Pontificia Universidad JAVERIANA Bogotá

Univ. Sci. 23 (3): 419-436, 2018.
doi: 10.11144/Javeriana.SC23-3.cpop

ORIGINAL ARTICLE

Cellulases production on paper and sawdust using native *Trichoderma asperellum*

Y-Maritza Zapata[1], Angelica Galviz-Quezada[1], Víctor-Manuel Osorio-Echeverri[2,*]

Edited by
Juan Carlos Salcedo-Reyes
(salcedo.juan@javeriana.edu.co)

1. Semillero de investigación SIFACS, Facultad de Ciencias de la Salud, Institución Universitaria Colegio Mayor de Antioquia, Carrera 78 # 65-46, Medellín, Colombia.

2. Grupo Biociencias, Facultad de Ciencias de la Salud, Institución Universitaria Colegio Mayor de Antioquia, Carrera 78 # 65-46, Medellín, Colombia.

* victor.osorio@colmayor.edu.co

Received: 26-07-2017
Accepted: 27-09-2018
Published on line: 02-11-2018

Citation: Zapata YM, Galviz-Quezada A, Osorio-Echeverri VM. Cellulases production on paper and sawdust using native *Trichoderma asperellum*, *Universitas Scientiarum,* 23 (3): 419-436, 2018. doi: 10.11144/Javeriana.SC23-3.cpop

Funding:
Research Incubator Program of the Health Sciences Faculty (SIFACS).

Electronic supplementary material:
N.A.

Abstract

Microbial cellulases are industrially used enzymes that catalyze the cleavage of the glycosidic bonds of cellulose. This hydrolysis yields sugars that can be used in processes such as bioethanol production. These enzymes are mainly produced by fungi belonging to the genus *Trichoderma* via submerged or solid state fermentation with cellulosic materials as substrates. Recent publications have increasingly demonstrated that alternatives to *T. reesei* enzymes in the production of second-generation biofuels exist. Here, cellulolytic activities of crude extracts obtained from a native isolate of *T. asperellum* from coffe pulp and a strain of *T. reesei* were evaluated. Solid state fermentations were performed using paper and sawdust as substrates. The activities were measured after 12 days of incubation. The extracts obtained from *T. reesei* showed higher cellulase and endoglucanase activities (6.5 and 5.8 U/g) than those obtained using *T. asperellum* (5.6 and 4.1 U/g) with paper as substrate. There were no significant differences between isolates when grown on sawdust. It was possible to verify that native *T. asperellum* was able to produce cellulases on lignocellulosic material such as moistened paper and sawdust without having undergone a chemical pretreatment.

Keywords: cellulases; cellulolytic extracts; solid state fermentation; *Trichoderma*.

Introduction

Cellulose is an important structural component of the plant cell wall and therefore, one of the most abundant biological materials on Earth. It is a polysaccharide consisting of a linear chain of several hundreds to many thousands of β (1→4) linked D-glucose units [1]. Cellulose, hemicellulose, and lignin are the main components of the lignocellulosic biomass, such as seed husks, bagasse, woodchips, straw, dry leaves, and sawdust. This lignocellulosic biomass represents an economical, plentiful, renewable energy source because it is generally waste material [2, 3].

These waste materials can be used to produce biofuels, the use of which can help reduce carbon dioxide emission as well as dependence on fossil fuels. For this process, polymers in the lignocellulosic biomass must be broken down into fermentable sugars. The enzymatic hydrolysis of these compounds is an environment-friendly process catalyzed by both types of cellulases (endo-1,4-b-D-glucanase, EC 3.2.1.4; exo-b-1,4-glucan cellobiohydrolase, EC 3.2.1.91; and b-glucosidase, EC 3.2.1.21) and hemicellulases (exo-1,4-b-xylosidase, EC 3.2.1.37 and endo-1,4-b-xylanase, EC 3.2.1.8) [4, 5]. Also, this hydrolysis can be performed under neutral pH and low temperature and with low by-product formation, thus being highly efficient [6].

Nevertheless, high concentrations of enzymes are required to scale-up cellulose hydrolysis to industrial levels. Therefore, the study of biotechnology-based approaches is important for their use in production of cellulase-producing microorganisms, which are of interest also in the textile, paper, pharmaceutical, food, and detergent industries [6, 7].

This study focussed on *Trichoderma*, one of the most studied cellulase-producing genera of fungi [4]. *T. reesei* is the most studied species for cellulolytic enzyme production at an industrial level. It is a common soil fungus found in the rhizospheres of crop plants, decaying wood, and other decomposing materials. It is characterized by rapid growth, mostly bright green conidia, and a repetitively branched conidiophore structure [8]. Although it is believed that *T. reesei* is the only and indispensable choice for enzymatic cellulose saccharification and mutant strains with high cellulolytic activity, such as *T. reesei* Rut C30 have been developed, recent publications have increasingly demonstrated that fungi other than *T. reesei* are used for cellulolytic enzymes production and it is necessary to optimize the culture conditions for these fungi as well as the technology required for efficient cellulase production in bioreactors [9-12].

Several strains of *Trichoderma* have been used in submerged fermentations to study cellulase production using substrates, such as cellulose [5], pulp mill lime mud [13], flower stems [14, 15], and crop residues [16]. Likewise, researchers have also used corncob [6], mushroom compost [17], oat straw [18], wheat bran [18, 19], rice husk and bran [20], rice straw [19, 21], cauliflower and legumes residues [21], and sugarcane bagasse [19, 22] as substrates in solid state fermentations (SSF). Additionally, enzymatic hydrolysis of corn stover, rice straw, sawdust, and paper has been performed with cellulases produced by *Trichoderma* to obtain fermentable sugars [2, 5, 6, 23]. However, these substrates must undergo pretreatment for lignin removal, because lignin constitutes a barrier to cellulose breakdown by microorganisms [6, 24].

Waste paper can be used as a substrate in SSF to produce cellulases with fungi of the genus *Trichoderma* [3]. This type of fermentation, in comparison to others, enables the use of low-cost substrates, recovery of enzymes with higher concentrations, and faster growth of aerobic microorganisms, such as the filamentous fungi. SSF also uses less energy and has lower sterility requirements than those of submerged fermentations [25]. Paper has low lignin and high cellulose contents and does not require any chemical pretreatment for its use in cellulase production. Hence, besides being environment-friendly, waste paper is an ideal substrate for fungal cellulase production.

The objective of this work was to evaluate cellulolytic enzyme production in SSF with a native isolate of *T. asperellum* using paper and sawdust as substrates and to ascertain the effect of lignin on enzyme production. A strain of *T. reesei* was used as reference organism.

Materials and methods

Microorganisms

T. reesei T114 was donated by the Environmental and Agricultural Biotechnology Unit of the Biological Research Corporation (CIB). *T. asperellum* and other fungi were isolated from coffee pulp after 60 days of ensilage by serial dilutions in saline solution and culturing on potato dextrose agar (PDA) supplemented with gentamicin. The colonies were screened for the characteristics reported for *Trichoderma* and subcultured to obtain the isolates.

To obtain monosporic cultures, conidia suspensions from each colony were serially diluted and inoculated onto agar-agar plates. Next, cultures were made from the plates containing conidia separated enough to transfer them individually to a new plate [26], which was then incubated at 25 °C for 6 days and further stored at 4 °C.

Morphological and molecular characterization

Isolates were observed after 6 days of incubation on PDA plates at 25 °C. Fungi were identified according to their macroscopic (color, texture, and appearance) and microscopic (appearance of hyphae, conidia, and conidiophores) features. Genomic DNA of native *T. asperellum* was extracted from the pure culture and the internal transcribed spacer (ITS4 and ITS5) regions of the ribosomal DNA were amplified by PCR and subsequently sequenced. Sequences were aligned and compared against available sequences in the databases GenBank, EMBL (European Molecular Biology Laboratory) and UNITE (https://unite.ut.ee) using the BLAST of NCBI (National Centre for Biotechnology Information, http://www.ncbi.nlm.nih.gov/).

Validation of fungal cellulolytic activity

To establish their cellulolytic activity, isolates were cultured in a medium containing the following components: carboxymethyl cellulose (CMC), 10 g/L; $(NH_4)_2SO_4$, 0.5 g/L; $CaCl_2$, 0.5 g/L; KH_2PO_4, 0.1 g/L; K_2HPO_4, 0.1 g/L; and agar-agar, 15 g/L. Additionally, three modifications of this culture medium were used to further evaluate fungal growth: i) use of sawdust with an average particle size of 0.15 mm instead of CMC, ii) addition of 2.5 g/L of yeast extract and peptone to the original composition, and iii) use of CMC without adding salts. Hydrolysis of cellulosic substrates was confirmed after incubation for 5 days at 30 °C.

Solid state fermentation

Sawdust and bond paper were used as substrates. Paper was cut into pieces with sizes of approximately 5 × 5 mm^2. Sawdust was ground to an average particle size of 2 mm. Both the substrates were sterilized in an autoclave at 121 °C for 15 min, followed by drying at 70 °C for 24 h.

SSF was performed in 250-mL Erlenmeyer flasks sealed using cotton balls, with each flask containing 10 g of dried substrate. The substrates were moistened with a sterile solution (yeast extract, 2.5 g/L; peptone, 2.5 g/L; $(NH_4)_2SO_4$, 0.5 g/L; $CaCl_2$, 0.5 g/L; KH_2PO_4, 0.1 g/L, K_2HPO_4, 0.1 g/L; pH, 6.0) to obtain an initial humidity content of 80 %. Conidia from PDA cultures were suspended in a solution of Tween 80 (0.1 % v/v) and inoculated into the above mentioned solution to obtain a final concentration of 1×10^7 conidia/mL. Erlenmeyer flasks were incubated at 28 °C with 80 % relative humidity for 12 days.

Enzyme assays

Crude enzymatic extracts were obtained by washing the cultures with 50 mM citrate buffer solution (1:2.5, w/v, pH 4.8) for 30 min. Solids were separated by centrifugation at 16 000 × g and 4 °C for 15 min. Supernatants were stored at -20 °C.

Total cellulolytic activity (FPase) was measured using the filter paper assay (FPA) according to Ghose, 1987 [27]. Whatman filter paper N1 was soaked in 1 mL of the enzyme extract that was diluted in 1 mL of 50 mM citrate buffer (pH 4.8). The reaction mixtures were incubated at 50 °C for 30 min. To measure endoglucanase activity (CMCase), 1 mL of the enzyme extract was added to 1 mL of CMC (2 % w/v) prepared in citrate buffer. The mixture was incubated at 50 °C for 30 min.

The concentration of reducing sugars released was measured using the dinitrosalicylic acid (DNS) method [28]. For this method, 0.5 mL of DNS solution (NaOH, 1.6 g; sodium and potassium tartrate, 30 g; 3,5-dinitrosalicylic acid, 1 g; in 100 mL of distilled water) was added to 0.5 mL of each sample and incubated in boiling water for 5 min. After the samples were cooled to room temperature, the absorbance at 540 nm was measured with a Nanocolor® spectrophotometer. A glucose solution (4 g/L) was used to plot the calibration curve. The extract obtained from uninoculated substrate was used as negative control.

One enzyme unit was defined as the amount of enzyme required to release 1 μmol of reducing sugars in 1 hour at 50 °C. The results were calculated using **Equation 1** [6].

$$EA = RS \cdot \frac{\upsilon_e}{E} \cdot \frac{1}{0.18 \cdot t}, \tag{1}$$

where EA is the enzyme activity (U/g), RS is the concentration of reducing sugars released (mg/mL), υ_e is the extract volume (mL), E is the mass of fermented substrate (g), and t is the reaction time (h).

Experimental design and statistical analysis

The solid state fermentation was performed through a randomized design and a factorial arrangement with three replicates for each treatment was followed. A two-way analysis of variance (ANOVA) for enzymatic activities with substrate and fungal isolate as factors was conducted with a significance level of 0.05. Significant differences were analyzed using Tukey's multiple comparison test. All tests were performed using R®.

Results and discussion

Fungi isolation and characterization

Besides *Trichoderma*, 12 other fungi were isolated from coffee pulp. After 2 months of ensilage, the coffee pulp from *Coffea arabica* contained ashes (14.68 %), lipids (1.49 %), proteins (19.91 %), fibers (29.47 %), soluble carbohydrates (34.47 %), nitrogen (3.19 %), phosphorus (0.23 %), potassium (6.55 %), calcium (0.75 %), and magnesium (0.18 %) [29]; these micro- and macronutrients are required for the growth of microorganisms. Hence, many microorganisms can use coffee pulp as a substrate for growth.

Microorganisms of the genera *Aspergillus*, *Candida*, *Enterobacter*, *Penicillium*, *Streptomyces*, *Fusarium*, *Geotrichum*, *Escherichia*, and *Pseudomonas* have been isolated from coffee pulp ensilaged for 2 months [29]; coffee pulp is an ideal medium for the growth of fungi and bacteria due to its high humidity content [30]. Likewise, some native fungi of the genus *Aspergillus* isolated from coffee husk can degrade caffeine and tannins [31]. In this study, we confirmed the presence of some already reported fungi in coffee pulp. In addition, a strain of *Trichoderma*, which can use cellulosic polymers as an energy source, was found.

One isolate was identified as specie belonging to the genus *Trichoderma* by observing the morphological features of his colony and his microscopic structures. This isolate presented the following features: hyaline tabicated microhyphae, regularly branched conidiophores, 3-5 hyaline bottle-shaped phialides on the conidiophore's edge, green ovoid unicellular conidia, fast-growing colonies with colorless, reverse, nonaerial mycelium at early stages, and tufty aerial mycelium at later stages. The native isolate was white-spotted green, whereas *T. reesei* exhibited white and green concentric rings [8, 32]. The results of ITS sequencing of the native *Trichoderma* indicated 99 % identity with *T. asperellum*.

Fungi growth on solid media with cellulosic substrates

After 5 days of incubation, native *T. asperellum* and *T. reesei* grew on solid culture media prepared with CMC and sawdust as substrates. The colonies were larger on media supplemented with yeast extract and peptone.

Culture medium composition modified *T. reesei* morphology as well as its cellulase production. Strains growing on media supplemented with yeast extract, peptone, and glucose showed denser and more highly branched mycelia, and thus, larger surface area, which enhanced their enzyme production due to higher enzyme-substrate interaction [32].

Microbial growth was limited in culture media that lacked salts, suggesting that salts are necessary for the production of cellulolytic enzymes; these results validated previous studies, which used pulp mill lime mud as a substrate for the growth of *T. asperellum* [13].

Enzyme production by SSF

After 12 days of incubation, fungal growth was higher on paper than on sawdust. FPase and CMCase activities were exhibited by both the isolates (**Fig. 1 and 2**), but these activities were significantly higher in case of *T. reesei* grown on paper. The concentrations of reducing sugars released in FPA with extracts produced by *T. reesei* growing on paper and sawdust were 2.82 and 0.96 g/L, respectively, while those of CMCase were 5.24 and 1.84 g/L, respectively.

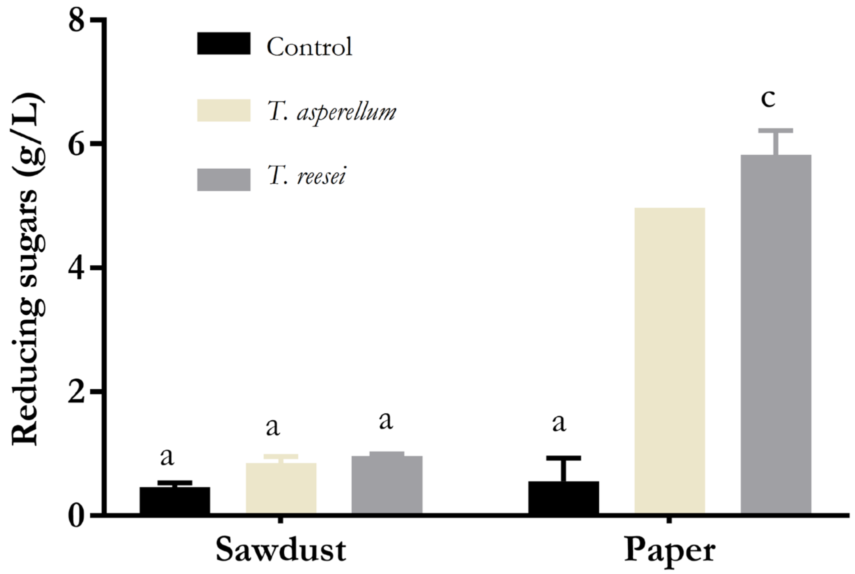

Figure 1. FPase activity of extracts obtained by SSF. Letters indicate significant differences ($p < 0.05$) between concentrations of released reducing sugars. Source: Authors.

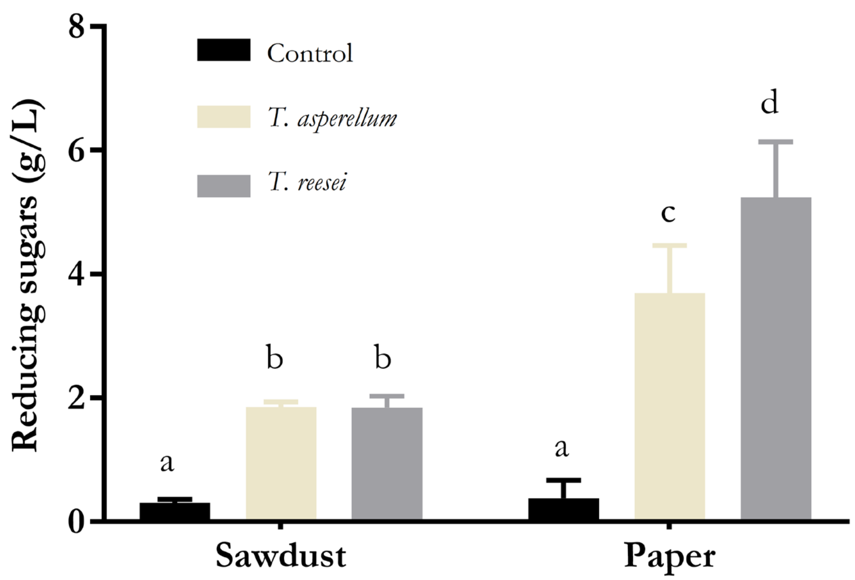

Figure 2. CMCase activity of extracts obtained by SSF. Letters indicate significant differences ($p < 0.05$) between concentrations of released reducing sugars. Source: Authors.

CMCase activity was also observed for isolates grown on sawdust, although the level was significantly lower than that observed for isolates grown on paper. Similar to other lignocellulosic materials, sawdust is composed of cellulose, hemicellulose, and pectin, forming a complex crystalline lattice with lignin, which makes the material refractory and blocks the interaction between cellulose and *Trichoderma* endo- and exoglucanases [10]. Other scholars have reported similar results. A considerably low enzyme activity using wood sawdust as a substrate for the production of cellulases with *T. harzianum* on SSF was found [23]. Similarly, more than 0.6 mg/mL of reducing sugars released using cellulolytic extracts from SSF of lignocellulosic materials with *T. reesei* could not be recovered [19].

In other studies, in contrast with this work, substrates were delignified before SSF to enhance cellulose production [6]. High cellulolytic activity in extracts from mixed and pure cultures of *T. reesei* and *A. niger* using wheat bran, rice straw or soybean hulls as substrates, which are much less refractory than sawdust, was found [21, 33]. Given that lignin is the main obstacle for enzyme production using sawdust as a substrate, further studies concerning delignification of sawdust before SSF with *Trichoderma* should be conducted [34, 35].

The ANOVA calculations (**Table 1**) showed that both the variables, i.e., the type of substrate and isolate, affected FPase and CMCase activities. Furthermore, interaction between these variables also affected enzyme production, suggesting that enzyme production is markedly different for each isolate depending on the substrate used.

Table 2 shows FPase and CMCase activities measured in enzyme units per gram of dried substrate (U/g). *T. asperellum* and *T. reesei* exhibited FPase activity that was higher than that of the negative control when using paper as the substrate. In contrast, no significant differences were found when using sawdust as the substrate, probably due to the barrier effect of lignin. FPase activity comprises the activity of endo- and exoglucanases, which can be produced by *Trichoderma* in the presence of an inducer, such as sophorose or cellulose, the latter being highly accessible in the fibers of paper pulp but not in sawdust [36].

Furthermore, significant differences were found in the CMCase activities between the two isolates and the negative control, which were higher on paper than on sawdust as the substrate (Table 2). *Trichoderma* endoglucanases cleave glycosidic bonds in a random manner, preferentially in portions of low molecular weight [23]. These enzymes may be induced by molecules present in both paper and sawdust.

Table 1. Two-factor ANOVA performed on the data of total cellulolytic (FPase) and endoglucanase (CMCase) activities ($p < 0.05$).

	Source	DF	SS	MS	F	P
FPase	Substrate	1	51.47	51.47	758.36	0.000
	Fungi	2	35.68	17.84	262.84	0.000
	Interaction	2	24.64	12.32	181.51	0.000
	Error	12	0.81	0.07		
	Total	17	112.60			
	$R^2 = 0.9928$					
CMCase	Substrate	1	17.45	17.45	55.86	0.000
	Fungi	2	41.28	20.64	66.07	0.000
	Interaction	2	10.25	5.12	16.40	0.000
	Error	12	3.75	0.31		
	Total	17	72.73			
	$R^2 = 0.9485$					

CMCase activity was higher than FPase activity when sawdust was used as the substrate. Other studies using isolates, such as those consistent with *Trichoderma*, SSF, and lignocellulosic substrates, such as wheat bran and straw, rice husk and straw, and sawdust, have reported similar results [18, 20, 21, 23]. This probably occurs because endoglucanases are produced in high quantities to reduce the size of cellulose chains, thus increasing the availability of energy sources to fulfill the energy demand in phases of higher biomass production [20].

On the contrary, FPase activity was higher than CMCase activity when paper was used as the substrate. Although this phenomenon is not common for *Trichoderma* cultures grown on lignocellulosic substrates, it has been observed in other studies a higher FPase activity with low concentration of nitrogen source and inoculum proportion in the cultures [37].

It should be considered that FPase activity was measured using filter paper in the enzymatic reaction; hence, it is possible that *Trichoderma* enzymes specific for this type of substrate were synthesized. As reported in other studies, *T. reesei* exhibited the highest cellulolytic activity. However, the native isolate of *T. asperellum* also produced cellulolytic enzymes that catalyzed the release of fermentable sugars using paper as the substrate. It will be noteworthy to

Table 2. FPase and CMCase activity of extracts obtained by SSF reported in enzyme units per gram of dried substrate. For each enzyme activity measure, letters indicate significant differences ($p < 0.05$).

Activity	Substrate	Control	*T. asperellum*	*T. reesei*
FPase	Sawdust	0.51 ± 0.08a	0.945 ± 0.12a	1.072 ± 0.04a
	Paper	0.61 ± 0.42a	5.594 ± 0.15b	6.468 ± 0.44c
CMCase	Sawdust	0.34 ± 0.06a	2.059 ± 0.09b	2.050 ± 0.21b
	Paper	0.43 ± 0.32a	4.106 ± 0.86c	5.827 ± 0.99d

investigate the ability of both the fungi to utilize different kinds of waste papers as an alternative source of reducing sugars and cellulolytic enzymes, which can hydrolyze other lignocellulosic materials.

Reliable comparisons with other studies cannot be made because there are only a few reports regarding the production of fungal enzymes by SSF using paper as a substrate, and those experiments were not conducted using similar temperature, incubation time, and buffer concentration as used in the current experiment.

Conclusions

This study confirmed that it is possible to isolate *Trichoderma*, widely reported as cellulolytic enzyme producers, from decomposing lignocellulosic materials. *T. reesei* isolate showed a higher production of endo- and exoglucanases than that by native *T. asperellum*. FPase and CMCase activities were very low when sawdust was used as a substrate in SSF with both the fungal isolates, probably because of the high lignin content, which acts as a barrier for fungi-cellulose interactions.

T. asperellum has been used for the biological control of some phytopathogenic fungi and although the cellulolytic activity of native *T. asperellum* in this study was lower than that by *T. reesei*, it was demonstrated that paper can be useful as a substrate for biomass and conidia production of *T. asperellum*.

Acknowledgements

We would like to thank the Research Incubator Program of the Health Sciences Faculty (SIFACS) for supporting this study and training young researchers. We are also grateful to the research group Biociencias and Research Department of the Institución Universitaria Colegio Mayor de Antioquia.

Conflict of interesest

There are no conflicts of interest with funding sources or institutions.

References

[1] Horton HR, Moran LA, Scrimgeour KG, Perry MD, Rawn JD. Principles of Biochemistry, 4th ed, Pearson Prentice Hall, New Jersey, USA, 2006.

[2] Rahnama N, Foo HL, Abdul Rahman NA, Ariff A, Md Shah UK. Saccharification of rice straw by cellulase from a local *Trichoderma harzianum* SNRS3 for biobutanol production, *BMC Biotechnology*, 14: 1-12, 2014.

doi: 10.1186/s12896-014-0103-y

[3] Damisa D, Sule EI, Moneme S. Cellulase production from waste paper using *Trichoderma* species isolated from rhizospheric soil, *African Journal of Biotechnology*, 11: 16342-16346, 2012.

doi: 10.5897/AJB12.2555

[4] Hansen GH, Lübeck M, Frisvad JC, Lübeck PS, Andersen B. Production of cellulolytic enzymes from ascomycetes: Comparison of solid state and submerged fermentation, *Process Biochemistry*, 50: 1327-1341, 2015.

doi: 10.1016/j.procbio.2015.05.017

[5] Fang H, Xia L. Cellulase production by recombinant *Trichoderma reesei* and its application in enzymatic hydrolysis of agricultural residues, *Fuel*, 143: 211-216, 2015.

doi: 10.1016/j.fuel.2014.11.056

[6] Sartori T, Tibolla H, Prigol E, Colla LM, Vieira Costa JA, Bertolin TE. Enzymatic saccharification of lignocellulosic residues by cellulases obtained from solid state fermentation using *Trichoderma viride*, *BioMed Research International*, 2015: 1-9, 2015.

doi: 10.1155/2015/342716

[7] Lopes Ferreira N, Margeot A, Blanquet S, Berrin J-G. Use of cellulases from *Trichoderma reesei* in the twenty-first century - Part I: Current industrial uses and future applications in the production of second ethanol generation, in Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG, *Biotechnology and Biology of Trichoderma*, Chapter 17: 245-261, 2014.

doi: 10.1016/B978-0-444-59576-8.00017-5

[8] Kredics L, Hatvani L, Naeimi S, Körmöczi P, Manczinger L, Vágvölgyi C, Druzhinina I. Biodiversity of the genus *Hypocrea/Trichoderma* in different habitats, in Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG, *Biotechnology and Biology of Trichoderma*, Chapter 1: 3-24, 2014.

doi: 10.1016/B978-0-444-59576-8.00001-1

[9] Ma L, Li C, Yang Z, Jia W, Zhang D, Chen S. Kinetic studies on batch cultivation of *Trichoderma reesei* and application to enhance cellulase production by fed-batch fermentation, *Journal of Biotechnology*, 166: 192-197, 2013.

doi: 10.1016/j.jbiotec.2013.04.023

[10] Do Vale LH, Filho EX, Miller RN, Ricart CAO, De Sousa MV. Cellulase Systems in *Trichoderma*: An Overview, in Gupta VK, Schmoll M, Herrera-Estrella A, Upadhyay RS, Druzhinina I, Tuohy MG, *Biotechnology and Biology of Trichoderma*, Chapter 16: 229-244, 2014.

doi: 10.1016/B978-0-444-59576-8.00016-3

[11] Li Y, Liu C, Bai F, Zhao, X. Overproduction of cellulase by *Trichoderma reesei* RUT C30 through batch-feeding of synthesized low-cost sugar mixture, *Bioresource Technology*, 216: 503-510, 2016.

doi: 10.1016/j.biortech.2016.05.108

[12] Zhang F, Zhao X, Bai F. Improvement of cellulase production in *Trichoderma reesei* Rut-C30 by overexpression of a novel regulatory gene Trvib-1, *Bioresource Technology*, 247: 676-683, 2018.

doi: 10.1016/j.biortech.2017.09.126

[13] Centeno Rumbos R, Pavone Maniscalco D. Producción de celulasas y biomasa del hongo *Trichoderma reesei* utilizando lodo papelero como fuente de carbono, *Revista de la Sociedad Venezolana de Microbiología*, 35: 40-46, 2015.

Available at: http://www.scielo.org.ve/pdf/rsvm/v35n1/art08.pdf

[14] Suesca Díaz A. Producción de enzimas celulolíticas a partir de cultivos de *Trichoderma* sp. con biomasa lignocelulósica, Master of Science thesis, Universidad Nacional de Colombia, Bogotá, Colombia. 2012.

Available at: http://www.bdigital.unal.edu.co/7843/1/300054.2012.pdf

[15] Zhao S, Liang X, Hua D, Ma T, Zhang H. High-yield cellulase production in solid-state fermentation by *Trichoderma reesei* SEMCC-3.217 using water hyacinth (*Eichhornia crassipes*), *African Journal of Biotechnology*, 10: 10178-10187, 2011.

doi: 10.5897/AJB10.748

[16] Chandra M, Kalra A, Sharma PK, Sangwan RS. Cellulase production by six *Trichoderma* spp. fermented on medicinal plant processings, *Journal of Industrial Microbiology & Biotechnology*, 36: 605-609, 2009.

doi: 10.1007/s10295-009-0544-9

[17] Grujic M, Dojnov B, Potocnik I, Duduk B, Vujcic Z. Spent mushroom compost as substrate for the production of industrially important hydrolytic enzymes by fungi *Trichoderma* spp. and *Aspergillus niger* in solid state fermentation, *International Biodeterioration & Biodegradation*, 104: 290-298, 2015.

doi: 10.1016/j.ibiod.2015.04.029

[18] Ortiz GE, Guitart ME, Cavalitto SF, Albertó EO, Fernández-Lahore M, Blasco M. Characterization, optimization, and scale-up of cellulases production by *Trichoderma reesei* cbs 836.91 in solid-state fermentation using agro-industrial products, *Bioprocess and Biosystems Engineering*, 38: 2117-2128, 2015.

doi: 10.1007/s00449-015-1451-2

[19] Maurya DP, Singh D, Pratap D, Maurya JP. Optimization of solid state fermentation conditions for the production of cellulase by *Trichoderma reesei*, *Journal of Environmental Biology*, 33: 5-8, 2012.

Available at: http://jeb.co.in/journal_issues/201201_jan12/paper_02.pdf

[20] Kupski L, Arnhold Pagnussatt F, Garda Buffon J, Badiale Furlong E. Endoglucanase and total cellulase from newly isolated *Rhizopus oryzae* and *Trichoderma reesei*: Production, characterization, and thermal stability, *Applied Biochemistry and Biotechnology*, 172: 458-468, 2014.

doi: 10.1007/s12010-013-0518-2

[21] Dhillon GS, Oberoi HS, Kaur S, Bansal S, Brar SK. Value-addition of agricultural wastes for augmented cellulase and xylanase production through solid-state tray fermentation employing mixed-culture of fungi, *Industrial Crops and Products*, 34: 1160-1167, 2011.

doi: 10.1016/j.indcrop.2011.04.001

[22] Marx IJ, van Wyk N, Smit S, Jacobson D, Viljoen-Bloom M, Volschenk H. Comparative secretome analysis of *Trichoderma asperellum* S4F8 and *Trichoderma reesei* Rut C30 during solid-state fermentation on sugarcane bagasse, *Biotechnology for Biofuels*, 6: 1-13, 2013.

doi: 10.1186/1754-6834-6-172

[23] Pathak P, Bhardwaj NK, Singh AK. Production of crude cellulase and xylanase from *Trichoderma harzianum* PPDDN10 NFCCI-2925 and its application in photocopier waste paper recycling, *Applied Biochemistry and Biotechnology*, 172: 3776-3797, 2014.

doi: 10.1007/s12010-014-0758-9

[24] Saratale GD, Kshirsagar SD, Sampange VT, Saratale RG, Oh S-E, Govindwar SP et al. Cellulolytic enzymes production by utilizing agricultural wastes under solid state fermentation and its application for biohydrogen production, *Applied Biochemistry and Biotechnology*, 174: 2801-2817, 2014.

doi: 10.1007/s12010-014-1227-1

[25] Singhania RR, Patel AK, Soccol CR, Pandey A. Recent advances in solid-state fermentation, *Biochemical Engineering Journal*, 44: 13-18, 2009.

doi: 10.1016/j.bej.2008.10.019

[26] Escudero Agudelo J, Daza Merchán ZT, Gil Zapata NJ, Mora Muñoz OY. Evaluación de las enzimas celulolíticas producidas por hongos nativos mediante fermentación en estado sólido (SSF) utilizando residuos de cosecha de caña de azúcar, *Revista Colombiana de Biotecnología*, XV: 108–117, 2013.

[27] Ghose TK. Measurement of cellulase activities, *Pure and Applied Chemistry*, 59: 257-268, 1987.

[28] Miller GL. Use of Dinitrosalicylic Acid reagent for determination of reducing sugar, *Analytical Chemistry*, 31: 426-428, 1959.

[29] Blandón Castaño G, Dávila Arias MT, Rodríguez Valencia N. Caracterización microbiológica y fisicoquímica de la pulpa de café sola y con mucílago, en proceso de lombricompostaje, *Cenicafé*, 50: 5-23, 1999.

[30] Daivasikamani S, Kannan N. Studies on post-harvest mycoflora of coffee cherry of robusta, *Journal of Coffee Research*, 16: 102-106, 1986.

[31] Brand D, Pandey A, Roussos S, Soccol CR. Biological detoxification of coffee husk by filamentous fungi using a solid state fermentation system, *Enzyme and Microbial Technology*, 27: 127-133, 2000.

doi: 10.1016/S0141-0229(00)00186-1

[32] Ahamed A, Vermette P. Effect of culture medium composition on *Trichoderma reesei's* morphology and cellulase production, *Bioresource Technology*, 100: 5979-5987, 2009.

doi: 10.1016/j.biortech.2009.02.070

[33] Boggione MJ, Allasia MB, Bassani G, Farruggia B. Potential use of soybean hulls and waste paper as supports in SSF for cellulase production by *Aspergillus niger*, *Biocatalysis and Agricultural Biotechnology*, 6: 1-8, 2016.

doi: 10.1016/j.bcab.2016.02.003

[34] Lo C-M, Zhang Q, Callow NV, Ju L-K. Cellulase production by continuous culture of *Trichoderma reesei* Rut C30 using acid hydrolysate prepared to retain more oligosaccharides for induction, *Bioresource Technology*, 101: 717-723, 2010.

doi: 10.1016/j.biortech.2009.08.056

[35] Madamwar D, Patel S. Formation of cellulases by co-culturing of *Trichoderma reesei* and Aspergillus niger on cellulosic waste, *World Journal of Microbiology and Biotechnology*, 8: 183-186, 1992.

doi: 10.1007/BF01195843

[36] Amore A, Giacobbe S, Faraco V. Regulation of cellulase and hemicellulase gene expression in fungi, *Current Genomics*, 14: 230-249, 2013.

doi: 10.2174/1389202911314040002

[37] Guoweia S, Man H, Shikai W, He C. Effect of some factors on production of cellulase by *Trichoderma reesei* HY07, *Procedia Environmental Sciences*, 8: 357-361, 2011.

doi: 10.1016/j.proenv.2011.10.056

Producción de celulasas en papel y aserrín usando *Trichoderma asperellum*

Resumen. Las celulasas microbianas son enzimas utilizadas industrialmente, que catalizan la ruptura de enlaces glicosídicos de celulosa. Esta hidrólisis produce azúcares que pueden utilizarse en procesos tales como la producción de bioteanol. Estas enzimas son producidas principalmente por hongos pertenecientes al género *Trichoderma* vía fermentación en estado sólido o sumergido, con materiales celulósicos como sustratos. Las publicaciones recientes han demostrado de forma creciente que existen alternativas a las enzimas de *T. reesei* en la producción de biocombustibles de segunda generación. En este estudio se evaluaron las actividades celulolíticas de extractos crudos obtenidos de un aislamiento nativo de *T. asperellum* de pulpa de café y una cepa de *T. reesei*. Las fermentaciones en estado sólido se llevaron a cabo usando como sustratos papel y aserrín. Las actividades se midieron después de 12 días de incubación. Los extractos obtenidos de *T. reesei* mostraron mayor actividad de celulasa y endoglucanasa (6.5 and 5.8 U/g) que los obtenidos usando *T. asperellum* (5.6 and 4.1 U/g) con papel como sustrato. No hubo diferencias significativas entre los dos aislamientos cuando crecieron en aserrín. Se pudo verificar que *T. asperellum* nativa fue capaz de producir celulasas en material lignocelulósico, como papel humedecido y aserrín, que no había pasado por un pretratamiento químico.

Palabras clave: celulasas; extractos celulolíticos; fermentación en estado sólido; *Trichoderma*.

Produção de celulases em papel e serragem usando *Trichoderma asperellum*

Resumo. As celulases microbianas são enzimas utilizadas industrialmente, que catalisam o rompimento das ligações glicosídicas da celulose. Esta hidrólise produze açúcares que podem ser utilizados em processos como a produção de bioetanol. Estas enzimas são produzidas principalmente por fungos pertencentes ao gênero *Trichoderma*, via fermentação em estado sólido ou submerso, com materiais celulósicos como substrato. As publicações recentes veem demonstrando de maneira crescente que existem alternativas as enzimas de *T. reesei* na produção de biocombustíveis de segunda geração. Neste estudo foram avaliadas as atividades celulolíticas de extratos brutos obtidos de um isolamento nativo de *T. asperellum* da polpa de café e uma cepa de *T. reesei*. As fermentações em estado sólido se realizaram usando como substrato papel e serragem. As atividades foram medidas depois de 12 dias de incubação. Os extratos obtidos de *T. reesei* mostraram maiores atividades de celulase e endoglicanase (6.5 e 5.8 U/g) que os obtidos usando *T. asperellum* (5.6 e 4.1 U/g) com papel como substrato. Não houve diferenças significativas entre os dos isolamentos quando cresceram em serragem. Foi possível verificar que *T. asperellum* nativa foi capaz de produzir celulases em material lignocelulósico, como papel humedecido e serragem, que não haviam passado por um pré-tratamento químico.

Palavras-chave: celulases; extratos celulolíticos; fermentação em estado sólido; *Trichoderma*.

Y-Maritza Zapata

BSc in Biotechnology in 2016 awarded by Institución Universitaria Colegio Mayor de Antioquia, Medellín, Colombia. She developed research projects whose results have been presented at different scientific events. She did her professional internship in Biotic Products Development Center (CEPROBI) at Instituto Politécnico Nacional de México. She is pursuing a master's degree in Development of Biotic Products.

Angelica Galviz-Quezada

BSc in Biotechnology in 2016 awarded by Institución Universitaria Colegio Mayor de Antioquia, Medellín, Colombia. She developed research projects whose results have been presented at different scientific events. She completed her professional internship in Laboratorio de Bioconversiones at Universidad Nacional de Colombia, Medellín.

Víctor-Manuel Osorio-Echeverri

BSc in Chemical Engineering and MSc in Biotechnology, both degrees awarded by Universidad Nacional de Colombia, where he served as a professor from 2004 to 2012. He is a professor at Institución Universitaria Colegio Mayor de Antioquia in the Biotechnology bachelor's degree program and coordinator of the graduate certificate program of Environmental Microbiology in Health Sciences Faculty.