XII Seminário Brasileiro de Tecnologia Enzimática
ENZITEC 2016

Temperature and pH Effects on the Activity and Stability of the Xylanases Produced by the Thermophilic Fungus *Rasamsonia emersonii* S10.

Jéssica de Araujo Zanoni[1], Eleni Gomes[2] and Gustavo O. Bonilla-Rodriguez[2]

[1]Graduate Program in Microbiology, IBILCE-UNESP, E-mail: jehzanoni@hotmail.com
[2] IBILCE-UNESP, Rua Cristovão Colombo 2265, São José do Rio Preto SP 15054-000, Brazil.

SUMMARY

The xylanolytic complex consists of extracellular enzymes, mainly produced by fungi and bacteria, which hydrolyze the β (1-4) linkages of xylan. The degradation of this polysaccharide constituent of hemicellulose results in monomers of xylo-oligosaccharides subsequently converted into xylose. These enzymes have many industrial applications, being potential targets for research. This project aimed to characterize the xylanases present in the crude extract produced by the thermophilic fungus Rasamsonia emersonii S10 in solid state cultivation with respect to temperature and pH. Assays were performed to evaluate the effect of pH and temperature on enzyme activity, determining the optimum conditions and the stability of the enzymes. Optimal pH values were obtained at 5.5 and 7, and 80 ° C as optimum temperature for a 4 minutes incubation. Regarding thermal stability, the highest stability values occurred in the range from 4 to pH 5.5, and for incubation times of 30 and 40 minutes at 50 °C in the absence of the substrate.

Keywords: Enzyme; xylanase; thermophilic fungi, bioethanol.

INTRODUCTION

Enzymes are obtained from different natural sources, including plants and animals, but the microorganisms capable of growing in a wide diversity of environments are the preferred source of enzymes for the industry. Among these stand out the fungus for this purpose because of their ease of production and enzymes that can be extracted without the need for cell lysis (HALTRICH et al., 1996).

The agro-industrial wastes are potential sources of substrates for enzymatic degradation, including bagasse from sugarcane, corn cobs, sawdust, and other lignocellulosic materials rich in cellulose and hemicellulose present in the plant walls, which are considered important sources of sustainable energy (PAYNE et al., 2015).

The wide use of enzymes from xylanolytic complexes in industrial processes (NIEHAUS et al., 1999) demands further studies to characterize these enzymes not only in the isolated or pure form, but also the enzyme complex present in the crude extract obtained after cultivation process of microorganisms.

MATERIALS AND METHODS

The strain of the thermophilic fungus *Rasamsonia emersonii* S10 was obtained at the Laboratory of Applied Biochemistry and Microbiology of Institute of Biosciences, Letters and Exact Sciences of the São Paulo State University (IBILCE-UNESP).

Solid State Cultivation (SSC) was performed in polypropylene bags, adapted to provide the conditions necessary for cultivation. Agar discs colonized by the fungus (one disk of 10 mm in diameter for every 10 mL of nutrient solution) was inoculated into 5 g of a mixture of sugarcane bagasse, wheat bran, corn stover (1:1:1 w/w). To this mixture was added 20 mL of nutrient solution (providing an initial moisture content of about 70%) consisting of (g/L): $(NH)_4SO_4$ (3.5); KH_2PO_4 (3.0); $MgSO_4.{}_7H_2O$ (0.5); $CaCl_2$ (0.5), Tween 80 (10.0) and adjusted to pH 5.0. Cultivation occurred in an incubator set at 55 °C for 144 hours. After reaching the cultivation time, enzymatic extraction was done by adding 50 mL of distilled water (1:10 w/v) to each culture vessel and transferred to an orbital shaker kept at 150 rpm for 40 minutes. The crude extract was clarified by vacuum filtration and by centrifuging at 10,000 x*g* at 4 °C for 30 minutes. The supernatant was used as enzyme solution and stored at -80 ° C. The enzyme assays used 225 µL of substrate (sodium acetate buffer 0.2M pH 5.0 + 1% Beechwood xylan) added to 25 µL of crude enzyme solution, and incubated in a water bath in test tubes at 50 °C for 4 minutes, in order to measure the initial rate Vo. After that time the reaction was stopped by adding 250 µL of DNS (3.5-dinitrosalicylic acid) measuring the release of reducing sugars (MILLER, 1959). One unit of enzyme activity (U) is the amount of enzyme required to release 1 µmol of reducing sugar per minute (NC-IUB, 1979).

For determination of the optimum pH, the xylanolytic activity was assessed by incubating 0.1 mL of crude enzyme solution in 0.9 mL of a xylan suspension (1 %) at different pH values at 50 °C for 10 minutes. The following buffers (0.2 M) were used: Citrate (pH 3 to 3.5), acetate (pH 4, 4.5, 5, 5.5), MES (pH 6, 6.5, 7), Tris (pH 7.5 and 8) and glycine (pH 8.5, 9 and 9.5) the pH of the buffer was adjusted when necessary to correct the displacement effect of the temperature on the pKa (APPLICHEM, 2008).

For optimum temperature determination, xylanolytic activity was tested as a function of the incubation temperature from 30 to 90 °C as described above at the optimum pH.

To analyze the stability of the enzymes in relation to pH, a final volume of 1 mL of the crude extract suitably diluted 1:1 (v/v) in a buffer was incubated for 24 hours at 24 °C in 0.2 M buffers in the absence of the substrate. After the incubation period, the enzyme activity was determined as described previously, compared to a control diluted 1:1 with deionized water.

Thermal stability was analyzed by incubating the enzyme solution in the absence of the substrate for 2, 5, 10, 20, 30 and 40 min. at temperatures between 30 and 90 °C, followed by the enzyme assay at the optimum pH.

RESULTS AND DISCUSSION

The crude extract display five xylanases when analyzed by zymography (results not shown). Concerning the effect of pH, within the range of pH values examined two peaks of

higher activity were evident at 5.5 and 7.0, which could be ascribed to functional heterogeneity among the isoenzymes (Figure 1A). In terms of the pH stability variation was observed that the enzyme retained 70 % or higher activities in the range from 4.0 to 8.0, showing values higher than 100 % between 4.0 and 5.5 (Figure 1B).

A) B)

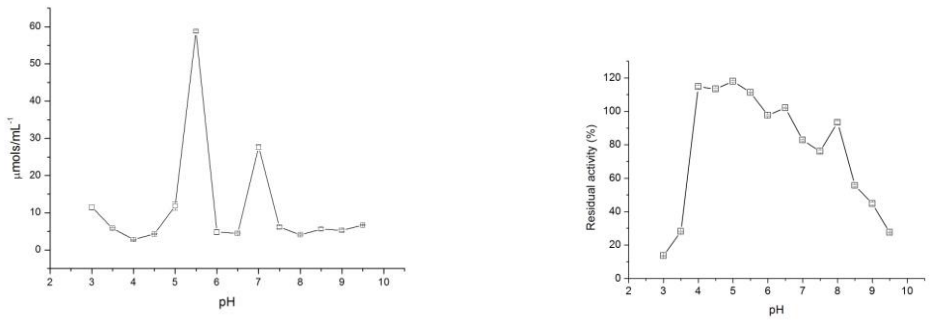

Figure 1. Effect of pH on xylanolytic activity. A) optimum pH; B) enzyme stability.

A xilanase produced by SSC by the mesophilic fungus *Aspergillus fumigatus* displayed an optimum pH of 5.5 (SILVA et al., 1999), and for a xylanase produced by *Aspergillus niger*, were described two peaks of pH, at 5.0, and 7.7.(PEREIRA, 2013).

The optimum temperature was achieved at 80 ^{o}C, decreasing at 90 ^{o}C (Figure 2A). Concerning thermal stability in the absence of the substrate, the highest activities were obtained at 50 ^{o}C for incubation times of 30 and 40 minutes (Figure 2B).

A) B)

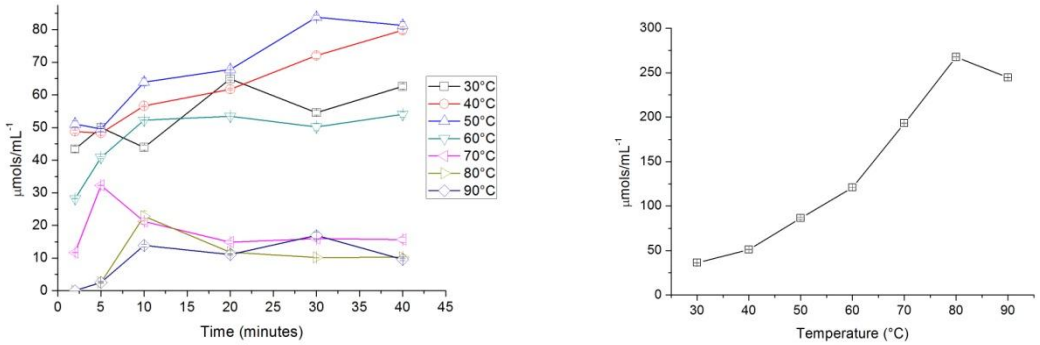

Figure 2. Effect of the temperature on xylanolytic activity. A) Optimum temperature. B) Thermal stability.

The same optimum temperature value was reported for the crude extract produced by the thermophilic fungus *R. emersonii* (then still classified as *Talaromyces emersonii*) (TUOHY; COUGHLAN, 1992) and for a xylanase produced by the fungus *Chrysoporthe cubensis* (GOMES, 2014), with the highest activity values for the thermal stability in the temperature range between 65 and 80 ^{o}C.

CONCLUSIONS

The results shown here displaying interesting characteristics for the crude extract that motivate us to carry out a study of the isolated forms.

REFERENCES

APPLICHEM. **Biological Buffers**. APPLICHEM**:** 18 p. 2008.

GOMES, S. K. **Purificação e Caracterização de Xilanases do Fungo Chrysoporthe cubensis e Utilização na Hidrólise de Bagaço de Cana-de-açucar**. 2014. 59 (Master). Universidade Federal de Viçosa, Viçosa, Brazil.

HALTRICH, D. et al. Production of fungal xylanases. **Bioresource Technology,** v. 58, n. 2, p. 137-161, 1996.

MILLER, G. L. Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar. **Analytical Chemistry,** v. 31, n. 3, p. 426-428, 1959.

NC-IUB. Units of Enzyme Activity. **European Journal of Biochemistry,** v. 97, n. 2, p. 319-320, 1979.

NIEHAUS, F. et al. Extremophiles as a source of novel enzymes for industrial application. **Appl Microbiol Biotechnol,** v. 51, n. 6, p. 711-729, 1999.

PAYNE, C. M. et al. Fungal Cellulases. **Chemical Reviews,** v. 115, n. 3, p. 1308-1448, 2015.

PEREIRA, P. E. D. **Análise de celulases e xilanases por fungo isolado a partir do Bioma Cerrado**. 2013. (Master). Universidade Federal de Goiás, Goiânia, Brazil.

SILVA, C. H. C. E. et al. Purification and characterization of a low molecular weight xylanase from solid-state cultures of Aspergillus fumigatus Fresenius. **Revista de Microbiologia,** v. 30, p. 114-119, 1999.

TUOHY, M. G.; COUGHLAN, M. P. Production of thermostable xylan-degrading enzymes by Talaromyces emersonii. **Bioresource Technology,** v. 39, n. 2, p. 131-137, 1992.

Acknowledgments: FAPESP and CAPES, for financial support.