranoside, *o*-nitrophenyl-β-D-galactopyranoside, lactose and cellobiose. The best synthetic and natural substrates were *p*-nitrophenyl-β-D-fucopyranoside and cellobiose, respectively. Enzyme activity was stimulated up to 2-fold by glucose or xylose at concentrations from 25 to 200 mM. Addition of β-glucosidase to a reaction medium containing *Trichoderma reesei* cellulases increased the saccharification of sugarcane bagasse by about 50%. These findings suggest that *Humicola grisea var. thermoidea* β-glucosidase has a potential for biotechnological applications in lignocellulosic materials bioconversion.

β-D-Glucosidase, *Humicola grisea* var. *Thermoidea,* cellulose saccharification

FAPESP, CNPq, CAPES