Production of amylases by *Aspergillus tamarii* in solid state fermentation at high initial glucose concentrations

Daniela Farani de Souza and Rosane Marina Peralta*

*Departamento de Bioquímica, Laboratório de Bioquímica e Fisiologia de Microrganismos, Universidade Estadual de Maringá, 87020-900, Maringá, Paraná, Brasil. *Author for correspondence. e-mail: rmperalta@uem.br*

ABSTRACT. The effect of glucose and other soluble sugars (xylose, fructose, maltose, cellobiose and lactose) in the production of amylases by *Aspergillus tamarii* was studied in solid state fermentation (SSF). Wheat bran solid state cultures were resistant to catabolite repression even at high concentration of glucose (10%). Results show the potential of solid state systems to overcome the adverse effects of high sugar concentrations in the media. The ability to prevent catabolite repression appear to be related with the content moisture of solid state systems: less content moisture of cultures means less catabolite repression caused by glucose.

Key words: amylase, *Aspergillus tamarii*, catabolite repression, glucose effect, solid-state fermentation.

RESUMO. Produção de amilases por *Aspergillus tamarii* em fermentação em estado sólido a altas concentrações iniciais de glucose. O efeito de glucose e outros açúcares solúveis (xilose, frutose, maltose, celobiose e lactose) na produção de amilases por *Aspergillus tamarii* foi estudado em fermentação em estado sólido (SSF). As culturas em estado sólido utilizando farelo de trigo como substrato foram resistentes à repressão catabólica, mesmo a altas concentrações iniciais de glucose (10%). Os resultados mostram o potencial dos sistemas em estado sólido em minimizar os efeitos adversos da repressão catabólica. A habilidade destes sistemas em evitar repressão catabólica parece estar relacionada com o conteúdo de umidade das culturas: quanto menor a umidade inicial das culturas em estado sólido, maior a sua resistência à repressão catabólica por glucose.

Palavras-chave: amilase, *Aspergillus tamarii*, repressão catabólica, efeito glucose, fermentação em estado sólido.

A variety of microorganisms, including bacteria, yeasts and filamentous fungi, have been reported to produce amylolytic enzymes (Guzman-Maldonado, 1995). Amylases are used in a variety of industrial processes which require efficient saccharification of raw starch (Bowles, 1996).

Solid state cultivation systems (SSF) and submerged liquid cultivation systems have been used for amylase production, although most research has used liquid culture, which allows greater control of culture conditions such as temperature and pH. However, solid state fermentation is gaining interest in recent years due to potential advantages in manufacturing products such as enzymes in high yield, at high concentrations and with high specificity (Pandey *et al.,* 1999). Some of the advantages of SSF over conventional submerged cultures involving fungi are simple equipment and low moisture content, which prevents bacterial contamination. The list of different substrates used for the cultivation of microorganisms to produce enzymes is long, but wheat bran is the most common substrate used in this type of cultivation (Pandey *et al*., 1999)

Catabolite repression caused by glucose and other easily metabolizable sugars in the production of amylase by microorganisms developed in submerged cultures is well documented (Murygina, 1988, Nandakumar *et al*., 1999, Ray *et al*., 1996, Sadhukhan *et al*., 1992). On the other hand, the ability of the solid state fermentation significantly minimizing catabolic repression has been described in the production of different hydrolytic enzymes, including amylolytic systems by some microorganisms (Babu and Satyanarayana, 1995, Gessesse and Mamo, 1999, Ramesh and Lonsane, 1991, Siqueira *et al*., 1997) In a previous work, *A. tamarii* has been found to grow well and to produce high levels of amylase in submerged fermentation using starch and maltose as substrate (Moreira *et al.,* 1999). The purpose of this research was to study the capability of *A. tamarii* to produce amylases in SSF in

the presence of glucose and other soluble and easily metabolizable sugars.

Material and methods

Microorganism. *Aspergillus tamarii* used in this research was isolated from soil and described previously as a good amylase producer (Moreira *et al*., 1999)

Enzyme production in solid-state fermentation. For production of enzymes in SSF, the fungi were grown at 30ºC in 250 ml Erlenmeyer flasks containing 5 g of wheat bran, enriched or not enriched with glucose (or other easily metabolizable sugar), at different concentrations. Mineral solution (Montenecourt and Eveileigh, 1977) was used to adjust the moisture content from 43 to 81%. Dry weight of the substrate and moisture content were determined gravimetrically after drying of samples at 60ºC.

Enzyme extraction. At certain periods, 50ml of cold water were added to the cultures, the mixtures were shaken for 1h at 4ºC and centrifuged. The supernatants were assayed for amylolytic activity. Results were expressed as the mean of at least three independent cultures.

Enzyme assay. Amylase activity was estimated by analysis of reducing sugars released during hydrolysis of 0.5% (w/v) starch in 0.05 M phosphate buffer, pH 6.0, at 50ºC for 30 min. by the dinitrosalicylic acid method (Miller, 1959). One unit of amylase activity was defined as the amount of enzyme that releases 1 μmol of reducing sugar as glucose per min under the assay conditions. Results were expressed as unit of amylolytic activity per g of dry substrate (U/g dry wet).

Determination of remaining glucose. The remaining glucose concentration in the media was determined by the glucose oxidase/peroxidase method (Bergmeyer and Bernt, 1974). Cultures without fungi were submitted to similar treatment as described for enzyme extraction to determine initial glucose concentration in time zero.

Results and discussion

The effect of enrichment of wheat bran solid state system with different easily metabolizable sugars in the production of amylase is shown in Figure 1. No catabolic repression was evidenced when soluble sugars were added at 1% (w/w). Previous results obtained in submerged cultures showed that the production of amylase was severely affected by glucose at 1% (Moreira *et al.,* 1999).

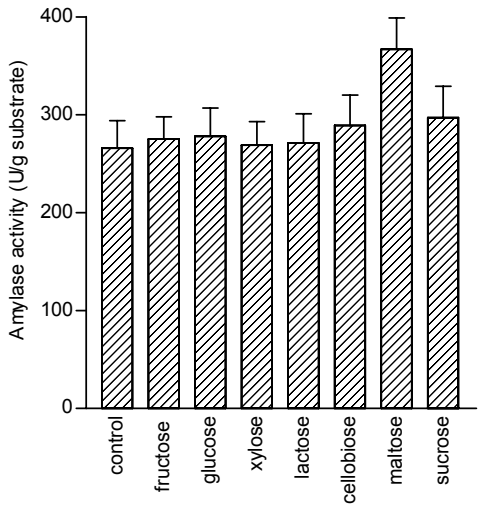

Figure 1. Effect of enrichment of solid state cultures with easily metabolizable sugars in the production of amylases. The cultures were developed for 5 days at 73% moisture content. Different soluble sugar were added in cultures to obtain 1% concentration (w/w). Error bars are S.D. from mean

Basal wheat bran cultures were enriched with increasing amounts of glucose to test the capacity of wheat bran cultures to produce amylase in the presence of high initial glucose concentrations. Cultures were monitored daily (Figure 2). Basal wheat bran cultures, in which no glucose was added, contained about 0.1% of glucose. Maximum amylase production was obtained after 5 days of cultivation and the enrichment of wheat bran cultures with glucose at 5 or 10% did not cause any catabolite repression (Figure 2A). Catabolite repression by glucose was observed only when higher amounts of glucose were added to the cultures (15% or more). The determination of residual glucose in the cultures showed that glucose was very efficiently consumed by fungal cells. Less than 10% of initial glucose was present after 5 days of cultivation (Figure 2B). However, it became clear in this experiment that the production of amylase was not affected by the high glucose concentration present in the media during the first four days of cultivation, considering that the production of amylase started on the second day.

The ability of solid-state fermentation significantly minimizing catabolic repression has been previously described. Some examples are the production of α-amylases from *Bacillus coagulans*

(Babu and Satyanarayana, 1995), *Aspergillus niger* (Nandakumar *et al*., 1999), *Picnoporus sanguineus* (Siqueira *et al*., 1997), xylanases from *Bacillus licheniformis* (Archana and Satyanarana, 1997), *Bacillus* sp. (Gessesse and Mamo, 1999), pectinases from *Aspergillus* sp (Aguilar and Huitrón, 1986) and *Aspergillus niger* (Solis-Pereyra *et al.,* 1996). However, all solid state systems described as resistant to catabolite repression were developed by the use of wheat bran as substrate. It is possible that some chemical component of wheat bran may be, at least in part, responsible for this factor.

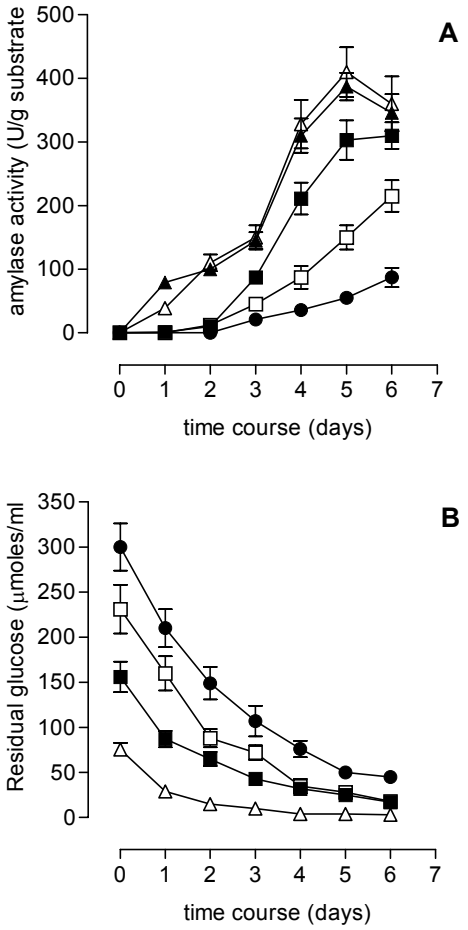

Figure 2. Time course of production of amylases in solid state cultures. Cultures were developed at 73% moisture content. Control cultures (▲); 5% glucose supplemented cultures (Δ); 10% glucose supplemented cultures (■); 15% glucose supplemented cultures (❒); 20% glucose supplemented cultures (●). Errors bars are S.D. from mean

Content moisture in the solid substrate is a critical factor in SSF (Ramadas *et al*., 1996). In order to study the effect of initial substrate moisture on enzyme production by the cultures, different initial moisture contents were established in the substrate (Figure 3). Maximum enzyme activity was obtained in cultures in which the initial moisture was equal to or higher than 73%. Too much water reduces substrate porosity, deforms the structure of wheat bran and causes stickiness of the substrate, leading to reduced O_2 transfer (Ramadas *et al*., 1996). However, the solubility of nutrients in wheat bran is less at low water levels. This may explain the reduction of production amylase when the initial moisture content is low (Lonsane and Ramesh, 1990). The ability to prevent catabolite repression appears to be also related with the content moisture of solid-state systems. Catabolite repression was more evident in cultures in which moisture content was superior to 77% and almost none at initial content moisture equal or inferior than 62% (Figure 3). Highest content moisture in solid-state systems could increase the processes of diffusion and, as a consequence, the glucose would be more accessible to the cells. Data agree with those obtained previously in the production of amylase by *Bacillus licheniformis* M27 (Ramesh and Lonsane, 1991), where the authors suggested that the absence of catabolite repression in solid state systems is due to several factors collectively. These include the slow and low processes of diffusion in solid state cultures due to the low water activity (Ramesh and Lonsane, 1990).

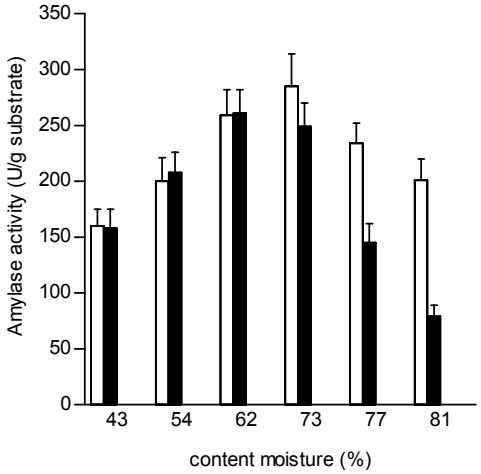

Figure 3. Effect of moisture content in the production of amylases and in the ability of the cultures to overcome catabolite repression by glucose. Cultures were developed for 5 days. Open bars: control cultures. Closed bars: 15% glucose supplemented cultures. Error bars are S.D. from mean

Results in current research confirm the ability of wheat bran solid state systems to overcome the

catabolite repression. However, further studies will be necessary to understand the ability of wheat bran solid-state systems to minimize catabolite repression by glucose. The production of amylases by *A. tamarii* in solid state fermentation at high initial glucose concentration may be improved by optimization of the culture conditions and extraction of enzyme.

Acknowledgements

This research had the support of the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Universidade Estadual de Maringá. R.M. Peralta is Research Fellows of CNPq. D.F. de Souza received a fellowship from CNPq (PIBIC-UEM)

References

AGUILAR, G.; HUITRÓN, C. Application of fed-batch cultures in the production of extracellular pectinases by *Aspergillus* sp. *Enzyme Microb. Technol.,* New York, v. 8, p. 541-545, 1986

ARCHANA, A.; SATYANARANA, T. Xylanase production by thermophilic *Bacillus licheniformis* A99 in solid-state fermentation. *Enzyme Microb. Technol.,* New York, v. 21, p. 12-17, 1997

BABU, K.R.; SATYANARAYANA, T. α-amylase production by thermophilic *Bacillus coagulans* in solid state fermentation. *Process. Biochem.,* Rickmansworth, v. 30, p. 305-309, 1995

BERGMEYER, H.U.; BERNT, E. D-glucose determination with glucose oxidase and isomerase. In: BERGMEYER, H.U. *Methods of enzymatic analysis*. New York: Verlag Chimie/Academic Press, 1974, p. 1205-1212

BOWLES, L.K. Amylolytic enzymes. *Food Sci. Technol.,* Zurich, v. 75, p. 105-129, 1996

GESSESSE, A.; MAMO, G. High-level xylanase production by an alkaliphic *Bacillus* sp using solid state fermentation. *Enz. Microb. Technol.,* New York, v. 25, p. 68-72, 1999

GUZMAN-MALDONADO, H.; PAREDES-LOPEZ, O. Amylolytic enzymes and products derived from starch: a review. *Crit. Rev. Food Sci. Nutr.,* Boca Raton, v. 35, p. 373-403, 1995

LONSANE, B.K.; RAMESH, M.V. Production of bacterial thermostable alpha amylase by solid-state fermentation, a potential tool for achieving economy in enzyme production and starch hydrolysis. *Adv. Appl. Microbiol.,* London, v. 35, p. 1-55,1990

MILLER, G.L. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.,* Washington, D.C., v. 31, p. 426-428, 1959

MONTENECOURT, B.S.; EVEILEIGH, D.E. Preparation of mutants of *Trichoderma reesei* with enhanced cellulase production. *Appl. Environ. Microbiol.,* Washington, D.C., v. 34 , p. 777-784, 1977

MOREIRA, F.G. *et al*. Production of amylases by *Aspergillus tamarii*. *Braz. J. Microbiol.,* v. 30, p. 157-162, 1999

MURYGINA, V.P. Regulation of alpha-amylase biosynthesis in *Bacillus diastaticus* mutants with various levels of enzyme synthesis. *Mikrobiologiia*, v. 57, p. 734-739, 1988

NANDAKUMAR, M.P. *et al*. Studies on catabolite repression in solid state fermentation for biosynthesis of fungal amylases. *Let. Appl. Microbiol.,* Oxford, v. 29, p. 380-384, 1999

PANDEY, A. *et al*. Solid state fermentation for the production of industrial enzymes. *Curr. Sci.,* Bangalore, v. 77, p. 149-162, 1999

RAMADAS, M. *et al*. Production of amyloglucosidase by *Aspergillus niger* under different cultivation regimens. *World J. Microbiol. Biotechnol.,* Dordrecht, v. 12, p. 267-271, 1996

RAMESH, M.V.; LONSANE, B.K. Critical importance of moisture content in alpha-amylase production by *Bacillus licheniformis* M27 in solid state fermentation. *Appl. Microbiol. Biotechnol.,* Berlin, v. 33, p. 501-505, 1990

RAMESH, M.V.; LONSANE, B.K. Ability of a solid state fermentation technique to significantly minimize catabolic repression of α-amylase production by *Bacillus licheniformis* M27. *Appl. Microbiol. Biotechnol.,* Berlin, v. 35, p. 591-593, 1991

RAY, R.R. *et al*. Induction and catabolite repression in the biosynthesis of β-amylase by *Bacillus megaterium* B6. Biochem. *Mol. Biol. Int.,* v. 38, p. 223-230, 1996

SADHUKHAN, R.K. *et al*. Induction and regulation of α-amylase synthesis in a cellulolytic thermophilic fungus *Myceliophthora thermophila* D14 (ATCC 48104). *Indian J. Exp. Biol.,* Nova Delhi, v. 30, p. 482-486, 1992

SIQUEIRA, E.M.D. *et al*. *Pycnoporus sanguineus*: a novel source of alpha-amylase. *Mycol. Res.,* Cambridge, v. 101, p. 188-190, 1997

SOLIS-PEREYRA, S. *et al*. Production of pectinases by *Aspergillus niger* in solid state fermentation at high initial glucose concentrations. *World J. Microbiol. Biotechnol.,* Dordrecht, v. 12, p. 257-260, 1996

Received on January 04, 2001.
Accepted on March 28, 2001.