Fibrolytic activity of four *Trichoderma* strains grown on agro-industrial residues

Actividad fibrolíticas de cuatro cepas de *Trichoderma* crecidas en residuos agroindustriales

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Abstract

The aim of this study was to compare the cellulolytic and xylanolytic activities of two strains of *Trichoderma viride*, one of *Trichoderma reesei* and one of *Trichoderma harzianum* grown on four different substrates. Each substrate contained 20% wheat bran and 80% agro-industrial waste (corn stover (CS), sugarcane bagasse (SCB), *Yucca schidigera* fiber (YS), or compost elaborated from solid waste generated in the university cafeteria (CSW)). An interaction ($P < 0.01$) between the substrate and strain was detected for both cellulolytic and xylanolytic enzyme activities. The highest cellulolytic activity ($P < 0.01$) was obtained with *T. reesei* grown on YS, CS, and SBC, and the lowest was from the two *T. viride* strains grown on most of the substrates. The highest xylanolytic activities ($P < 0.01$) were detected for *T. harzianum* with YS and SCB and *T. reesei* with CSW and CS, while one *T. viride* strain exhibited intermediate and the other showed the lowest activity. In conclusion, *T. reesei* CDBB356 showed the highest fibrolytic activity for most of the tested substrates, a finding that suggests it has the highest potential for fibrolytic enzyme production. There is a potential application for *T. reesei* CDBB356 enzymes on ruminant feed supplements to improve forage digestibility.

Keywords
cellulolytic activity • xylanolytic activity • enzymes • agro-industrial waste • *Trichoderma*

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Resumen

El objetivo de este estudio fue comparar las actividades celulolíticas y xilanolíticas de dos cepas de *Trichoderma viride*, una de *Trichoderma reesei* y una de *Trichoderma harzianum* cultivadas en cuatro diferentes substratos. Cada substrato contenía 20% de salvado de trigo más 80% de residuos agroindustriales (rastrojo de maíz (CS), bagazo de caña de azúcar (SCB), fibra de *Yuca shidigera* (YS) o composta, elaborada a partir de residuos sólidos de la cafetería universitaria (CSW)). Se detectó una interacción (P <0,01) cepa por sustrato en las actividades enzimáticas celulolíticas y xilanolíticas. La mayor actividad celulolítica (P <0,01) se obtuvo con *T. reesei* en YS, CS y BC y la más baja con las dos cepas de *T. viride,* en la mayoría de los sustratos. Las actividades xilanolíticas más altas (P <0,01) se detectaron en *T. harzianum* con YS y SCB y *T. reesei* con CSW y CS, mientras que una cepa *de T. viride* fue intermedia y la otra tuvo las actividades más bajas. En conclusión, *T. reesei* CDBB356 mostró la actividad fibrolítica más alta en la mayoría de los sustratos, confirmando el mayor potencial para producir esas enzimas. Existe una aplicación potencial de las enzimas de *T. reesei* CDBB356 en suplementos alimenticios para rumiantes para mejorar la digestibilidad del forraje.

Palabras clave
actividad celulolítica • actividad xilanolítica • enzimas • residuo agroindustrial • *Trichoderma*

Introduction

Trichoderma reesei has important industrial applications because of its cellulolytic and hemicellulolytic enzymes (19). Among the cellulase-producing fungi, the genus *Trichoderma* shows a high capacity to produce both exoglucanases and endoglucanases, and media components along with the solid substrate used for culturing under solid-state fermentation help determine the type of enzyme(s) produced (9).

Trichoderma species are widely distributed and there is high biodiversity with a variety of biological activities in agricultural fields (16). In a study in east China, Jiang *et al.* (2016) identified 17 species, among which *Trichoderma harzianum* was dominant, whereas, in a similar study in central Europe, *Trichoderma viride* was the most abundant among 15 species found. One of the most studied strains of *T. reesei* was originally isolated on the Solomon Islands in the US; this strain was modified by metabolic engineering, and mutants of this strain are used in several industrial bioprocesses (6).

Fibrolytic enzymes can be used to improve digestibility, an important need with regards to feed utilization for ruminant production. Several fibrolytic enzymes have been developed and commercially used (20), most of them based on celullases and hemicellulases produced by *Trichoderma* and other fungi (5). The global market for feed enzymes was estimated at $899.19 million in 2014 and is expected to reach nearly $1.3 billion by 2020, a compound annual growth rate of 7.3% from 2015 to 2020 (28). In many

countries, commercial enzyme products have not been implemented as strategy to improve feed utilization because imported products are expensive and its inclusion is not profitable (20). A country that develops and produces its own fibrolytic enzymes represents an opportunity to improve forage digestibility at a low cost. Hence, identification of strains able to degrade agricultural lignocellulosic residues to develop new enzymatic products locally is important in underdeveloped countries. Thus, the objective of this study was to compare the cellulolytic and xylanolytic activities of two strains of *T*. *viride*, one of *T*. *reesei*, and one of *T*. *harzianum* grown on four agro-industrial wastes. Each culture was composed of 20% wheat bran and 80% corn stover (CS), sugarcane bagasse (SCB), *Yucca schidigera* fiber (YS), or compost elaborated from university cafeteria solid waste (CSW). This design produced four different substrates. Since the most extensively studied cellulose-secreting microorganism is the filamentous fungus *T*. *reesei*, and its fibrolytic activity has been characterized (28), the hypothesis was that *T*. *reesei* CDBB356 would exhibit the greatest potential to produce fibrolytic enzymes.

Materials and methods

Substrates and chemical composition

Composite representative samples of CS, SCB, and wheat bran were obtained from the experimental dairy farm at the University of Chapingo, Mexico; residual fiber from *Y*. *schidigera* (YS) from commercial production was obtained from Alltech de Mexico S.A. de C.V.; CSW was obtained from anaerobic and aerobic fermentation of organic residues from the Autonomous Metropolitan University cafeteria. All samples were oven dried at 45°C and milled to pass a 2 mm screen using a Wiley Mill (Standard model 4; Arthur H. Thomas Co., Philadelphia, PA). The dry matter and ashes in substrates were analyzed according to the AOAC (2), and neutral detergent fiber (NDF) and acid detergent fiber (ADF) content was determined; analyses were conducted according to Van Soest *et al.* (1991). Nitrogen content was determined with the Dumas procedure using a Leco FP-428® instrument.

Microorganisms and inoculum preparation

T. *viride* 1 (Culture Collection of CINVESTAV, México), *T*. *viride* 2 (Culture Collection of UAM-Xochimilco Phytopathology, México), *T*. *harzianum* Rifai (Genetic Resource Center strain collection at the Science Institute BUAP Center of Agroecology, México), and *T*. *reesei* CDBB356 (Culture Collection of CINVESTAV, México) were cultivated in Petri dishes that contained potato-dextrose agar (PDA) and incubated for 7 days at 27°C. The spores were harvested and counted as described by Escamilla-Alvarado *et al.* (2013).

Solid fermentation and enzymatic activity

Substrates were milled to pass a 2.38 mm screen. Subsequently, a mixture of 80% each substrate and 20% wheat bran was assembled (3). Culture media were prepared by placing 3 g of each substrate (the mix of agro-industrial waste and wheat bran) separately in 250 ml Erlenmeyer flasks and then adding 12 ml sterile water. All culture media were autoclaved (121°C, 15 psi, 25 min) and inoculated with 1 x 10^6 conidia/g dry substrate (gds) for each strain tested (26). Cultures were incubated for 7 days. To obtain the crude enzymatic extracts (CEE), each flask was placed in an ice bath on top of a stirrer.

The flask contents were resuspended and filtered with medical gauze, centrifuged (4°C, 7740 g, 15 min), and the supernatants (CEE) were stored at 4°C until its use for enzymatic determinations (4).

Xylanase and cellulase activities were determined by reducing sugars release with 3,5-dinitrosalicylic acid (DNS) (21), using Birchwood xylan (0.5%; Sigma-Aldrich) and 1% carboxymethylcellulose (Sigma-Aldrich) in sodium citrate buffer (0.5 M, pH 5.3).

The reactions were performed as described by Loera and Córdova (2003), using xylose and glucose for a standard curve; readings were taken with a Cary spectrophotometer at 540 nm.

An enzyme unit (U) was defined as the amount of enzymatic extract that released 1 µmol of reduced sugars per min per gds (15). Protein concentrations of the extracts were assayed according to the Bradford method (7), using bovine serum albumin as a standard. All determinations were made in triplicate.

Statistical design

Results were analyzed as a completely randomized design with a 4 x 4 factorial arrangement, where the factors were *Trichoderma* strains and substrates. Means were compared with the Scheffe test (30), and data was analyzed with JMP software (27).

Results and discussion

Table 1 presents the substrate chemical compositions. Lignocellulosic results showed a typical composition of high cell wall content and low protein from agricultural residues (1, 18), whereas CSW was similar to other restaurant wastes analyzed, with high water and protein content and usually low fiber content (14, 22).

An interaction between substrate and strain was detected for the enzyme activities (table 2, page 196; $P < 0.0001$). The highest cellulolytic activity was obtained with *T. reesei* grown in YS, CS, and SCB, while the lowest activity was for the two *T. viride* strains grown in most of the substrates. The cell wall composition in agro-industrial residues is usually high in cellulose and lignin (10, 33); lignin is the major factor in recalcitrance of cell walls to saccharification, particularly during enzymatic hydrolysis (10).

Table 1. Chemical composition (%) of the substrates used in solid-state fermentation.
Tabla 1. Composición química (%) de sustrato usado en fermentación sólida.

Item	CS	SCB	YS	CSW	Wheat bran
Dry matter	95.65 ± 0.15	97.66 ± 0.01	96.31 ± 0.39	26.8 ± 0.761	93.71 ± 1.22
Ash	7.12 ± 0.38	1.8 ± 0.025	2.35 ± 0.5	3.35 ± 0.160	5.38 ± 0.79
Crude protein	3.29 ± 0.20	------	0.75 ± 0.01	15.35 ± 1.72	15.9 ± 0.3
NDF	79.66 ± 1.62	86.41 ± 6.11	67.5 ± 3.17	82.73 ± 1.86	53.39 ± 2.78
Hemicellulose	25.91 ± 0.92	17.2 ± 2.46	10.35 ± 0.65	16.99 ± 0.43	33.49 ± 1.38
ADF	53.75 ± 2.05	69.21 ± 3.86	57.15 ± 2.85	65.74 ± 1.52	19.90 ± 2.87

NDF: neutral detergent fiber; ADF: acid detergent fiber.
NDF: fibra detergente neutro; ADF: fibra detergente ácido.

Table 2. Comparison of enzymatic activities and crude extract protein content of four *Trichoderma* strains after fermentation with four substrates.

Tabla 2. Comparación de las actividades enzimáticas y contenido de proteína del extracto crudo de cuatro cepas de *Trichoderma* después de la fermentación con cuatro sustratos.

Strain	Substrate	Activity		Protein, mg/ mL
		Cellulolytic, U/gds	Xylanolytic, U/gds	
T. harzianum	YS	36.09^{de}	385.01 ^{b}	0.263 ^{cde}
	SCB	71.49^{de}	358.56 ^{bc}	0.758 ^{e}
	CSW	48.81^{de}	306.03 ^{c}	0.384^{bcde}
	CS	94.95^{cd}	186.43 ^{de}	0.744 ^{ab}
T. viride 1	YS	33.18^{de}	132.53 ^{efg}	0.187 ^{e}
	SCB	39.10^{de}	65.83 ^{ghi}	0.156 ^{e}
	CSW	19.00 ^{e}	33.26 ^{i}	0.721 ^{abc}
	CS	71.50^{de}	329.67 ^{bc}	0.26 ^{de}
T. viride 2	YS	43.60^{de}	145.78 ^{def}	0.393^{bcde}
	SCB	63.35^{de}	91.56^{fghi}	0.278 ^{cde}
	CSW	31.79^{de}	43.05 ^{hi}	0.15 ^{e}
	CS	69.01^{de}	314.3 ^{bc}	1.12 ^{a}
T. reesei	YS	215.99 ^{a}	213.47 ^{d}	0.711^{abcd}
	SCB	191.74^{ab}	111.3 ^{fgh}	0.095 ^{e}
	CSW	134.78^{bc}	460.18 ^{a}	0.468^{bcde}
	CS	205.40 ^{a}	474.86 ^{a}	0.454^{bcde}
SEM		3.54	4.14	0.025
P-value interaction		0.0001	0.0001	0.0001
P-value strain		0.0001	0.0001	0.0008
P-value substrate		0.0001	0.0001	0.0001

Different letters within a row indicate significant differences (P < 0.01).
SEM: Standard error of the mean; gds: grams dry substrate; CSW: cafeteria solid waste.
Diferentes letras dentro de una fila indican diferencias significativas (P < 0,01).
SEM: error estándar de la media; gds: gramos de sustrato seco; CSW: residuos sólidos de cafetería.

The highest cellulolytic activity can be associated with cellulose content; for example, SCB contains at least 33% cellulose (33) and YS contains more than 40% (11). The cellulose content in CS is also high and varies from 35 to 42% (31).

The highest cellulolytic activities obtained from *T. reesei* (table 2) are similar to those reported by Vyas and Vyas (2005) for *T. viride* cultivated on groundnut shell waste, higher or similar to values reported with *Trichoderma asperellum* grown on agricultural straws (wheat straw, rice bran, wheat bran, and corn cob). Some of the values were greater than 145 U/gds (9), higher than cellulolytic activities found in commercial products used for ruminants (20, 25).

The highest xylanolytic activities were detected in *T. harzianum* grown on YS and SCB and *T. reesei* cultured with CSW and CS. Both *T. viride* strains showed low xylanolytic activity; strain 2 activity was slightly higher than strain 1. SCB and CS are characterized by high hemicellulose content (30, 32); however, xylanolytic activity was higher when the fungus was grown with YS, even though this substrate has a lower hemicellulose content (table 1, page 195).

The complex cell wall structure may induce different types of enzymes simultaneously. Indeed, studies with enzymes and treatments indicate that xylan and lignin removal enhances cellulose availability (5). *T. reesei* strains can modify their enzymatic activity according to the sugar(s) present as carbon sources, as demonstrated by Dondelinger *et al.* (2016) using different combinations of lactose, glucose, xylose, and a hemicellulosic hydrolysate.

Sipos *et al.* (2010) compared xylanolytic activities in *T. reesei* Rut C30 using different carbon sources (Solka Floc 200, lactose, and steam-pre-treated CS); some sources of lactose and Solka Floc 200 resulted in low specific activities, whereas CS promoted the highest activity.

In addition to substrates, there are differences in strains; an enzymatic extract obtained by solid-state fermentation of peach palm waste by *Trichoderma stromaticum* had an activity of 1440 U/g (8).

The highest xylanase activities in the present study were in the same range as commercial enzymes used for ruminants, values that have spanned from 134 to 222 UI/g (25).

The interaction between substrate and strain can be explained because the cell wall composition is different when grown in each substrate. Therefore, fungi are stimulated for different metabolic pathways. Dondelinger *et al.* (2016) used various carbon sources (lactose, glucose, xylose, and hemicellulosic hydrolysate) with two *T. reesei* strains, and the enzymatic activities were as different as those observed in this study. Raghuwanshi *et al.* (2014) also showed that a mutant *T. asperellum* strain duplicated enzymatic activities compared with the wild strain. Enzyme production can be optimized by changing pH, wheat bran level, protein sources, substrate concentration, and other factors (9). Ortiz Robledo, *et al.* (2017) concluded that the cutting age of the substrates also influences its fermentative characteristics. This results confirmed that the *Trichoderma* strains evaluated, especially *T. reesei* CDBB356, generate competitive levels of xylanolytic and cellulolytic activities compared to previous reports and even the enzymatic activities reported for commercial products. Moreover, since *Trichoderma* species grow fast enough to prevent contamination in solid-state cultures and produce enzymes that can break down the complex cell wall (35), the strains investigated in this work can be used as reference for further investigations that focus on elaborate enzymatic products as ruminant feed supplements. In special applications, the addition of supplementary enzymes such as α-xylosidase, endo-arabinase, and pectinases enhances the ability to degrade *Trichoderma* extract lingnocellulose, since these enzyme activities only occur at low levels in this fungus (6).

Conclusions

The *Trichoderma* strains used in this study showed versatility to produce fibrolytic enzymes in solid fermentation in all the evaluated substrates, with a marked variation in enzymatic profiles according to the combination of strain and type of substrate.

T. reesei CDBB356 stood out with the highest cellulolytic activity on CS and YS, while the highest xylanolytic activity was obtained on CSW and CS. Considering the relative low cost of the substrates used and the fact that *Trichoderma* species grow fast enough to prevent contamination in solid-state cultures, there is a potential application of *T. reesei* CDBB356 enzymes on ruminant feed supplements to improve food digestibility.

References

1. Aceves, O. J.; Ortega, C. M. E.; Mendoza, M. G.; Herrera, H. J.; Zetina, C. P.; Bárcena, G. R. 2014. Nutritional evaluation of wheat straw upgraded by *Pleurotus ostreatus* in growing lambs. Anim Nutr Feed Technol. 14: 593.
2. AOAC. 2012. Official Methods of Analysis. 17th ed. Arlington: Association of Official Analytical Chemists.
3. Arce-Cervantes, O.; Mendoza, G.; Miranda, L. A.; Meneses, M.; Loera, O. 2013. Efficiency of lignocellulolytic extracts from thermotolerant strain *Fomes* sp. EUM1: stability and digestibility of agricultural wastes. J Agr Sci Technol. 15: 229-40.
4. Arce-Cervantes, O.; Saucedo-García, M.; Leal-Lara, H.; Ramírez-Carrillo, R.; Cruz-Sosa, F.; Loera, O. 2015. Alternative supplements for *Agaricus bisporus* production and the response on lignocellulolytic enzymes. Sci Hortic. 192: 375-80.
5. Beauchemin, K. A.; Colombatto, D.; Morgavi, D. P.; Yang, W. Z. 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J Anim Sci. 81: 37-47.
6. Bischof, R. H.; Ramoni, J.; Seiboth, B. 2016. Cellulases and beyond: the first 70 years of the enzyme producer *Trichoderma reesei*. Microb Cell Fact. 15: 106.
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: 248-54.
8. Carvalho, E. A.; dos Santos. Góes L. M.; Uetanabaro, A. P. T.; da Silva, E. G. P.; Rodrigues, L. B.; Pirovani, C. P.; da Costa, A. M. 2017. Thermoresistant xylanases from *Trichoderma stromaticum*: application in bread making and manufacturing xylo-oligosaccharides. Food Chem. 221: 1499-506.
9. Chakraborty, S.; Gupta, R.; Jain, K. K.; Kuhad, R. C. 2016. Cost-effective production of cellulose hydrolysing enzymes from *Trichoderma* sp. RCK65 under SSF and its evaluation in saccharification of cellulosic substrates. Bioproc Biosyst Eng. 39: 1659-70.
10. Chen, F.; Dixon, R. A. 2007. Lignin modification improves fermentable sugar yields for biofuel production. Nat Biotechnol. 25: 759-61.
11. Davis, S. C.; Dohleman, F. G.; Long, S. P. 2011. The global potential for *Agave* as a biofuel feedstock. GCB Bioenergy. 3: 68-78.
12. Dondelinger, E.; Aubry, N.; Ben. Chaabane, F. B.; Cohen, C.; Tayeb, J. 2016. Contrasted enzymatic cocktails reveal the importance of cellulases and hemicellulases activity ratios for the hydrolysis of cellulose in presence of xylans. AMB Express. 6: 24.
13. Escamilla-Alvarado, C.; Poggi-Varaldo, H. M.; Ponce-Noyola, M. T. 2013. Use of organic waste for the production of added-value holocellulases with *Cellulomonas flavigena* PR-22 and *Trichoderma reesei* MCG 80. Waste Manag Res. 31: 849-58.
14. García, A. J.; Esteban, M. B.; Márquez, M. C.; Ramos, P. 2005. Biodegradable municipal solid waste: characterization and potential use as animal feedstuffs. Waste Manage. 25: 780-7.

15. Hernández, P. A.; Bárcena, J. R.; Mendoza, G. D.; Montes, M. C.; González, S. S.; Rojo, R. 2011. Xylanase activity from *Cellulomonas flavigena* extracts as affected by temperature and its degradation under in vitro ruminal conditions. Afr J Microbiol Res. 5: 961-4.
16. Jiang, Y.; Wang, J. L.; Chen, J.; Mao, L. J.; Feng, X. X.; Zhang, C. L.; Lin, F. 2016. *Trichoderma* biodiversity of agricultural fields in East China reveals a gradient distribution of species. PLoS One. 11: 1-14.
17. Loera, O.; Córdova, J. 2003. Improvement of xylanase production by a parasexual cross between *Aspergillus niger* strains. Braz Arch Biol Technol. 46: 177-81.
18. Manríquez, O. M.; Montano, M. F.; Calderon, J. F.; Valdez, J. A.; Chirino, J. O.; Gonzalez, V. M.; Zinn, R. A. 20016. Influence of wheat straw pelletizing and inclusion rate in dry rolled or steam-flaked corn-based finishing diets on characteristics of digestion for feedlot cattle. Asian-Australas J Anim Sci. 29: 823-9.
19. Martinez, D.; Berka, R. M.; Henrissat, B.; Saloheimo, M.; Arvas, M.; Baker, S. E.; Chapman, J.; Chertkov, O.; Coutinho, P.; Cullen, D.; Danchin, E.; Grigoriev, I.; Harris, P.; Jackson, M.; Kubicek, C.; Han, C.; Ho, I.; Larrondo, L; De Leon, A; Magnuson, J; Merino, S; Misra, M.; Nelson, B.; Putnam, N.; Robbertse, B.; Salamov, A.; Schmoll, M.; Terry, A.; Thayer, N.; Westerholm-Parvinen, A.; Schoch, C.; Yao, J.; Barabote, R.; Nelson, M.; Detter, C.; Bruce, D.; Kuske, C.; Xie, G.; Richardson, P.; Rokhsar, D.; Lucas, S.; Rubin, E.; Dunn-Coleman, N.; Ward, M.; Brettin, T. S. 2008. Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). Nat Biotech. 26: 553-60.
20. Mendoza, G. D.; Loera-Corral, O.; Plata-Pérez, F. X.; Hernández-García, P. A.; Ramírez-Mella, M. R. 2014. Considerations on the use of exogenous fibrolytic enzymes to improve forage utilization. Sci World J. 2014: 247437.
21. Miller, G. L; 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 31: 426-8.
22. Myer, R. O.; Brendemuhl, J. H.; Johnson, D. D. 1999. Evaluation of dehydrated restaurant food waste products as feedstuffs for finishing pigs. J Anim Sci. 77: 685-92.
23. Ortiz Robledo, F.; Reyes Estrada, O.; Carrete Carreón, F. O.; Sánchez Arroyo, F.; Herrera Torres, E.; Murillo Ortiz, M.; Rosales Serna, R. 2017. Nutritional and fermentative quality of maralfalfa (*Pennisetum* sp.) silages at different cutting ages and ground corn levels. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 49(2): 345-353.
24. Raghuwanshi, S.; Deswal, D.; Karp, M.; Kuhad, R. C. 2014. Bioprocessing of enhanced cellulase production from a mutant of *Trichoderma asperellum* RCK2011 and its application in hydrolysis of cellulose. Fuel. 124: 183-9.
25. Ramırez, L.; Aranda, E.; Mendoza, G.; Landois, L.; Miranda, L.; Crosby, M. 2005. Characterization of commercial fibrolytic products for ruminant diets. Vet Méx. 36: 1-9.
26. Sainos, E.; Díaz-Godínez, G.; Loera, O.; Montiel-González, A.; Sánchez, C. 2006. Growth of *Pleurotus ostreatus* on wheat straw and wheat-grain-based media: biochemical aspects and preparation of mushroom inoculum. Appl Microbiol Biotechnol. 72: 812-5.
27. Sall, J.; Lehman, A.; Stephens, M. L.; Creighton L. 2012. JMP start statistics: a guide to statistics and data analysis using JMP. SAS Institute.
28. Singh, R.; Kumar, M.; Mittal, A.; Mehta, P. K. 2016. Microbial enzymes: industrial progress in 21st century. 3 Biotech. 6: 174.
29. Sipos, B.; Benkö, Z.; Dienes, D.; Réczey, K.; Viikari, L.; Siika-aho, M. 2010. Characterisation of specific activities and hydrolytic properties of cell-wall-degrading enzymes produced by *Trichoderma reesei* Rut C30 on different carbon sources. Appl Biochem Biotech. 161: 347-64.
30. Steel, R. G. D.; Torrie, J. H.; Dickey, D. A. 1997. Principles and procedures of statistics: A Biological Approach. New York: McGraw-Hill.
31. Templeton, D. W.; Sluiter, A. D.; Hayward, T. K.; Hames, B. R.; Thomas, S. R. 2009. Assessing corn stover composition and sources of variability via NIRS. Cellulose. 16: 621-39.

32. Van Soest, P. J.; Robertson, J. B.; Lewis, B. A. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci. 74: 3583-97.
33. Vargas- Betancur, G. J.; Pereira, N. 2010. Sugar cane bagasse as feedstock for second generation ethanol production. Part I: diluted acid pretreatment optimization. Electron. J Biotechnol. 13: 1-9.
34. Vyas, A.; Vyas, D. 2005. Production of fungal cellulases by solid state bioprocessing of groundnut shell wastes. J Sci Ind Res India. 64: 767-70.
35. Zeilinger. S.; Gruber. S.; Bansal. R.; Mukherjee. P. K. 2016. Secondary metabolism in *Trichoderma*-Chemistry meets genomics. Fungal Biol Rev. 30: 74-90.

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