American Journal of Agricultural and Biological Sciences 7 (2): 129-134, 2012
ISSN 1557-4989

Inulinase Production by *Penicillium Citrinum* ESS in Submerged and Solid-State Cultures

[1]Cynthia Lafuente-Castaneda, [1]Gabriela Martinez, [1]Juan C. Contreras-Esquivel, [1]Raul Rodriguez-Herrera, [2]Arely Prado-Barragan and [1]Cristobal N. Aguilar
[1]Department of Food Science and Technology, School of Chemistry, Universidad Autonoma de Coahuila. Blvd. Venustiano Carranza. Col. Republica, CP 25280, Saltillo, Coahuila, Mexico
[2]Departament of Biotechnology, Universidad Autonoma Metropolitana. Unidad Iztapalapa, CP 09340, D.F, Mexico

Abstract: Problem statement: This work reports the *Penicillium citrinum* ESS strain as a new source of inulinase (2,1-β-D fructanhydrolase, E.C. 3.2.1.7). **Approach:** The enzyme was produced in both, Solid-State (SSC) and Submerged Culture (SmC) using a basal medium added either with inulin or sucrose (5 gL^{-1}). **Results:** The culture grew faster in SmC than in SSC with both carbon sources; however, yields of inulinase activity per gram of biomass were higher in SSC (1,658,237.28 for inulin and 66598.96 for sucrose) than in SmC (441.05 for inulin and 183.91 for sucrose). **Conclusion:** The highest inulinase production was obtained by SSC, either with inulin or sucrose, reaching values of 6650 and 2970 U/L respectively. In contrast, in SmC were obtained higher levels of inulinase activity (2241 U/L) with sucrose as carbon source (24 h), while with inulin, the enzyme production was negatively affected and retarded.

Key words: *Penicillium citrinum* ESS, inulinase production, Solid-State Culture (SSC)

NTRODUCTION

Inulin is a linear β (2-1) linked fructose polymer with terminal glucose unit, and it can be found as a reserve carbohydrate in a composite family of plant tubers (Ertan *et al*., 2003a; 2003b). Inulin is used for production of ultra-high fructose syrup, ethanol, aceto-buthanol, gluconic acid, sorbitol and fructooligosacchrides. Fructose obtained from inulin comes from a single enzymatic step and represent yields up to 95% fructose (Vandamme and Deryke, 1983; Pandey *et al*., 1999). Inulin can be hydrolysed by two different types of inulinases: Exoinulinase (β-D-fructan fructohydrolase, EC 3.2.1.80) that liberates fructose; and endoinulinase (2-1- β-D-fructan fructanohydrolase, EC 3.2.1.7) that produces oligosaccharides (Skowronek and Fiedurek, 2004; Shuichi and Norio, 1992).

Inulinases have been extracted and characterized from inulin-storing tissues (Pandey *et al*., 1999; Gupta *et al*., 1994), however production of microbial inulinases have recently draw attention as they offer several industrial advantages. Many organisms, including filamentous fungi, yeast and bacteria have been claimed as inulinases producers (Wei *et al*., 1999). Microbial inulinases are potentially effective for preparing high fructose syrups from inulin (Ettalibi and Baratti, 1987; Pessoni *et al*., 1999). These hydrolases are usually inducible and their exo-acting forms may hydrolyse fructose polymers (inulin) and oligosaccharides such as sucrose and raffinose. Fungal inulinases extracts came often as stable mixture of highly active fructanhydrolases.

The best known inulinases are those produced by species of *Penicillium* (Shuichi and Norio, 1992), *Aspergillu*s and *Kluyveromyces* (Pandey *et al*., 1999). Inulinases have been shown to be produced by *Penicillium janczewskii* isolated from the rhizosphere of *Vernonia. Herbacea* (Pessoni *et al*., 1999), *Aspergillus niveus* Blochwitz 4128 URM isolated from sunflower rhizosphere(Souza-Motta *et al*., 2005).

Our group reported the isolation and characterization of several xerophilic fungal strains with the ability to grow in rich-tannin plants (Cruz-Hernandez *et al*., 2005). When these fungi were molecular and biochemically characterized, *P. citrinum*

Corresponding Author: Cristobal N. Aguilar, Department of Food Science and Technology, School of Chemistry, Universidad Autonoma de Coahuila. Blvd. Venustiano Carranza. Col. República, CP 25280, Saltillo, Coahuila, Mexico

ESS revealed its capacity to grow in a minimal medium with inulin as sole carbon source (Lafuente-Castaneda *et al*., 2004), being selected as a fungus with potential for industrial use (Lafuente-Castaneda *et al*., 2005). Lafuente-Casteneda *et al*. (2006) demonstrated that *P. citrinum* ESS was able to produce high levels of inulinase.

In the present study, the activity of extracellular inulinase from *P. Citrinum* ESS was studied in both, Submerged Culture (SmC) and Solid-State Culture (SSC) using either sucrose or inulin as carbon source inducer and inulinase inducer.

MATERIALS AND METHODS

Microorganism and inoculums preparation: *Penicillium citrinum* ESS was isolated from plants of the semiarid region of Mexico (Cruz-Hernandez *et al*., 2005). Strain spores were propagated, harvested in a cryoprotector system and stored at -20°C. The strain was activated in Potato Dextrose Agar (PDA) and incubated at 30°C for 72 h; spores were suspended Tween 80 (0.01%) and counted in a Neubauer chamber.

Culture conditions: For SmC, the culture medium was prepared with the following composition (g/L): $NaNO_3$ (7.65), KH_2PO_4 (3.04), $MgSO_4$ (1.52) and KCl (1.52); and the carbon source was inulin or sucrose (5.0), pH value was adjusted to 5.5. Erlenmeyer flasks (500 mL) containing 100 mL of medium were inoculated with 1.0×10^7 spores mL^{-1} and incubated (30°C, 96 h) in a rotatory shaker (250 rpm). Samples (three individual flasks) were taken at 12 h intervals, and the mycelium was separated by filtration (Whatman No. 41) and the filtrated was considered as the enzymatic extract.

For SSC, Erlenmeyer flasks (250 mL) containing 10 g of polyurethane foam (0.5 mm) impregnated at 70% (v/w) with the culture medium described above and inoculated with 2.0×10^7 spores/g were statically incubated at 30°C. Samples (three individual flasks) were taken at 12 h intervals, and the fermented mass was mechanical squeezed; the slurry resulted was considered as the inulinase extract.

The enzymatic extracts (SSC and SmC) were analysed for pH, total sugars content, biomass, protein and extracellular inulinase activity.

Biomass determination: For SmC, mycelial mass was washed with distilled water, thenbiomass was recovered by filtration(Whatman No. 41). Thereafter, it was dried at 60°C up until constant weight was reached. For SSC, 0.5 g of the fermented mass were mixed with phosphoric acid (5.0 mL, 0.15 M) and boiled for 5 min; the mixture was then centrifuged and filtered. Liquid phase was used for protein determination.

Total sugars content: The total sugars content was determined according to the method proposed by Miller (1959), after total acid hydrolysis of the samples using fructose or glucose as standard.

Inulinase assay: For the assay, 5 µL of diluted supernatant were mixed with 245 µL of inulin (0.1%) solution (0.1 M sodium acetate buffer, pH 4.5). Samples were incubated at 37°C for 12 h and then, the reaction was stopped by boiling the mixture (10 min). The reducing sugars released during the reaction were assayed following the Somogyi-Nelson methodology (Somogyi, 1952; Nelson, 1944). One unit of enzymatic activity was defined as the amount of enzyme required to liberate 1 µ mol of reducing sugars per minute under the assay conditions.

Protein determination: Total protein was determined according to the method suggested by Bradford (1976), using bovine serum albumin as standard protein. All the assays were performed in duplicate for each analysed flask.

Determination of kinetic parameters: The kinetic parameters used in this study were determined as described below.

Growth kinetics: biomass production, determined as × (g/L) was modelled by the Gompertz growth model Eq. 1:

$$X = \frac{X\max}{\frac{X\max - Xo}{Xo}} e^{-e^{-\mu(t-Z)}} \quad (1)$$

Where:

µ = The specific growth rate (1/h)
Z = The retardation constant (h)
Xmax = The equilibrium value for X
Xo = The value of X when t = 0

Kinetic parameters were obtained for the indicated initial values of sucrose and inulin. Specific growth rate, µ (1/h), was calculated from Eq. 2. Biomass yield, Y x/s (gX/gS), was calculated using the following Eq. 2:

$$Y_{X/S} = \frac{X_{max} - X_0}{S_0 - S_f} \quad (2)$$

Where:

S_0 = The value of S when t = 0
S_f = The value of S when P = P_{max}

Enzyme yield, Yp (U/g), was calculated using the following Eq. 3:

$$Y_P = \frac{P_{max} - P_0}{X_0 - X_{max}} \quad (3)$$

Enzyme formation quotient, q_p (U/gXh), was obtained from the next Eq. 4:

$$q_P = Y_P \mu \quad (4)$$

Substrate uptake quotient, qs (gS/gXh) was obtained from the next Eq. 5:

$$q_S = \frac{\mu}{Y_{X/S}} \quad (5)$$

Finally, Productivity, P (U/Lh), was calculated when $P = P_{max}$,using the Eq. 6:

$$P = \frac{P_{max}}{t_f} \quad (6)$$

Where:

t_f = The value of time when $P = P_{max}$
U = The enzyme unit
Gx = The grams of biomass
gS = The grams of substrate

Experimental design and analysis data: Inulinase production by *P. citrinum* ESS was statistically evaluated using a completely randomized designwith factorial fix with three independent variables: carbon source, culture system and time of culture. Mean values were also compared by Duncan test.

RESULTS AND DISCUSSION

In the present study, *P. citrinum* ESS growth and inulinase production were kinetically evaluated when cultured in SSC and SmC with either inulin or sucrose (5 gL^{-1}) as a carbon source.

The growth curves of *P. citrinum*ESS, in SSC and SmC with different carbon sources, are shown Fig. 1. In SSC, a maximum biomass production (0.055 gL^{-1}) was obtained at 72 h culture when inulin was used, while with sucrose, the maximum biomass value (0.045 gL^{-1}) was reached at 48 h. In SmC, maximal biomass values were obtained at 96 h with inulin (12.5 gL^{-1}) and at 36 h with sucrose (1 gL^{-1}).

The fungal inulinase production profile in SSC and SmC, both at 30°C and initial pH 5 are shown Fig. 2. Depending of the culture system, the effect of substrate on inulinase production is different. The highest inulinase production (6650.0 U/L) was obtained in SSC when inulin was used as carbon source. A low enzyme production level was obtained in this kind of culture when sucrose was used. In contrast, in SmC, the inulinase production levels were 1538.0 U/L with inulin (96 h) and 2241.0 U/L (24 h) with sucrose. It has been reported that sucrose exhibit a repression effect on inulinase production in SmC, however in the present study it is showed that inulinase production by *P. citrinum* ESS in SmC is higher when sucrose rather than inulin, is used as a carbon source. This phenomenon of inulinase induction by sucrose has been also evidenced by (Gouda, 2002) for *Aspergillus fumigatus*, Tohamy (2006) for *Streptomyces grisenus* and Pessoni *et al*. (2007) for *Penicillium janczewskii*. However, it is well known that inulin is better inducer for inulinase production than levan, sucrose and raffinose. All these molecules increase production of InuE gene while other metabolizable hexoses are molecules of strong catabolic repression (Burne *et al*., 1999; Yuan *et al*., 2006). The reason for obtaining high levels of inulinase production with sucrose in SmC can be explained considering that this substrate causes a similar strong regulation than inulin (Yuan *et al*., 2006) inducing a rapid up take or conversion of the available sucrose. However further studies are needed to compare the induction ratios and patterns of inulinase by *P. citrinum* ESS in both culture systems employing different initial concentrations of carbon sources and evaluating at least the products of *suc* A, *inu* A and*inuE* genes.

Table 1 shows the values of the kinetic parameters. Growth kinetic parameters were higher with sucrose in SSC than those in SmC, while enzyme kinetic parameters were higher with inulin in SSC than those obtained in SmC. Values reported for P were found at E_{max} (maximum level of enzyme activity). Under the culture conditions tested, it was possible to reach a production value of 1.6 times lower than that reported by Skowronek and Fiedurek (2006) whom evaluated the biosynthesis of inulinase by *A. niger* mycelium which was immobilized in polyurethane foam, in this case it was reported an inulinase production value of 0.15 U mL^{-1} h at 48 h of culture. However, our values were slightly higher than those reported by Ashokkumar *et al*. (2001) for *A. niger* inulinase produced in SSC and SmC.

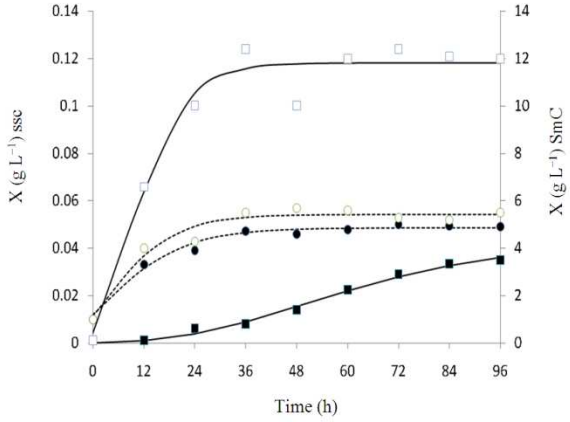

Fig. 1: Growth curves of *P. citrinum* ESS, in SSC (dotted lines) with inulin (black squares) and sucrose (white squares) and SmC (solid lines) with inulin (black circles) and sucrose (white circles). Lines represent calculated values and pointed markers are the experimental values

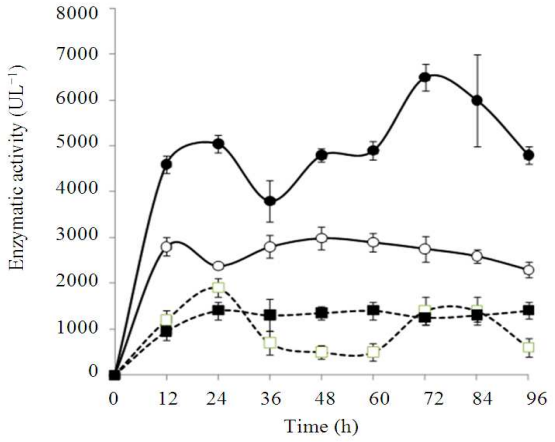

Fig. 2: Inulinase production by *P. citrinum* ESS in SSC (solid lines) and SmC (dotted lines) using sucrose (white circles) and inulin (black circles) as carbon sources

Table 1: Kinetic parameters associated to inulinase production in SSC and SmC

Kinetic parameters*	SSC Inulin	SSC Sucrose	SmC Inulin	SmC Sucrose
μ (1/h)	0.10	0.12	0.03	0.140
Yp (U/g)	1658237.28	66598.96	441.05	183.910
q_p(U/gh)	165823.73	7991.87	13.23	25.750
P (U/Lh)	92.367 (72 h)	34.38 (72 h)	16.02 (96 h)	93.38 (24 h)

Fructose formation from inulin comes from a single enzymatic step and represent yields up to 95% fructose (Vandamme and Deryke, 1983; Pandey *et al*., 1999). In the present study, *P. citrinum* ESS grew faster and higher biomass production was obtained with sucrose as carbon source in both culture systems, however production was greater in SmC than in SSC. The biomass and inulinase production patterns obtained were slightly different among the culture systems. In SSC, the maximal inulinase production was obtained at 72 h of culture when inulin was used; however, after this time the enzyme value decreased, probably due to proteolytic activity. In contrast, in SmC this reduction pattern did not occur.

The substrates commonly used for inulinase production, inulin and sucrose have been preferred as the carbon source. In general, if the microbial strain shows only inulinase activity, inulin is the best substrate, but if the microorganism exhibits inulinase activity coupled with invertase activity, sucrose is a better source for enzyme production (Lafuente-Castaneda *et al*., 2006). In a study carried out to evaluate the effect of different molecules as carbon source production using either individually or in combination with inulinase, inulin was found to be the most suitable substrate for enzyme production. Also, the results showed that inulinase production by fungal culture is inducible and that it is subject to catabolic repression. In the same study, maximum inulinase level (80 U/L) was reached at 60 h; thereafter, enzyme activity decreased to about 28 U/L in 120 h (Poorna and Kulkarni, 1995).

The results reported by Poorna and Kulkarni (1995) indicate that inulin induces higher inulinase activity than sucrose. In our study we were able to demonstrate that inulinase activity was higher in SSC than in SmC using *P. Citrinum* ESS. Although inulin is the typical substrate used for inulinase production, a variety of substrates (carbon and energy sources) have been tested (Pandey, 2004). Ertan *et al*. (2003b) reported an inulinase title of 1.25 U/L at 24 h in SmC for *Penicillium spinulosum*, the maximum activity value varies with culture time and culture conditions (Nelson, 1944). Higher levels of inulinase activity have been reported for yeasts rather than for filamentous fungi (Ertan *et al*., 2003a; Souza-Motta *et al*., 2005; Mazutti *et al*., 2006; Jing *et al*., 2003).

Inulinase production is also influenced by factors such as: presence of metal ions, pH, air flux, and temperature. Although more research is needed, the positive influence of KCl (0.01 M), $MgSO_4$ (0.01 M), and $FeSO_4$ (0.001 M) has been observed in *Penicillium* sp. 1. The highest extracellular inulinase production by *Debaryomyces castelli* and *Arthrobacter ureafacien*

soccurs at pH 7.5, while with *Penicillium* sp 1 maximal production occurs at pH 5.0. *Kluyveromyces fragili* sproduces comparable cell and enzyme yields in a range of pH from 3.5- 6.0. Other research papers have pointed a specific pH value were the maxima inulinase production is obtained: *K. marxianus*, pH 5.6 (Rouwenhorst *et al*., 1990); *Chrysosporium pannorum*, pH 4.5-5.5 (Xiao *et al*., 1988); *P. rugulosum*, pH 5.0-6.0 and *Fusarium oxisporum*, pH 5.5. (Gupta *et al*., 1990). In general, fungi have been seen to prefer a pH of 5.0-6.0 for inulinase production. Shaker conditions have also reported for inulinase production by *Penicillium* sp.

Although inulinase industrial production, as well as the most of the research on microbial inulinases deals with submerged fermentation (bath, fed-batch, continuous regimen), attempts have also been made to use solid state fermentation for this purpose. In our study, the biomass yield of *P. Citrinum* ESS was higher with sucrose in SmC than in SSC; however, the inulinase yield obtained either with inulin or sucrose in SSC was higher than that obtained in SmC. These results are in agreement with those reported by Aguilar *et al.* (2001) whom observed higher enzyme titles with low levels of biomass in SSC, compared with the low enzyme titles found with high biomass levels in SmC. This can be due to diffusion struggle of substrates in SSC.

Penicillium citrinum ESS isolated from a semiarid region in Mexico produces an extracellular inulinase extract by both SSC and SmC when using either inulin or sucrose as carbon sources. The highest enzyme production (6650 U/L) was obtained at 72 h of culture by SSC when inulinase was used as a carbon source. Nevertheless, optimisation of SSC parameters is required in order to increase the enzyme production.

CONCLUSION

The highest inulinase production was obtained using SSC either with inulin or sucrose, reaching values of 6650 and 2970 U/L respectively. In contrast, in SmC higher levels of inulinase activity (2241 U/L) were obtained with sucrose as carbon source (24 h), while with inulin the enzyme production was negatively affected and retarded.

ACKNOWLEDGMENT

This study was supported by The Food Research Department of Universidad Autonoma de Coahuila. Cynthia Lafuente-Castaneda thanks to UNIVERSIA-Santander program by the graduate student scholarship. Authors want to thank to PP4 at Universidad Autonoma Metropolitana for the technical support.

REFERENCES

Aguilar, C.N., C. Augur, E. Favela-Torres and G. Viniegra-Gonzalez, 2001. Production of tannase by *Aspergillus niger* Aa-20 in submerged and solid-state fermentation: Influence of glucose and tannic acid. J. Ind. Microbiol. Biotechnol., 26: 296-302. DOI: 10.1038/sj.jim.7000132

Ashokkumar, B., N. Kayalvizhi and P. Gunasekaran, 2001. Optimization of media for β-fructofuranosidase production by *Aspergillus niger* in submerged and solid state fermentation. Proc. Biochem., 37: 331-338. DOI: 10.1016/S0032-9592(01)00204-7

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254. DOI: 10.1016/0003-2697(76)90527-3

Burne, R.A., Z.T. Wen, Y.Y. Chen and J.E. Penders, 1999. Regulation of expression of the fructan hydrolase gene of Streptococcus mutans GS-5 by induction and carbon catabolite repression. J. Bacteriol., 181: 2863-2871. PMID: 10217779

Cruz-Hernandez, M., J.C. Contreras-Esquivel, F. Lara, R. Rodriguez-Herrera and C.N. Aguilar, 2005. Isolation and evaluation of tannin-degrading fungal strains from the Mexican desert. Z. Naturforsch. C., 60: 844-848. PMID: 16402543

Ertan, F., F. Ekinci and T. Aktac, 2003b. Production of inulinases from *Penicillium spinulosum*, *Aspergillus parasiticus* NRRL2999 and Trichoderma viride. Pak. J. Biol. Sci., 6: 1332-1335.

Ertan, F., T. Aktac, A.C. Kaboglu, F. Ekinci and E. Bakar, 2003a. Determination of optimum cultivation conditions on the production of inulinase fron *Rhizoctonia solani*. Pak. J. Biol. Sci., 6: 1386-1388.

Ettalibi, M. and J.C. Baratti, 1987. Purification, properties and comparison of invertase, exoinulinases and endoinulinases of *Aspergillus ficuum*. Applied Microbiol. Biotechnol., 26: 120-126. DOI: 10.1007/BF00282143

Gouda, M.K., 2002. Some properties of inulinase from *Aspergillus fumigatus*. Pak. J. Biol. Sci., 5: 589-593.

Gupta, A.K., A. Gill, N. Kaur and R. Singh, 1994. High thermal stability of inulinases from *Aspergillus* species. Biotechnol. Lett., 16: 733-734. DOI: 10.1007/BF00136480

Gupta, A.K., P. Rathore, N. Kaur and R. Sing, 1990. Production, thermal stability and immobilisation of inulinase from *Fmarium oxysporum*. J. Chem. Technol. Biotechnol., 47: 245-257. DOI: 10.1002/jctb.280470306

Jing, W., J. Zhengyu, J. Bo and A. Augustine, 2003. Production and separation of exo- and endoinulinase from *Aspergillus ficuum*. Process Biochem., 39: 5-11. DOI: 10.1016/j.foodres.2004.04.011

Lafuente-Castaneda C., C.N. Aguilar, R. Rodriguez-Herrera and J.C. Contreras-Esquivel, 2005. Evaluacion del uso potencial de cepas fúngicas extremofilas aisladas del semidesierto mexicano. Actividades de Ciencia y Tecnologia UAdeC, 1: 57-66.

Lafuente-Castaneda C., R. Rodriguez-Herrera, F. Lara-Victoriano, J.C. Contreras-Esquivel and L. Prado-Barragan *et al*., 2006. Inulinase production by a xerophilic *Penicillium citrinum*. Proceedings of the 2nd International Congress on Bioprocesses in Food Industries (ICBF' 06), Rio Patras, Greece.

Lafuente-Castaneda, C., R. Rodriguez-Herrera, J.C. Contreras-Esquivel and C.N. Aguilar, 2004. Biochemical characterization and potential evaluation of fungal strains isolated from Mexican semidesert plants. Proc. Int. Con. Food Sci. Technol. Dev. Countries, 1: 23-38

Mazutti, M., J.P. Bender, H. Treichel and M.D. Luccio, 2006. Optimization of inulinase production by solid-state fermentation using sugarcane bagasse as substrate Enz. Microb. Technol., 39: 56-59. DOI: 10.1016/j.enzmictec.2005.09.008

Miller, G.L., 1959. Protein determination of large numbers of samples Anal. Chem., 31: 426-428. DOI: 10.1021/ac60149a611

Nelson, N., 1944. A photometric adaptation of the somogyi method for the determination of glucose J. Biol. Chem., 153: 375-380.

Pandey, A., 2004. Concise Encyclopedia of Bioresource Technology. 1st Edn., Routledge, ISBN: 1560229802, pp: 735.

Pandey, A., C.R. Soccol, P. Selvakumar, V. Soccol and N. Krieger *et al*., 1999. Recent developments in microbial inulinases Its production, properties, and industrial applications. Applied Biochem, Biotechnol., 81: 35-52. DOI: 10.1385/ABAB:81:1:35

Pessoni, R.A.B., M.R. Braga and R.D.C.L. Figueiredo-Ribeiro, 2007. Purification and properties of exo-inulinases from *Penicillium janczewskii* growing on distinct carbon sources. Mycologia, 99: 493-503. DOI: 10.3852/mycologia.99.4.493

Pessoni, R.A.B., R.C.L. Figueiredo-Ribeiro and M.R. Braga, 1999. Extracellular inulinases from *Penicillium janczewskii*, a fungus isolated from the rhizosphere of *Vernonia herbacea* (Asteraceae). J. Applied Microbiol., 87: 141-147. DOI: 10.1046/j.1365-2672.1999.00805.x

Poorna, V. and P.R. Kulkarni, 1995. A study of inulinase production in *Aspergillus niger* using fractional factorial design. Biores. Technol., 54: 315-320. DOI: 10.1016/0960-8524(95)00156-5

Rouwenhorst, R.J., M. Hensing, J. Verbakel, W.A. Scheffers and J.P.V. Duken, 1990. Structure and properties of the extracellular inulinase of *Kluyveromyces marxianus* CBS 6556. Applied Environ. Microbiol., 56: 3337-3345.

Shuichi, O. and S. Norio, 1992. Purification and subsite affinities of exo-inulinase from *Penicillium trzebinskii*. Biosci. Biotech. Biochem., 56: 1443-1447.

Skowronek, M. and J. Fiedurek, 2004. Optimisation of inulinase production by *Aspergillus niger* using simplex and classical method technol. Biotechnology, 42: 141-146.

Skowronek, M. and J. Fiedurek, 2006. Inulinase biosynthesis using immobilized mycelium of *Aspergillus niger*. Enz. Microb. Technol., 38: 162-167. DOI: 10.1016/j.enzmictec.2005.05.011

Somogyi, M., 1952. Notes on sugar determination J. Biol. Chem., 195: 19-23. PMID:14938350

Souza-Motta, C.M.D., M.A.D.Q. Cavalcanti, A.L.F. Porto, K.A. Moreira and J.L.D.L. Filho, 2005. *Aspergillus niveus* Blochwitz 4128URM: new source for inulinase production Braz. Arch. Biol. Technol., 48: 343-350. DOI: 10.1590/S1516-89132005000300003

Tohamy, E.Y., 2006. Purification and Characterization of Exoinulinase Enzyme from *Streotomyces grisenus*. Pak. J. Biol. Sci., 9: 911-916.

Vandamme, E.J. and D.G. Deryke, 1983. Microbial inulinases: Fermentation process, properties, and applications and applications. Adv. Applied Microbiol., 29: 139-176. DOI: 10.1016/S0065-2164(08)70356-3

Wei, W., S. Wang, X. Zhu and W. Wan, 1999. Isolation of a mutant of *Kluyveromyces* sp. Y-85 resistant to catabolite repression. J. Biosci. Bioeng., 87: 816-818. DOI: 10.1016/S1389-1723(99)80159-5

Xiao, R., M. Tanida and S. Takao, 1988. Inulinase from *Chrysosporium pannorum*. J. Ferment. Technol., 66: 553-558. DOI: 10.1016/0385-6380(88)90088-X

Yuan, X.L., C. Goosen, H. Kools, M.J. Maarel and C.A. Hondel *et al*., 2006. Database mining and transcriptional analysis of genes encoding inulin-modifying enzymes of *Aspergillus niger*. Microbiology, 152: 3061-3073. DOI: 10.1099/mic.0.29051-0