academicJournals

Vol. 10(16), pp. 565-571, 28 April, 2016
DOI: 10.5897/AJMR2016.7924
Article Number: F9383E158218
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Tannase production by *Aspergillus* spp. UCP1284 using cashew bagasse under solid state fermentation

Tatiana P. S. L. Liu[1], Tatiana S. Porto[2], Keila A. Moreira[2], Galba M. C. Takaki[3], Romero Brandão-Costa[4]*, Polyanna N. Herculano[1] and Ana L. F. Porto[1]

[1]Animal Morphology and Physiology Department, Federal Rural University of Pernambuco, Dois Irmãos, CEP: 52171-900, Recife-PE, Brazil.
[2]Academic Unit of Garanhuns, Federal Rural University of Pernambuco, CEP: 55292-901, Garanhuns-PE, Brazil.
[3]Catholic University of Pernambuco - NPCIAMB-UNICAP CEP: 50050-900, Recife, PE, Brazil.
[4]Department of Biochemistry, Center of Biological Sciences, Federal University of Pernambuco, (UFPE), Av. Prof. Moraes Rego s/n, CEP 50.670-420 Recife, PE, Brazil.

Received 13 January, 2016, Accepted 17 March, 2016

Production of tannase by *Aspergillus* species UCP1284 was studied using solid state fermentation and cashew bagasse as substrate. Amount of 35 strains of fungi species isolated from soil of Caatinga were used for qualitative selection of strains with potential for production of tannase. Through the selected fungi, a complex study was achieved about the influence of the variables: substrate amount, initial moisture content and tannic acid concentration on the production of tannase by solid state fermentation, using a factorial design (2^3). The maximum activity was 12.26 U/g of dry substrate obtained at the time of 48 hours using 10.0 g of substrate with initial moisture content of 40% and using 2.0% of tannic acid. This first design for enzyme production demonstrated the influence of the studied parameters, mainly, substrate amount on the enzyme yield, and its relevance in the process for the subsequent optimization protocol.

Key words: Tannase, *Aspergillus* species, Caatinga, cashew bagasse, solid fermentation.

INTRODUCTION

Tannin acyl hydrolase (CE 3.1.1.20) commonly known as tannase, catalyses ester bonds hydrolysis of hydrolysable tannins. The reaction produces glucose and gallic ácid as final products (Noha et al., 2014). Tannins are secondary metabolites of plants. Beside vegetable source, tannase can be obtained from animal source. However, the most important source is microbiological, since the enzymes obtained that way are more stable than others produced by alternative sources (Selwal and Selwal, 2011; Singh et al., 2011). Among microorganisms used to produce

*Corresponding author. E-mail: romero_brandao@outlook.pt or romero.bcosta@ufpe.br. Tel: +55.81.3320.6345.

tannase, others researchers highlight the importance of filamentous fungi, yeasts and bacteria (Renovato et al., 2011). According to Aguilar et al. (2007), most of tannase producer microorganisms are from fungus of genus *Penicillium* and *Aspergillus.*

Tannase applications are diversified among food, pharmaceuticals and rations industries. Other uses of tannase might include leather tanning, instant tea manufacturing and clarifying agent for juices, beers, some wines and sodas that have coffee as component (Selwal et al., 2011).

There's ongoing search for new sources to produce tannase, with higher catalytic stability and lower production cost (Batra and Saxena, 2005). Solid State Fermentation (SSF) is one of the alternatives to lower cost production, which could be defined as a bioprocess held on absence or near absence of substrate free water (Viniegra-González et al., 2003). However, the substrate must contain enough moisture for microbian growth (Thomas et al., 2013). This process has as one of advantages, the high concentrated enzyme production using agro industrial residues (Manjit et al., 2008).

The cashew (*Anacardium occidentale* L.) belongs to Anacardiadeae family. This fruit is native from South America and is cultivated on America (North, South and Central), Africa and Asia. In Brazil, cashew farming is highly concentrated on Northeastern region mainly on Ceará, Piauí and Rio Grande do Norte states. These locations are responsible for 97% of internal production (Carneiro et al., 2012).

In Northeastern Brazil, cashew's agro industries occupy an important place on economic and social context. Cashew's nut is the main product for exportation. However, the pseudo fruit is commercialized as sweet creams, beverages and dehydrated forms. Also, they are consumed *in natura*. Paiva et al. (2000) estimate that juice production is the most representative usage of the fruit.

Accordingly, FAO (2012) has estimated that the world production of cashew was 2.001.301 t. Brazil was responsible for 90.19% of the total, which demonstrates the importance of the country against the world market of these agro products. Fonteles et al. (2016) report that the cashew bagasse is the residue generated peduncle processing. Despite being rich in nutrients, bagasse is treated as waste. However, this disposal could be used as raw material in various industrial processes such as the production of ethanol as a substrate for solid fermentation in order to produce the enzymes.

It is estimated that 20 to 25% of cashew's pseudo fruit turns to bagasse or residual fiber, being used on animal feeding or simply being discarded, which causes damages to environment (Abreu et al., 2013). Wrong disposal of agro industrial residues might represent one of the main causes of environmental pollution and also an important loss of biomass, which could be used to produce different high-valued compounds such as enzymes.

Ernest et al. (2003) reports that after antibiotics, enzymes are the main product explores by biotechnological industries, once they are used on a large scale. Filamentous fungi have an important role on enzyme production, because of their fast growth on many substrates, easy handling and the ability to produce a lot of biotechnological applicable metabolites.

The aim of this study was to evaluate the production of tannase by *Aspergillus* species UCP1284 in solid-state fermentation (SSF) using cashew bagasse (*A. occidentale* L.) as a substrate and to evaluate the influence of variables on the enzyme production.

MATERIALS AND METHODS

Microorganisms

The 35 strains of filamentous fungi among the genera *Aspergillus*, *Penicillium* and some species of the group of Zygomycota were part of the Project Network North East of filamentous fungi in soils of Caatinga and Amazonia (RENNORFUN) collection and were kindly provided by Profª. Drª. Galba Maria de Campos Takaki. The culture medium used for the maintenance of the fungi was Malt Extract Agar and for the sporulation of the culture Potato Dextrose Agar (PDA) or Czapek were used.

Chemical reagents

The reagents tannic acid and methanolic rhodanina were purchased from Sigma (St. Louis, Mo, USA). Sodium phosphate and sodium hydroxide were purchased from Merck (Darmstad, Germany). All the other reagents were in analytical grade.

Screening of tannase producing fungi

The species of fungi were subjected to a screening in solid medium, in order to select strains with higher potential for tannic acid degradation. For selection, fragments of the culture with seven days of growth in malt extract Agar (Klich, 2002) were transferred to the center of the culture medium proposed by Murugan et al. (2007) containing agar Czapek Dox minimal medium using as substrate 0.5% tannic acid solution (in sterile Millipore filter, 0.22 µm) as the only one carbon source. The plates were incubated on Biochemical Oxygen Demand (BOD TECNAL TE-401) at 30°C for 72 h. The potential for tannic acid degradation were evidenced by the formation of halo around the colony. The potential was determined according to the methodology described by Teather and Wood (1982) and adapted to the enzyme studied. The Tannase Index (TI) was determined and expressed by the ratio of the diameter of the halo of degradation (mm) and the diameter growth of the colony (mm).The fungi that exhibited potential for tannase index equal to or greater than 1.8 mm and showed no potential for mycotoxin production were selected for solid state fermentation.

Table 1. Variables levels used in factorial design (2^3) for tannase production by Solid state fermentation (SSF).

Variable	Levels		
	Lower (-1)	Central (0)	High (+1)
Sa (g)[a]	5.0	7.5	10.0
Im (%)[b]	40	50	60
Ta (%)[c]	0	1	2

[a]Sa: Substrate amount (g); [b]Im: initial moisture (%), [c] Ta: Tannic acid.

Detection of mycotoxins

The fluorescence technique described by Lin and Dianese (1976) using the coconut agar (MAC) was used. Fungi were centrally peaked in Petri dishes containing the MAC medium with pH adjusted to 6.9 and incubated in the dark at 30°C for six days. The potential for mycotoxin production was verified by observing the presence of a halo of bluish violet fluorescent color on the reverse of the colony, when exposed to long (365 nm) wave ultraviolet light in the darkroom.

Substrate fermentation

The agro industrial substrate cashew used in the fermentation was kindly provided by Natural Pulp, which was washed and kept for an hour in a solution of sodium hypochlorite 2%, then washed and dried at 65°C to constant weight and then stored in sealed plastic containers.

Solid state fermentation (SSF)

For tannase production, the bagasse cashew was used as a substrate with a particle size between 3.0 and 8.0 mm to provide improved absorption and porosity to facilitate transport of oxygen as well as nutrients during SSF (Spier et al., 2008). The fermentation was performed in 250 ml Erlenmeyer flasks sterilized, containing 5 g of cashew substrate, initially sterilized at 60°C for 120 min and then in the ultraviolet (UV) light for 120 min. The inoculum was prepared by suspending the spores present on the malt extract agar plates in sodium phosphate buffer (10 mM, pH 5.5). The number of spores was determined in a Neubauer counting chamber and the inoculum of 10^7 spores per gram and nutrient solution containing 0.5% yeast extract and 1% dextrose in sodium phosphate buffer (10 mM, pH 5.5) containing 0.5% of tannic acid, was inoculated in the substrate used for SSF. The initial moisture of 40% the substrate was determined in accordance with the standards of the Institute Adolfo Lutz (2005). The flasks were incubated at 30°C in Biochemical Oxygen Demand (BOD) (TECNAL TE401). The fermentation was carried out for 48 h.

Experimental design to tannase production

In order to evaluate the amount of substrate influence over, initial moisture content and the concentration of tannic acid was performed full factorial design (2^3) (Table 1). All statistical analyses were carried out using *Statistica* 8.0 software (2008). The SSF utilized for planning is described in earlier.

Extraction of enzyme

The time of fermentation for the experiment was 96 h. The contents of the flasks were harvested at regular intervals (24 h). A mass of 3.0 g of the fermented mixture was mixed with 18 ml of sodium phosphate buffer (10 mM, pH 5.5). After maceration, extraction was performed with filter paper (Whatman no. 1) under vacuum. The extract was clarified by filtration and centrifugation at 2000 rpm for 10 min. The supernatant was used as an enzymatic extract for subsequent analytical determinations.

Tannase activity

The tannase activity was determined according to the methodology proposed by Sharma et al. (2000) and modified by Ordonez et al. (2011). The activity was performed using 100 µl of the enzyme extract and 100 µl of tannic acid solution (0.3 mM) in sodium phosphate buffer (10 mM, pH 5.5), incubated for 30 min at 30°C. Then were added 300 µl of solution of methanolic rhodanina (0.667% w/v) and 100 µl of sodium hydroxide (500 mM) for sample dilution was added 900 µl of distilled water, after incubation for 10 min at 30°C and the color was measured at 520 nm. One unit of enzyme activity was defined as the amount of enzyme required to catalyze the production of 1 µmol of gallic acid per minute on these conditions. The enzyme activity was expressed in units per gram of substrate in dry basis (gds).

RESULTS AND DISCUSSION

From the 35 fungal cultures isolated from Caatinga's soil, 64.57% were acknowledged as potential producers of tannase, while 31.42% did not grow (Table 2). The fungi growth absence in all Zygomycota species, in medium containing tannic acid, this could be due to sensibility or incapacity to use such substance as nutritive font. On similar screening, Pinto et al. (2001) worked with 30 species of *Aspergillus.* The authors report that the direct measurement of colony diameter is a good capacity index of degradation of tannic acid as sole source of carbon. After 72 h of growth, the best results attained by degradation index were from *Aspergillus* spp. UCP1284, UCP1290 and *Penicillium* species UCP1285. Darah et al. (2011) report that *Aspergillus*, *Penicillium*, *Paecilomyces* and *Rhizopus* species are widely studied for tannase production, obtained a good enzyme activity. In our study, the presence of halo was observed using 0.5% (w/v) of tannic acid. This value is similar to others published by literature (Purohit et al., 2006; Murugan, 2007; Ordoñez et al., 2011).

In 42.85% of cultures grown in MAC medium screening to detect mycotoxin showed production potential, while 57.14% were producers. Yazdani et al. (2010) state that HPLC and TLC tests are the most precise for mycotoxin detection. Although, other tests, as used in the present study, can be used as a preliminary detection indicator of mycotoxin. *Aspergillius* spp. UCP1284 produced the best solid state fermentation result (6.77 U/mL), for fungal

Table 2. Result of screening for tannase potential on solid medium and potential producers of mycotoxins.

Access number[1]	Culture	Øh	Øc	Ti	Access number[1]	Culture	Øh	Øc	Ti
UCP1266	*Lichtheimia hyalospora*	Nc	-	-	UCP1284	*Aspergillus* spp.	9	5	1.8
UCP1267	*Lichtheimia hyalospora*	Nc	-	-	UCP1285	*Penicillium* spp.	10	4	2.5
UCP1268*	*Aspergillus* spp.	15	10	1.50	UCP1286*	*Penicillium* spp.	14	11	1.27
UCP1269*	*Aspergillus* spp.	13	9	1.44	UCP1287*	*Aspergillus* spp.	12	7	1.71
UCP1270*	*Aspergillus* spp.	23	10	2.30	UCP1288*	*Penicillium* spp.	13	9	1.44
UCP1271	*Aspergillus* spp.	12	10	1.25	UCP1289	*Penicillium* spp.	11	8	1.37
UCP1272*	*Aspergillus* spp.	11	7	1.57	UCP1290	*Aspergillus* spp.	19	10	2.00
UCP1273*	*Aspergillus* spp.	10	4	2.50	UCP 1291	*Penicillium* spp.	11	9	1.22
UCP1274*	*Aspergillus* spp.	22	17	1.30	UCP 1292	*Penicillium* spp.	10	8	1.25
UCP1275	*Aspergillus* spp.	Nc	-	-	UCP 1293	*Fennellomyces heterothallicus*	Nc	-	-
UCP1276	*Aspergillus* spp.	17	12	1.41	UCP 1294*	*Apophysomyces elegans*	Nc	-	-
UCP1277*	*Aspergillus* spp.	12	7	1.71	UCP 1295	*Rhizopus arthizus var. arthizus*	Nc	-	-
UCP1278*	*Aspergillus* spp.	11	6	1.83	UCP 1296	*Rhizopus microsporus var. chinensis*	Nc	-	-
UCP1279	*Aspergillus* spp.	23	18	1.27	UCP 1297	*Mucor prayagensis*	Nc	-	-
UCP1280	*Aspergillus* spp.	21	15	1.40	UCP 1298	*Lichtheimia corymbifera*	Nc	-	-
UCP1281	*Aspergillus* spp.	21	12	1.75	UCP 1299*	*Cunninghamella echinulata var. achinulata*	Nc	-	-
UCP1282*	*Aspergillus* spp.	12	8	1.50	UCP 1300	*Rhizopus stolonifer*	Nc	-	-
UCP1283*	*Aspergillus* spp.	15	12	1.25	-	-	-	-	

[1]Access number in the Cultures Collection of Environmental Science Nucleus, Catholic University of Pernambuco; [2](Ti): Tannase index = ratio between the (Øh) ring diameter (mm)/(Øc) of the colony diameter (mm). (Nc) = did not grow (*) potential mycotoxin producer.

selection. In fermentation using experimental design, 12.26 (U/gds) maximum of tannase was achieved after 48 h of fermentation, using 10 g of cashew substrate, 40% of initial moisture and 2% of tannic acid (Table 3).

In a study using apple cashew bagasse as substrate for solid state fermentation, Rodrigues et al. (2008) evaluated the effects of inoculum concentration, temperature and supplementation of the medium using other carbon sources, in the tannase production. The previous authors worked with *Aspergillus oryzae* and obtained a maximum activity of 4.63 (U/gds) after 48 h of fermentation using 10^7 (spores/g) of inoculum, 30°C of temperature, 40% of initial moisture, 1% (p/v) of saccharose supplementation, 2.5% tannic acid addition and 40 g of substrate.

Some authors reported different tannase production time. Chhokar et al. (2010) reported that maximum production of tannase was achieved after 48 h of fermentation using *Aspergillus heteromorphus* MTCC 8818. However, Selwal et al. (2011) observed maximum production only after 98 h using *Penicillium atramentosum.* Some works from literature related that the tannase production time ranges between 24 and 120 h and depend of microorganism and substrate used, which corroborate with our results.

According to Banerjee and Pati (2007), the tannase is produced during the first phase of the growth process, wherein the tannic acid does not penetrate cell membranes due to its high molecular weight; however, the microorganisms producing of tannase are able to hydrolyze tannic acid in the gallic acid and glucose. The available

Table 3. Experimental design results 2^3 for tannase production at 96 h of SSF by *Aspergillus* spp. SIS19.

Run	Sa (g)[a]	Im (%)[b]	Ta (%)[d]	Tannase (U/gds)			
				24 h	48 h	72 h	96 h
1	5.0	40	0.0	0.63	1.12	7.18	4.24
2	10.0	40	0.0	0.53	5.94	7.07	5.71
3	5.0	60	0.0	0.48	5.59	5.68	4.10
4	10.0	60	0.0	0.40	6.32	6.16	4.39
5	5.0	40	2.0	1.84	6.40	8.68	5.65
6	10.0	40	2.0	1.02	12.26	8.09	4.39
7	5.0	60	2.0	2.22	10.00	9.48	4.76
8	10.0	60	2.0	1.23	10.86	7.15	3.64
9[(c)]	7.5	50	1.0	2.38	6.58	7.35	5.78
10[(c)]	7.5	50	1.0	1.82	7.06	9.30	4.52
11[(c)]	7.5	50	1.0	1.14	5.79	6.50	5.99
12[(c)]	7.5	50	1.0	1.20	7.42	6.38	5.31

For the tannase cultivation time is given in hours. [a]As: Substrate amount (g), [b]Im: initial mousture (%), [d]Ta: tannic acid and (C): central points.

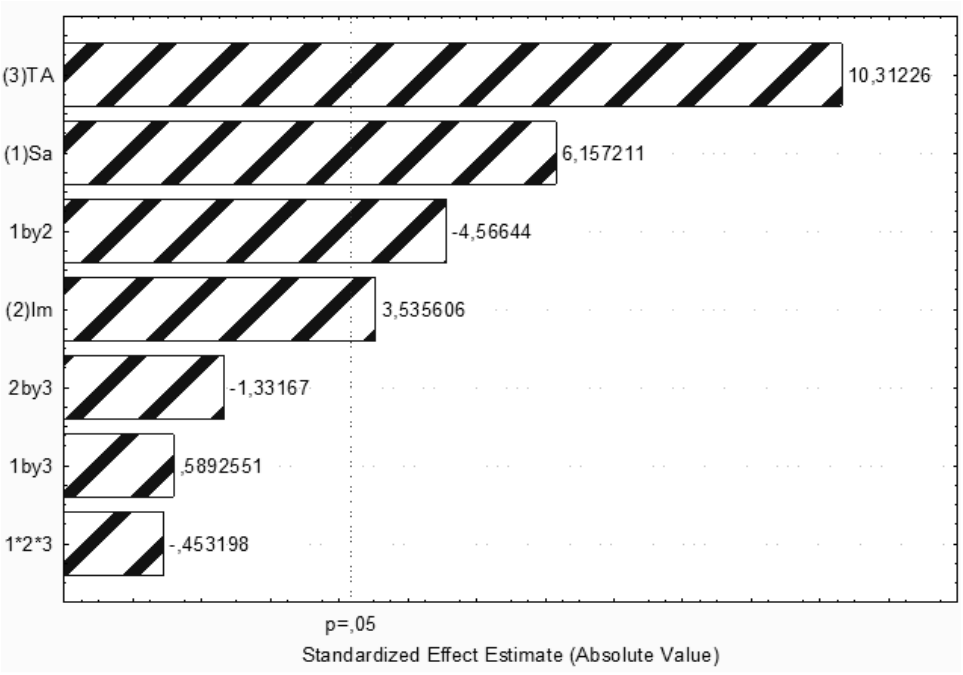

Figure 1. Pareto chart of main effects, with the response variable tannase activity (U/gds) at 48 h of SSF, using factorial design (2^3). The meanings of the symbols sorted in the figure are: (1) the quantity of substrate (g), (2) the initial moisture (%), (3) Tannic acid (%).

glucose is assimilated when glucose concentration falls and the gallic acid is also consumed in parallel. These concentrations might vary according to the species of microorganism and the type of substrate used.

The variable that most influenced positively tannase production was tannic acid concentration. Thus, higher tannase activities were obtained when higher concentrations of tannic acid were used. The initial moisture and substrate amount were also influenced significantly, both in isolated and combined variables as shown in Figure 1.

Several studies report inductive effects of different

tannic acid concentrations for tannase maximum production. Chhokar et al. (2010) reports a yield of 19.29% when purified derived from 1% concentration of tannic acid while using *A. heteromorphus* MTCC 8818. Raaman et al. (2010) used a tannic acid concentration of 1.5% (89 U/mL) along with *Paecilomyces variotii.* Abdel-Nabey et al. (2011) report production using 2% (p/v) of tannic acid as inductor. These same authors worked with *A. oryzae* and *Aspergillus japonicus* and achieved 23.7 and 14.6 U/mL, respectively. Higher values than 2.5% were reported by Madeira Jr. et al. (2011), whom obtained maximum production (2.292 U/g) using 6% of tannic acid. Even though tannase production might occur on tannic acid absence, some species of *Aspergillus* can tolerate higher concentrations than 20% without showing any deleterious effect neither over growth nor for enzyme production (Cruz-Hernández et al., 2006).

Prommajak et al. (2014) report using cashew as sole substrate, with different types of microorganisms and processing conditions, it can be obtain a wide biomolecular spectrum with pectinase and tannase enzymes amongst them.

The results obtained with this study show promising potential for *Aspergillus* spp. UCP1284, isolated from Caatinga's soil, to produce tannase using cashew bagasse as substrate. This current work also shows ideal circumstances to further studies for tannase production optimization.

Conflict of Interests

The authors have not declared any conflict of interest.

ACKNOWLEDGEMENTS

The authors would like to thank the Project RENNORFUN/CNPq process nº 563382/2010-4 and SISBIOTA/FACEPE process nº APQ-0086-2.12/11 for financial support on present work. The authors also appreciate FACEPE-CNPq for the scholarship granted; the Laboratory of Bioactive Technology (LABTECBIO/UFRPE) who provided the necessary structure for the research and the Natural Pulp for providing the substrate.

REFERENCES

Abreu FP, Dornier M, Dionisio AP, Carail M, Caris-Veyrat C, Dhuinque-Mayer C (2013). Cashew apple (*Anacardium occidentale* L.) extract from by-product of juice processing: A focus on carotenoids. Food Chem. 138:25-31.

Aguilar CN, Rodrigues R, Gutiérrez-Sánchez G, Augur C, Favela-Torres E, Prado-Barragan LA, Ramirez-Coronel A, Contreras-Esquivel JC (2007). Microbial tannases: Advances and perspective. Appl. Microbiol. Biotechnol. 76:47-59.

Banerjee D, Pati BR (2007). Optimization of tannase production by *Aureobasidium pullulans* DBS 66. J. Microbiol. Biotechnol. 17:1049-1053.

Batra A, Saxena RK (2005). Potential tannase producers from the genera *Aspergillus* and *Penicillium*. Proc. Biochem. 40:1553-1557.

Carneiro PT, Fernandes PD, Gheyi HR, Soares FAL, Santos MAL, Feitosa LATF (2012). Evapotranspiração do cajueiro anão precoce sob estresse salino em diferentes fases fenológicas. Irriga, Botucatu, Ed Especial, pp. 351-367.

Chhokar V, Sangwan M, Beniwal V, Nehra K, Nehra KS (2010). Effect of Additives on the Activity of Tannase from *Aspergillus awamori* MTCC9299. Appl. Biochem. Biotechnol. 160:2256-2264.

Darah I, Sumathi K, Hong LS (2011). Involvement of physicalparameters in medium improvement for tannase production by *Aspergillus niger* fetl ft3 in submerged fermentation. Biotechnol Res. Int. doi:10.4061/2011/897931.

Ernest M, Mendgen WK, Wirsel W (2003. Endophytic Fungal Mutualists: Seed- Borne *Stagonospora* spp. Enhance reed biomass production in axenic microosms. Mol Plant Microbe Interact 16:580-587.

Fonteles TV, Leite AKF, Silva ARA, Carneiro APG, Miguel E, de C, Cavada BS, Rodrigues S (2016). Ultrasound processing to enhance drying of cashew apple bagasse puree: Influence on antioxidant properties and *in vitro* bioaccessibility of bioactive compounds. Ultrason. Sonochem. 31:237-249.

Lin MT, Dianese JC (1976). A coconut-agar medium for rapid detection of aflatoxin production by *Aspergillus* spp. Phytopathology 66:1466-1469.

Manjit YA, Aggarwal NK, Kumar K, Kumar A (2008). Tannase production by *Aspergillus fumigatus* MA under solid-state fermentation. World J. Microbiol. Biotechnol. 24:3023-3030.

Murugan K, Saravanababu S, Arunachalam M (2007). Short Communication Screening of tannin acyl hydrolase (E.C.3.1.1.20) producing tannery efluent fungal isolates using simple agar plate and SmF process. Biores. Technol. 98:946-949.

Noha DO, Choib HS, Suhb HJ (2014). Catechine biotransformation by tannase with sequential addition of substrate. Process Biochem. 49:271-276.

Ordoñez RM, Colombo I, Alberto MR, Isla MI (2011). Production of tannase from wood-degrading fungus using as substrate plant residues: Purification and characterization. World J. Microbiol. Biotechnol. 27:2325-2333.

Paiva FFA, Garruti DS, Silva Neto RM (2000). Aproveitamento industrial do caju. Embrapa Agroindústria Tropical: Sebrae, Fortaleza- CE, P 88.

Pinto GAS, Leite SGF, Terzinha SC, Couri S, (2001). Selection of tannase producing *Aspergillus niger* strains. Braz. J. Microbiol. 32:24-26.

Purohit JS, Dutta JR, Nanda RK, Banerjee R (2006). Strain improvement for tannase production from coculture of *Aspergillus foetidus* and *Rhizopus oryzae*. Bioresour. Technol. 97:795-801

Raaman N, Mahendran B, Jaganathan C, Sukumar S, Chandrasekaran V (2010). Optimisation of extracellular tannase production from *Paecilomyces variotii.* World J. Microbiol. Biotechnol. 26:1033-1039.

Renovato J, Gutiérrez-Sánchez L, Rodríguez-Durán LV, Bergman C, Rodríguez R, Aguilar CN (2011). Differential Properties of *Aspergillus niger* Tannase Produced Under Solid-State and Submerged Fermentations. Appl. Biochem. Biotechnol. 165:382-395.

Rodrigues THS, Pinto GAS, Gonçalves LRB (2008). Effects of inoculum concentration, temperature, and carbon sources on tannase production during solid state fermentation of cashew apple bagasse. Biotechnol. Bioprocess. Eng. 13:571-576.

Selwal MK, Yadav A, Selwal KK, Aggarwal NK, Gupta R, Gautam, SK (2011). Tannase production by *Penicillium atramentosum* km under ssf and its applications in wine clarification and tea cream solubilization. Braz. J. Microbiol. 42:374-387.

Selwal MK, Selwal KK (2011). High-level tannase production by *Penicillium atramentosum* KM using agro residues under submerged

fermentation. Ann. Microbiol. 62:139-148.
Sharma S, Bhat TK, Dawra RK (2000). A spectrophotometric method for assay of tannase using rhodanine. Anal. Biochem. 279:85-89.
Singh B, Bhat TK, Sharma OP, Kanwar SS, Rahi PA, Gulati (2011). Isolation of tannase-producing *Enterobacter ludwigii* GRT-1 from the rumen of migratory goats. Small Rumin. Res. 102:172-176.
Spier MR, Greiner R, Rodriguez-León JA, Woiciechowski AL, Pandey A, Soccol VT (2008). Phytase production using citric pulp in SSF. Food Technol. Biotechnol. 46:178-182.
Statistica Software (2008). Inc. STATISTICA (data analysis software systems) version 8.0.
Teather RM and Wood PJ (1982). Use of Congo red polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. Appl. Environ. Microbiol. 43:777-780.
Thomas L, Larroche C, Pandeya A (2013). Review Current developments in solid-state fermentation. Biochem. Eng. J. 81:146-116.
The Food and Agriculture Organization Corporate Statistical Database (FAOSTAT). http://faostat.fao.org/
Viniegra-González G, Favela-Torres E, Aguilar CN, Rómero-Gomez SJ, Díaz-Godínez G, Augur C (2003). Advantages of fungal enzyme production in solid state over liquid fermentation systems. J. Biochem. Eng. 13:157-167.
Yazdani D, Zainal AMA, Tan YH, Kamaruzaman S (2010). Evaluation of the detection techniques of toxigenic *Aspergillus* isolates. Afr. J. Biotechnol. 9:7654-7659.