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Purification and Characterization of an Acid β-galactosidase from *Aspergillus foetidus*

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ABSTRACT

A novel extracellular β-galactosidase (EC 3.2.1.23) from the fungus Aspergillus foetidus grow in soybean residue 2% was partially purified by ultrafiltration, gel filtration (Sephacryl S-200 resin) chromatography and ion exchange (DEAE) chromatography. The purified enzyme showed optimum pH of 3.0 and optimum temperature of 50°C. Furthermore, the enzyme was stable at pH 3.0 at 50°C for 15h, retaining 65% of its activity after the study period. The β-galactosidase hydrolyzed orthonitrophenyl-β-D-galactopyranoside (ONPG) and lactose. The kinetic constants Km and Vmax measured for ONPG and lactose were 1.83 mM, 256.65 IU/mL and 40mM, 2.86 IU/mL, respectively. Thus, the enzyme can be considered interesting for application in dairy products to produce low lactose products.

Keywords: *Aspergillus foetidus*; β-galactosidase; purification; characterization; soybean residue.

INTRODUCTION

The β-galactosidases are widely distributed in nature. These enzymes are widely used for industrial applications, both in hydrolysis reactions and transgalactosylation (Ruiz-Matute et al., 2012). Lactose intolerance is characterized by disorder in absorption of lactose carbohydrate, which affects thousands of people worldwide and is associated with a deficiency or absence of intestinal β-galactosidase enzyme, restricting at first the consumption of dairy products by these individuals. Enzymatic hydrolysis of lactose by β-galactosidase plays an important role in the processing of dairy products with low lactose content for consumption by intolerant individuals. Microorganisms such as filamentous fungi are effective producers of enzymes from agro industrial waste. Due to the potential of fungi of the genus *Aspergillus* in producing β-galactosidase, this study aimed to purify and characterize a β-galactosidase from *Aspergillus foetidus* grown in soybean residue 2%.

MATERIAL AND METHODS

Microorganism and cultivation. *Aspergillus foetidus* was grown in liquid medium (0.4% peptone, 0.4% yeast extract, 0.2% KH_2PO_4, 0.8% NaH_2PO_4 and 0.25% $MgSO_4$) containing 2% soybean residue (w/w) for 168 hours at pH 7.0. Cultivation was carried out in Erlenmeyer flask containing 50 mL medium and kept on shaker at 28°C and 120 rpm.

Enzyme assay. The enzyme assay for the determination of β-galactosidase activity was based on Nagy et al. (2001). An aliquot of the fermented broth or crude extract (0.5 mL) was incubated with substrate (0.5 mL ONPG (Sigma-Aldrich) 3 mM diluted in 0.1M sodium phosphate buffer pH 7.0) for 30 minutes at 30°C. 2 mL of 0.1M Na_2CO_3 was used as stop solution. Absorbance reading was performed with a spectrophotometer (Shimadzu Spectrophotometer UV-1800) using a wavelength of 410 nm.

Enzyme Purification. The *Aspergillus foetidus* culture was filtered and the supernatant was ultrafiltered through a membrane of 100 kDa and 30 kDa. Subsequent assays were performed in purification system (AKTA PURE - GE Healthcare). Protein concentration was determined as described by Bradford (Bradford, 1976).**Gel filtration chromatography (Sephacryl S-200).** The obtained enzyme solution was eluted on Sephacryl S-200 column (16cm x 60cm) equilibrated with 0.1 M sodium phosphate buffer pH 7.0. The eluted sample with fixed flow rate of 0.5 mL/mL was collected into 2 mL fractions. Fractions with βgalactosidase activity were pooled and lyophilized. **Ion exchange chromatography (DEAEFF).** The lyophilized sample of the gel filtration column was dissolved in 0.1 M sodium phosphate buffer pH 7.0. The sample was eluted on the DEAE Fast Flow anion exchange column (DEAE FF-GE Healthcare 1mL) equilibrated with the same buffer at a flow rate of 0.25 mL/minute. A linear gradient of sodium chloride (0.0 to 1M) was applied.

Electrophoresis (SDS-PAGE) and zymogram. The protein profile of the denatured samples was analyzed in 10% polyacrylamide gel stained with Comassie blue solution (Laemmli, 1970). For zymogram, the gel obtained by electrophoretic run with non-denatured sample was immediately incubated in buffer containing a solution of 5-bromo-4-chloroindolyl-β-D-galactopyranoside (X-GAL) 0.02% (w/v) (O'Connell and Walsh, 2008).

Effect of pH. The effect of pH on β-galactosidase activity was estimated in the presence of buffer solutions: sodium acetate 50 mM (pH 2.0 to 6.0), 50 mM sodium phosphate (pH 7.0 to 9.0) and potassium chloride - hydrochloric acid (pH 1.0 to 1.5).

Effect of temperature. The optimum temperature of the enzyme was measured at temperatures of 4, 10, 15, 20, 30, 40, 50, 60, 70 and 80°C.

Thermostability. The sample was incubated at 50 ° C for 20 hours. The assay was performed at pH 3.0. The residual enzyme activity was determined every 60 minutes, as the enzymatic assay described above.

Determination of the kinetic parameters. For the determination of MichaelisMenten constant (Km) and maximum velocity (Vmax) tests were performed with substrate concentrations (ONPG) ranging from 1 mM to 50 mM and the substrate lactose concentrations ranging from 1

mM to 300 mM. Activity assays were performed as described above by replacing the substrate with the substrates at the concentrations mentioned above. The results for the kinetic parameters were determined by Enzfitter program (Leatherbarrow, 1999).

RESULTS AND DISCUSSIONS

β-galactosidase purification. The purification process is summarized on Table 1. After the ultrafiltration steps, β-galactosidase activity was identified in the >30kDa fraction presenting specific activity of 360.61 IU/mg. The chromatographic profile of the S-200 fraction revealed the presence of two peaks, in which one of them was detected βgalactosidase activity of 6.57 IU/mL. The peak with the highest activity for the enzyme of interest was concentrated and eluted in anion exchange column - DEAE. The β-galactosidase was eluted at approximately 0.09M sodium phosphate buffer with NaCl. This peak has resulted in a fraction with activity of 4.5 IU/mL and purification factor of 8.66.

Table 1 - Summary of the purification steps of β-galactosidase produced by *Aspergillus foetidus* grown in soybean residue as carbon source.

Fractions	Protein (mg/mL)	Activity (IU/mL)	Specific activity (IU/mg)	Yield (%)	Purification factor
Crude extract	0.150	26.000	173.333	100.000	1.000
<100	0.144	24.000	166.667	92.308	0.962
>30	0.065	23.440	360.615	90.154	2.080
S-200	0.0075	6.570	876.000	25.269	5.054
DEAE	0.003	4.506	1502.000	17.331	8.665

The protein profile of the purification steps was evaluated on polyacrylamide gel (SDS-PAGE) which revealed the presence of three bands in the S-200 fraction and two bands in the DEAE fraction with an estimated molar mass of 76.58 and 70.25 kDa, shown on Figure 1A. It is suggested that in the partially purified fraction there are still two proteins with very close molecular mass with similar physicochemical characteristics. This single purified peak was active for β-galactosidase in specific zymogram, Figure 1 B.

Physico-chemical characterization. The β-galactosidase was stable for 15h, with activity retention of 65% at pH 3.0. The optimum pH of the enzyme was pH 3.0 and temperature at the 50°C. The results are consistent with fungal β-galactosidase literature data (Nath et al., 2014). The hydrolytic activity was higher for ONPG (22.12 IU/mL) compared to lactose (5.62 IU/mL). Furthermore, the kinetic parameters (Km and Vmax) of β-galactosidase at 50°C for the substrates ONPG and lactose were analyzed according to the MichaelisMenten model. The values of Km and Vmax constants for ONPG were 1,83mM and 256.65 IU/mL, respectively. While for lactose the Km value obtained was 40mM and Vmax 2.86 IU/mL. These results suggest that the enzyme is more specific for ONPG substrate than to lactose.

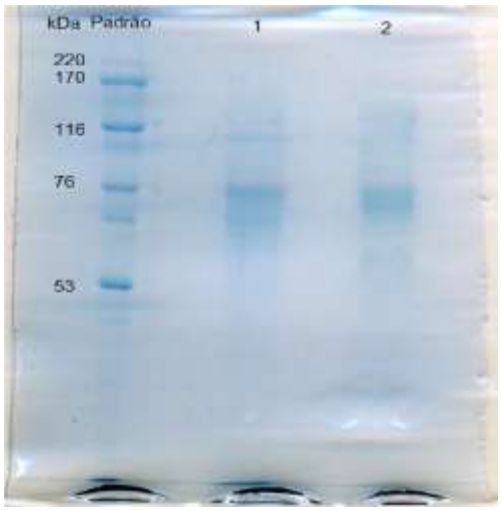

Figure 1 - A. Electrophoresis profile on polyacrylamide gel under denaturing conditions (SDS-PAGE 12%). Peak 1 S-200 (1) and peak 1 DEAE (2). Molecular weights of molecular markers used (HMW kit - GE Healthcare) are shown on the left. Figure B. Zymogram for β-galactosidase activity (peak 1 DEAE).

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CONCLUSIONS

The *Aspergillus foetidus* β-galactosidase cultured in soybean residue was partially purified. The enzyme characterization showed that the enzyme has potential biotechnological applicability, being active at acidic pH and 50°C temperature.

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