XII Seminário Brasileiro de Tecnologia Enzimática
ENZITEC 2016

L-asparaginase production by *Aspergillus terreus* CCT7693 through solid-state fermentation (SSF) using sugarcane bagasse as support

J. J. Muso Cachumba[1], L. P. Brumano[1], F. A. F. Antunes[1], J. C. Santos[1] e S. S. da Silva[1].

[1]Universidade de São Paulo, Escola de Engenharia de Lorena, Departamento de Biotecnologia
E-mail para contato: silviosilverio@gmail.com

ABSTRACT

The enzyme L-asparaginase (EC3.5.1.1) can be applied as agent for reducing acrylamide concentration in foods processed at high temperatures and as antileukemic drug. The present work evaluated the influence of different carbon and nitrogen sources in the production of extracellular L-asparaginase through solid-state fermentation (SSF) by Aspergillus terreus CCT7693. Supplemented sugarcane bagasse was used as support for SSF. Four carbon sources were tested: starch, xylose, glucose and maltose, as well as four nitrogen sources: asparagine, proline, glutamine and urea were evaluated. The highest enzymatic activities were found in the test containing starch (1%) and L-asparagine (2%), reaching around of 33.02 U/g of starch after 96 hours. Considering the periplasmic nature of fungal L-asparaginase, extracellular production of this enzyme could be important to make further purification steps easier.

Key words: L-asparaginase; solid-state fermentation; *Aspergillus terreus*.

INTRODUCTION

The enzyme L-asparaginase catalyzes the hydrolysis of L-asparagine into L-aspartic acid and ammonia. Among different uses, L-asparaginase has two main important applications. The first one as an alternative for treating some types of cancer such as acute lymphoblastic leukemia, lymphoid system malignant and Hodgkin's lymphomas (Appel et al., 2007; Broome, 1961; Duval, 2002). However, most of the treatments with this enzyme may lead to immunological sensitivity and immune inactivation (Narta et al., 2007). The second one is preventing the acrylamide formation, a carcinogenic substance, in fried and baked foods (Anese et al., 2011; Mohan Kumar et al., 2013; Medeiros Vinci, Mestdagh, & De Meulenaer, 2012).

Actually, the production of L-asparaginase is usually performed by submerged fermentation; however, this technique has many disadvantages that make the process highly expensive, with low production yield and generates large volumes of wastewater (El-Bessoumy et al., 2004). Talking this into account, one interesting alternative is the solid-state fermentation (SSF), a simple and effective technique that can increase the production yield with a lower costs and energy consumption (Couto & Sanromán, 2006; Thomas et al., 2013). One of the mains advantages of SSF is the possibility to use agricultural residues as the main component of the fermentation (Martinello et al., 2006).

Moreover, L-asparagine production is influenced by multiple factors such as the composition of the fermentation medium, temperature, pH, incubation time, etc. (Hymavathi

et al., 2009; Wei & Liu, 1998). Few investigators have reported studies about carbon and nitrogen sources for optimization process (Kumar et al., 2010). Therefore, in order to investigate and understand better this process, this study aims to evaluate the influence of four different carbon (starch, xylose, glucose and maltose) and nitrogen source (asparagine, proline, glutamine and urea) on the production of extracellular L-asparaginase through solid-state fermentation, using the filamentous fungus *Aspergillus terreus* CCT7693.

MATERIAL AND METHODS

For the reactivation stage, *A. terreus* CCT7693 (kindly provided by Coleção de Culturas Tropical Fundação André Tosello) was placed in solid PDA medium during 7 days at 30°C. Subsequently, spores were collected in a solution of 0.9% NaCl and 0.1% Tween 80. Finally, the obtained solution was standardized by counting spores, until concentration of 10^7-10^8 spores/mL. Sugarcane bagasse was first milled and washed in water, both warm and cold, in order to remove any residual sugar from the material. The material was dried until, approximately, 8% moisture and its particle size was standardized using a 20 Mesh sieve.

For the production of L-asparaginase extracellular by SSF, the sugarcane bagasse was used as support, supplemented with modified Czapek-Dox medium. The influence of different carbon (starch, xylose, glucose and maltose) and nitrogen (asparagine, proline, glutamine and urea) sources in the production of extracellular L-asparaginase was evaluated using 1% and 2% for carbon and nitrogen source, respectively. The culture was performed in 50 mL Erlenmeyer flasks having 2 g of bagasse and 9 mL of culture medium containing the corresponding nitrogen and carbon source for each experiment. 1 mL of standardized inoculum was added in each of the treatments except blank tests. The culture was incubated at 25°C, with initial relative moisture of 80% for 96.

In the enzymatic extraction was used buffer solution Tris-HCl 50 mM (pH 7.6) containing 0.5% of Tween 80. The extracting solution was added in each flask and this suspension was remained on agitation (180 rpm) at 25°C for 2 hours. Thus, the extracts were filtered with gauze and tested for enzymatic activity assay using hydroxamate method.

RESULTS AND DISCUSSION

This work evaluated 16 experiments in duplicates. The figure 1 shows the average of enzyme activity measurements by hydroxamate method, performed for each experiments. The blanks are not shown in the figure, however, as expected; they showed 0.0 U/g of carbon source of enzyme activity.

According to the Figure 1, the highest enzymatic activities were observed in the medium containing 1% starch and 2% L-asparagine (A-Asp), reaching 37,49 and 28,54 U/g of starch after 96 hours. In the others experiments, only that with maltose and L-glutamine (M-Glut) or L-asparagine (M-Asp) also showed positive results, reaching an average of the enzymatic activity of 20,86 and 3,31 U/g of maltose, respectively. Currently, there are few reported studies regarding the optimization of L- asparaginase by FES, despite its advantages. On the other hand, several studies showed proline as the best inductor of the production of L-asparaginase, however all these works were performed by submerged fermentation (Baskar; Renganathan, 2009; Tippani; Sivadevuni, 2012). Thus, probably metabolism in these two fermentation processes are different once according to results shown in this work, the best

inductor was L-asparagine followed by L-glutamine and for proline the enzyme activity was nil. Contradicted results also occurred related to carbon sources once in the enzyme production process by submerged fermentation, the most widely used source is glucose (Baskar; Renganathan, 2009; Tippani; Sivadevuni, 2012). In addition, Akilandeswari, et al. (2012) evaluated different carbon sources (glucose, sucrose and starch) in the production of L-asparaginase from *Aspergillus niger* by submerged fermentation. In this work, authors verified that the starch was the best substrate presenting enzymatic activity of 3.60 U/mL. However, in another similar study, Farag et al., (2015) investigated seven different substrates, such as soluble starch, fructose, maltose, dextrose, lactose and sucrose, in the production of L-asparaginase by *Aspergillus terreus*. Among these different substrates, the starch presented lower enzyme activity (1.98 U/mg protein).

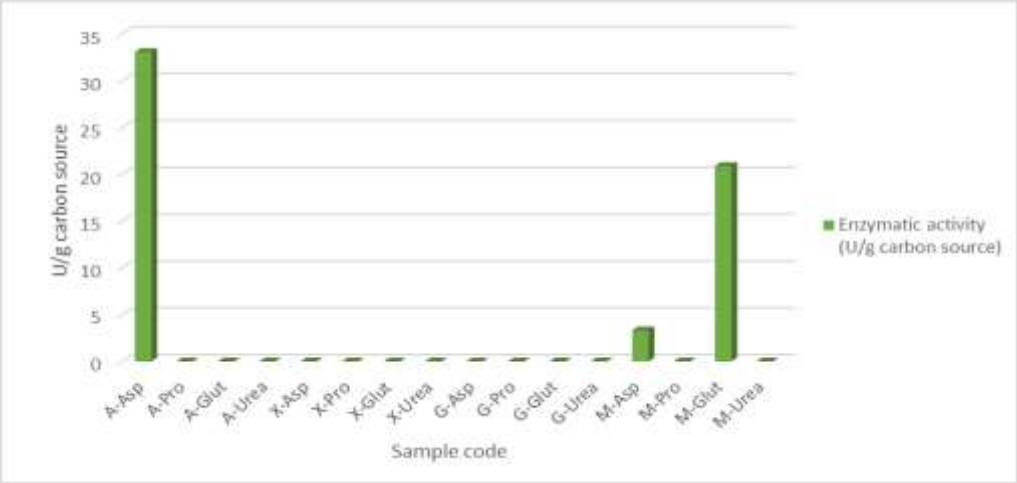

Figure 1 – Enzymatic activity of L-asparaginase for each treatment at 96 hours.

By this work, it is possible to mention that the use of sugarcane bagasse as support in SSF for producing extracellular L-asparaginase, would make the process sustainable and could reduce significantly its costs.

CONCLUSIONS

It was possible to produce a significant amount (33,02 U/ g starch) of extracellular L-asparaginase from the culture of *A. terreus* in solid-state fermentation using sugarcane bagasse as support, 1% starch as carbon source and 2% L-asparagina as nitrogen source. These results are relevant because of its contribution to the optimization process for producing this important enzyme. Also, this work showed SSF as an interesting alternative for the production of extracellular L-asparaginase, that currently is produced by bacteria in industrial scale and its periplasmic nature difficult extraction and purification phase.

ACKNOWLEDGEMENTS

The authors would like to thank FAPESP, CAPES and CNPq for financial support and Coleção de Culturas Tropical Fundação André Tosello for providing the fungus evaluated in the present work.

REFERENCES

Akilandeswari, K.; Kavitha, K.; Vijayalakshmi, M. Production of bioactive enzyme L-asparaginase from fungal isolates of water sample through submerged fermentation. International Journal of Pharmacy and Pharmaceutical Sciences, v. 4, n. SUPPL. 4, p. 363–366, 2012.

Anese, M., Quarta, B., Peloux, L., & Calligaris, S. (2011). Effect of formulation on the capacity of l-asparaginase to minimize acrylamide formation in short dough biscuits. Food Research International, 44(9), 2837–2842. http://doi.org/10.1016/j.foodres.2011.06.025

Appel, I. M., Van Kessel-Bakvis, C., Stigter, R., & Pieters, R. (2007). Influence of two different regimens of concomitant treatment with asparaginase and dexamethasone on hemostasis in childhood acute lymphoblastic leukemia. Leukemia, 21(11), 2377–80. http://doi.org/10.1038/sj.leu.2404793

Baskar, G.; Renganathan, S. Evaluation and Screening of Nitrogen Source for L-asparaginase Production by Aspergillus terreus MTCC 1782 using Latin Square Design. Journal of Mathematics and Statistics, v. 1, n. 2, p. 55–58, 2009.

Broome, J. D. (1961). Evidence that the L-Asparaginase Activity of Guinea Pig Serum is responsible for its Antilymphoma Effects. Nature, 191(4793), 1114–1115. http://doi.org/10.1038/1911114a0

Couto, S. R., & Sanromán, M. Á. (2006). Application of solid-state fermentation to food industry—A review. Journal of Food Engineering, 76(3), 291–302. http://doi.org/10.1016/j.jfoodeng.2005.05.022

Duval, M. (2002). Comparison of Escherichia coli-asparaginase with Erwinia-asparaginase in the treatment of childhood lymphoid malignancies: results of a randomized European Organisation for Research and Treatment of Cancer---Children's Leukemia Group phase 3 trial. Blood, 99(8), 2734–2739. http://doi.org/10.1182/blood.V99.8.2734

El-Bessoumy, A. A, Sarhan, M., & Mansour, J. (2004). Production, isolation, and purification of L-asparaginase from Pseudomonas aeruginosa 50071 using solid-state fermentation. Journal of Biochemistry and Molecular Biology, 37(4), 387–393. http://doi.org/10.5483/BMBRep.2004.37.4.387

Farag, A. M. et al. Optimization of production of anti-tumor l-asparaginase by free and immobilized marine Aspergillus terreus. The Egyptian Journal of Aquatic Research, v. 41, n. 4, p. 295–302, 2015.

Hymavathi, M., Sathish, T., Subba Rao, C., & Prakasham, R. S. (2009). Enhancement of L-asparaginase production by isolated Bacillus circulans (MTCC 8574) using response surface methodology. Applied Biochemistry and Biotechnology, 159(1), 191–8. http://doi.org/10.1007/s12010-008-8438-2

Kumar, S., Dasu, V. V., & Pakshirajan, K. (2010). Localization and production of novel l-asparaginase from Pectobacterium carotovorum MTCC 1428. Process Biochemistry, 45(2), 223–229. http://doi.org/10.1016/j.procbio.2009.09.011

Martinello, T., Kaneko, T. M., Velasco, M. V. R., Taqueda, M. E. S., & Consiglieri, V. O. (2006). Optimization of poorly compactable drug tablets manufactured by direct compression using the mixture experimental design. International Journal of Pharmaceutics, 322(1-2), 87–95. http://doi.org/10.1016/j.ijpharm.2006.05.034

Medeiros Vinci, R., Mestdagh, F., & De Meulenaer, B. (2012). Acrylamide formation in fried potato products – Present and future, a critical review on mitigation strategies. Food Chemistry, 133(4), 1138–1154. http://doi.org/10.1016/j.foodchem.2011.08.001

Mohan Kumar, N. S., Shimray, C. A., Indrani, D., & Manonmani, H. K. (2013). Reduction of Acrylamide Formation in Sweet Bread with l-Asparaginase Treatment. Food and Bioprocess Technology, 7(3), 741–748. http://doi.org/10.1007/s11947-013-1108-6

Narta, U. K., Kanwar, S. S., & Azmi, W. (2007). Pharmacological and clinical evaluation of l-asparaginase in the treatment of leukemia. Critical Reviews in Oncology/Hematology, 61(3), 208–221. http://doi.org/10.1016/j.critrevonc.2006.07.009

Thomas, L., Larroche, C., & Pandey, A. (2013). Current developments in solid-state fermentation. Biochemical Engineering Journal, 81, 146–161. http://doi.org/10.1016/j.bej.2013.10.013

Tippani, R.; Sivadevuni, G. Nutritional factors effecting the production of L-asparaginase by the Fusarium sp. African Journal of Biotechnology, v. 11, n. 15, p. 3692–3696, 2012.

Wei, D. ZH., & Liu, H. (1998). Promotion of L-asparaginase production by using n-dodecane. Biotechnology Techniques, 12(2), 129–131. http://doi.org/10.1023/A:1008888400727