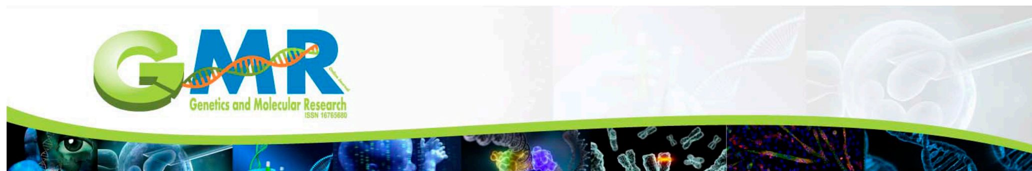

Expression of genes encoding cellulolytic enzymes in some *Aspergillus* species

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ABSTRACT. Fermentation is an important industrial process for microbial metabolite development and has wide applications in various fields. *Aspergillus* is the most important genus of fungi used for the production of microbial enzymes such as cellulase. The *Aspergillus* genome encodes various cellulolytic enzymes. In this study, we assayed the gene expression and cellulolytic enzyme production of three isolates: *A. niger* (KSU009), *A. terreus* (KC462061), and *A. flavus* (KSU014). Two fermentation systems, submerged fermentation and biofilm fermentation (BF), were used for this purpose. Gene expression analysis by RT-PCR showed that *cbhB*, *exo*, *eglA*, *eglB*, *eglC*, and *β-actin* genes were differentially expressed in the two fermentation systems for these three isolates during enzyme production. Furthermore,

the expression of all genes was found to be higher in the BF system. The six genes were not expressed in the isolates with no cellulolytic enzyme production. The isolates were identified by morphological and molecular methods, which were based on macroscopic characteristics and sequence analysis of ITS1, ITS2, and the 5.8S regions of rDNA.

Key words: *Aspergillus* spp; Cellulolytic enzyme genes; RT-PCR; Fermentation

INTRODUCTION

The global market for industrial enzymes was estimated to be $3.3 billion in 2010, and was expected to reach $4.4 billion by 2015 (Parameswaran et al., 2013). The difficulty in producing cellulase at an industrial scale has been a bottleneck in the development of novel bioprocesses and production of biofuels and bioproducts from renewable sources (Sørensen et al., 2013). Cellulases can be classified into three major types, endoglucanases (EG) (EC 3.2.1.4), exoglucanases (EXG) (cellobiohydrolase) (EC 3.2.1.91), and β-glucosidases (βG) (EC 3.2.1.21) (Rabinovich et al., 2002). Basic and applied research on cellulolytic enzymes has elucidated their roles in various industrial applications including food processing, animal feeds, fermentation, biofuels, agro-waste management, textiles, and the paper production (Kuhad et al., 2011).

Cellulase is primarily produced by microorganisms in nature (fungi, bacteria, and protozoa) (Watanabe and Tokuda, 2001). Various species of the *Aspergillus* genus (primarily *Aspergillus niger*) produce enzymes such as cellulase (Sridevi et al., 2015), lipase (Osuna et al., 2015), and estrase (Zhou et al., 2015). The four fermentation systems used for cellulose production are: submerged fermentation (SmF), solid-state fermentation (SSF), biofilm fermentation (BF), and surface-adhesion fermentation (Gutiérrez-Correa and Villena, 2003; Villena and Gutiérrez-Correa, 2006). SmF is based on the culture of microorganisms in a liquid nutrient broth, whereas other fermentation systems use a solid substrate surface for microorganism growth (Gutiérrez-Correa and Villena, 2003). In the BF system, filamentous fungi naturally attaches to the growth surface by cell adhesion or chemical bonding that induces changes in its physiology (Ghigo, 2003).

Genome*s* of several species of the *Aspergillus* genus often contain multiple cellulolytic enzyme genes that exhibit differential expression based on culture conditions and other factors (Ward et al., 2006). Genetic, transcriptomic, and proteomic studies have revealed that several genes and regulatory circuits are activated during enzyme production (Al-Sheikh et al., 2004).

Transcription patterns of cellulolytic enzymes have been examined via various molecular biological methodologies such as northern blotting (Marui et al., 2002), RT-PCR (Mahmood et al., 2014), expressed sequence tag analysis (Todaka et al., 2010), DNA microarray analysis (Ogawa et al., 2013) and quantitative real-time PCR (Bak, 2015). The gene profiles of EGs (*eglA*, *eglB*, and *eglC*), EXGs (*cbhA*, *cbhB*, and *exo*), and βGs (*β-actin*) were found to vary in different fungi, especially in *Trichoderma reesei* and *A. niger* (Villena et al., 2009; Mahmood et al., 2014).

However, very few studies have examined the effect of fermentation systems on the expression of genes encoding cellulolytic enzymes. Thus, the aim of the current study was to

assess differential gene expression of the *Aspergillus* spp (*A. niger* KSU009, *Aspergillus terreus* KC462061, and *Aspergillus flavus* KSU014) during enzyme production in two fermentation systems, BF and SmF.

MATERIAL AND METHODS

Aspergillus isolates

Six fungal isolates belonging to three different species of the *Aspergillus* genus were used in this study (Table 1). The six isolates were obtained from grains, seeds, and soil of agricultural crops. They were maintained under conditions as previously described (Mahmoud et al., 2014).

Table 1. *Aspergillus* isolates used in the study.

No.	Accession	Origin	Source	Taxon
1	KSU009	Saudi Arabia	Corn	*A. niger*
2	KC462061*	Saudi Arabia	Soil	*A. terreus*
3	KSU014	Saudi Arabia	Wheat	*A. flavus*

*GenBank accession number.

Identification of *Aspergillus* isolates

Morphological and molecular identification of *Aspergillus* isolates were based on macroscopic characteristics as well as gene sequences of ITS1, ITS2, and the 5.8S regions of rDNA (Raper and Fennell, 1965; Gehlot et al., 2011). PCR was performed using the PCR T Personal Thermo Cycler (Biometra, Germany). The PCR products were sequenced using an automated ABI-Prism 377 DNA Sequencer (Applied Biosystems Inc., CA, USA). The MEGA 6.01 software was used to align the sequences of *Aspergillus* isolates with nucleotide sequences provided by the GenBank. The neighbor-joining method was used to create phylogenetic trees (Saitou and Nei, 1987). Identification at the species level was based on the percentage of ITS similarities (Higgins et al., 2007).

Cellulolytic enzyme activity and fermentation system

The two fermentation systems, BF and SmF, were used to measure cellulolytic enzyme activities. The three *Aspergillus* genus isolates, *A. niger* (KSU009), *A. terreus* (KC462061), and *A. flavus* (KSU014), were inoculated and cultured under both fermentation systems. Specifications and assays of the two fermentation systems are summarized in Table 2. The experiment was repeated for the same isolates, and mercury(II) sulfate was added at a concentration of 500 ppm to stop enzymatic activities in the isolates.

Extraction of total RNA and synthesis of cDNA

Cultured *Aspergillus* isolates (100 mg) were extracted at 24, 48, 72, and 96 h. The mycelial pellets were grounded in liquid nitrogen to isolate total RNA using the RNeasy plant mini kit (QIAGEN, Germany), according to manufacturer instructions. cDNA synthesis was

carried out by reverse transcription using the ProtoScript First Strand cDNA Synthesis Kit (New England Biolabs, UK), according to the manufacturer instructions.

Table 2. Summary of fermentation systems used in the study.

Item	Biofilm fermentation	Submerged fermentation
inoculums formation	2 mL spore suspension (1 x 10^6)	The same
Culture medium	Duff media	The same
Support media	Polyester cloth 100/1	No support media
Experimental steps	Incubation, shaker bath, washing and Incubation	The same
Assays	Cellulase and xylanase were measured by one international unit (IU) of enzyme	The same
References	(Mahmood et al., 2014, Villena et al., 2009)	

Transcriptional analysis of selected cellulolytic genes

The synthesized cDNA of each gene was amplified via PCR by using the combination of forward and reverse primers outlined in Table 3 (Mahmood et al., 2014). The reaction mixture contained 2.25 μL 10 X buffer, 1.8 μL dNTP mix (5 mM each), 0.5 μL each forward and reverse primer (10 μM), 1 μL Taq DNA polymerase (New England BioLabs, UK), 2.25 μL cDNA sample, and 11.7 μL dH_2O. The amplification protocol was as follows: initial denaturation at 95°C (4 min); 40 cycles of: 95°C (30 s), 55°C (30 s), 72°C (30 s); final extension at 72°C (5 min); hold at 4°C. The PCR products were separated on a 1.5% agarose gel containing ethidium bromide, and were electrophoresed (75 V) and visualized using a gel documentation system (UVP).

Table 3. Primer list (adapted from Mahmood et al., 2014).

Primer pair	Gene	Sequence (5'-3')	Amplified region	MW	Optimum AT (°C)	RT-PCR product size (bp)
cbhB-F	*cbhB*	GCAAGTGTACCCACTCACACA	Celobiohydrolase	6344.2	58	587
cbhB-R		AAGCGGTTGTACGTGCAAGA		6206.1		
Exo-F	*Exo*	TGTGCTCTCGTTGCCCTCTTG		6346.2	60	598
Exo-R		AGTGCATTGGCGCCTTCCTC		6060.0		
eglA-F	*eglA*	TCCCCGTGTCACTTGCTATG	Endoglucanase	60.35.0	58.5	391
eglA-R		CAGTTCATAGTCGCCGCTAGA		6406.2		
eglB-F	*eglB*	ATCTCAACCAAGCAGCCATT		6030.0	56.5	470
eglB-R		CCAGGATATCCAGCATACCC		6031.0		
eglC-F	*eglC*	TGGTGTTACCGGTCTCTTCAAAACCGA		8250.4	61.5	515
eglC-R		GCTATACCAGGGATAGACTTACACTGCA		8581.7		
β-actin-F	*β-actin*	AGCGTGGTATCCTCACGCTC	3β-glucosidase gene	6069.0	57.5	625
β-actin-R		CTTCATGATGGAGTTGAACG		6172.1		

RESULTS

Identification of *Aspergillus* isolates

A. niger KSU009

A phylogenetic tree was constructed (Figure 1A), which included eight isolates related to *A. niger*. Four isolates were found to be closely related to *A. niger* (KSU009). As shown by the phylogenetic tree, *A. niger* KU681408, *A. niger* KR296852, and *A. niger* JN587346

belonged to the same clade. *A. niger* KSU009 was found to be very closely related to *A. niger* KU681408, with an ITS sequence similarity of 99.9%.

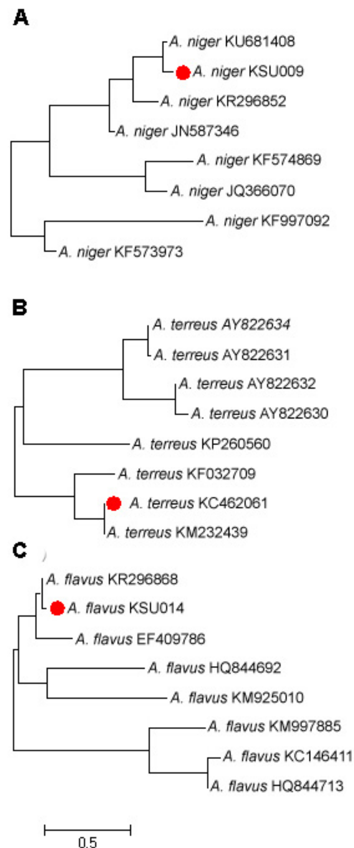

Figure 1. Phylogenetic tree of five *Aspergillus* species based on 5.8S and ITS sequences *A. niger* KSU009 (**A**); *A. terreus* KC462061 (**B**); *A. flavus* KSU014 (**C**).

A. terreus KC462061

The phylogenetic tree (Figure 1B) included eight isolates related to *A. terreus* KC462061. *A. terreus* KC462061 was found to be very closely related to *A. terreus* AY822634, with an ITS sequence similarity of 99.9%.

A. flavus KSU014

The phylogenetic tree constructed (Figure 1C) consisted of eight isolates related to *A. flavus*. Three isolates were closely related to *A. flavus* KSU014. The phylogenetic tree indicated that *A. flavus* KSU014 was classified into the same clade as *A. flavus* KR296868 and *A. flavus* EF409786. *A. flavus* KSU014 was found to be very closely related to *A. flavus* KR296868, with an ITS sequence similarity of 99.9%.

Cellulolytic enzyme activity

A. niger KSU009

The effect of the two fermentation systems on enzyme production by *A. niger* KSU009 is represented in Figure 2. For the BF system, cellulase activity increased linearly with reaction time, up to a maximum of 72 h, after which a decline in the activity was observed. Similar trend was also found for the SmF system. The effect of the BF and SmF systems on βG production by *A. niger* KSU009 was also examined. The BF system led to higher enzyme yield as well as stronger correlation between enzymatic activity and incubation time, as compared to the SmF system. As expected, *A. niger* KSU009 treated with mercury (II) sulfate failed to produce any detectable amount of enzymes.

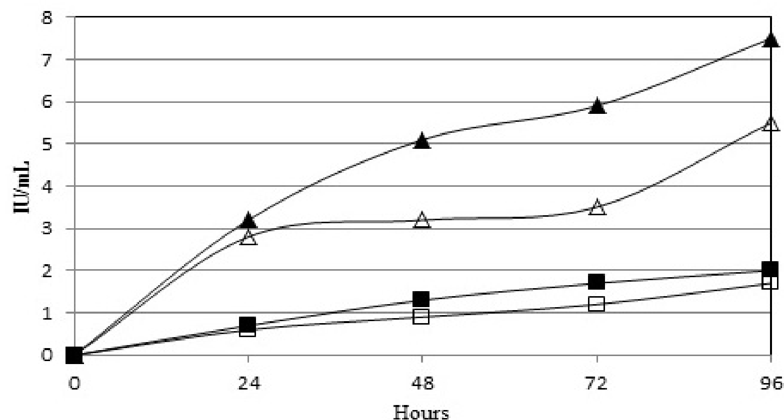

Figure 2. Activity of cellulase and βG in *Aspergillus niger* KSU009. Cellulase activity is represented by squares; βG activity is represented by triangles. *A. niger* KSU009 was grown in submerged fermentation (SmF, open symbols) and biofilm fermentation (BF, filled symbols).

A. terreus KC462061

The cellulase and βG activities were monitored for up to 96 h using the BF and SmF systems (Figure 3). *A. terreus* KC462061 produced cellulase under both BF and SmF systems, with higher yield found in the BF system as compared to that in the SmF system. βG activity in the BF and SmF systems showed steady increase with time during the incubation period. The activities of both the enzymes were higher under the BF system at the end of the incubation period. Mercury (II) sulfate treatment terminated enzyme production in *A. terreus* KC462061.

A. flavus KSU014

Production of cellulase and βG in the BF and SmF systems were also investigated in *A. flavus* KSU014 (Figure 4). The enzyme activity was monitored for 96 h, and both the enzymes exhibited a gradual linear rise in enzyme production. The enzyme production was found to be higher under the BF system as compared to the SmF system. Mercury (II) sulfate treatment terminated enzyme production in *A. flavus* KSU014.

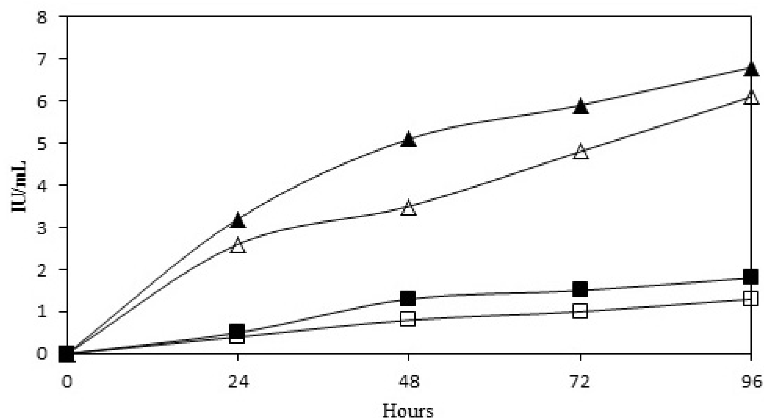

Figure 3. Activity of cellulase and βG in *Aspergillus terreus* KC46206. Cellulase activity is represented by squares; βG activity is represented by triangles. *A. terreus* KC46206 was grown in submerged fermentation (SmF, open symbols) and biofilm fermentation (BF, filled symbols).

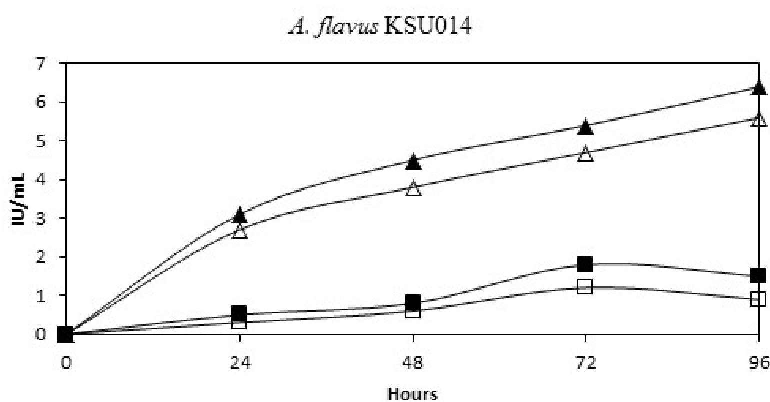

Figure 4. Activity of cellulase βG in *Aspergillus flavus* KSU014. Cellulase activity is represented by squares; βG activity is represented by triangles. *A. flavus* KSU014 was grown in submerged fermentation (SmF, open symbols) and biofilm fermentation (BF, filled symbols).

Transcriptional analysis of cellulolytic enzyme genes

Transcriptional analysis of six cellulolytic enzyme genes (*cbhB*, *exo*, *eglA*, *eglB*, *eglC*, and *β-actin*) for the six isolates was performed via RT-PCR.

A. niger KSU009

Results showed differential expression of *A. niger* KSU009 enzyme genes under the BF and SmF systems (Figure 5). Following 24 h incubation, all the genes were weakly expressed under both the fermentation systems, with the exception of *cbhB* and *eglB*. Strong gene expression was observed in BF (*Exo*, *cbhB*, and *eglA*) and SmF (*exo*) at 48 h. At 72

h, higher transcriptional rate was observed for all the cellulolytic enzyme genes in the BF system, with the exception of *eglA*; the SmF system displayed medium transcriptional levels for all the tested genes. At 96 h, gene expression in the BF system ranged from medium to high. In general, gene expression was higher in the BF system as compared to that in the SmF system. No gene amplification was observed when *A. niger* KSU009 was treated with mercury (II) sulfate.

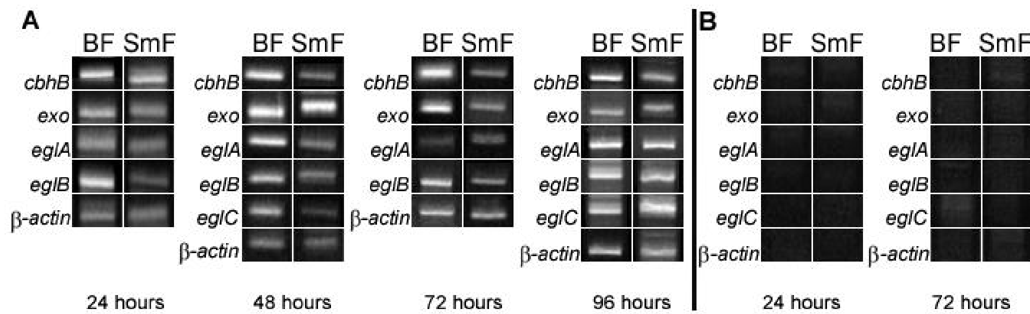

Figure 5. Expression of selected cellulolytic enzyme genes. **A.** *Aspergillus niger* KSU009 at 24, 48, 72, and 96 h, grown in BF and SmF systems; **B.** *A. niger* KSU009 treated with mercury at 24 and 72 h, grown in BF and SmF systems.

A. terreus KC462061

Transcriptional analysis of six *A. terreus* KC462061 cellulolytic enzyme genes is shown for the BF and SmF systems (Figure 6). At 24 and 48 h, all the enzyme genes, with the exception of *cbhB* and *eglA* (48 h-BF), were weakly transcribed under both the fermentation systems. At 72 h, all the genes under both the systems exhibited medium to high transcriptional levels. At 96 h, gene expressions in the BF system were found to be higher as compared to that in the SmF system. The transcription was terminated with mercury treatment.

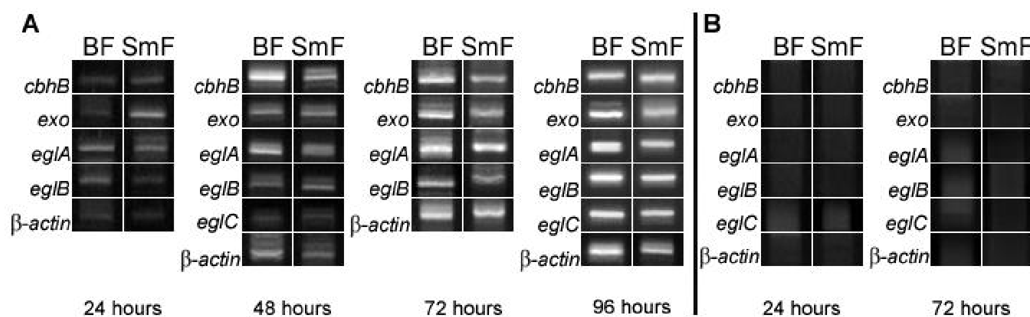

Figure 6. Expression of selected cellulolytic enzyme genes. **A.** *Aspergillus terreus* KC462061 at 24, 48, 72, and 96 h, grown in BF and SmF systems; **B.** *A. terreus* KC462061 treated with mercury at 24 and 72 h, grown in BF and SmF systems.

A. flavus KSU014

Expression levels of the six cellulolytic enzyme genes were assessed by RT-PCR under the BF and SmF systems (Figure 7). At 24 and 48 h, all the genes were weakly expressed under both the fermentation systems. At 72 and 96 h, higher gene expression was observed under the BF system, as compared to that under the SmF system. Similarly, no gene transcription was observed with mercury treatment.

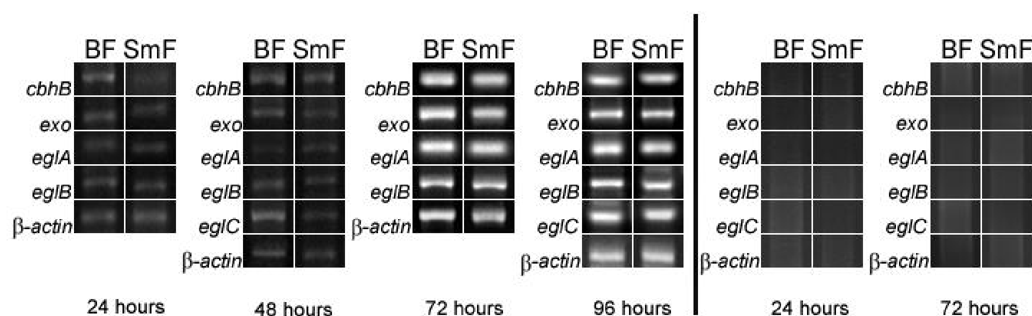

Figure 7. Expression of selected cellulolytic enzyme genes. **A.** *Aspergillus flavus* KSU014 at 24, 48, 72, and 96 h, grown in BF and SmF systems; **B.** *A. flavus* KSU014treated with mercury at 24 and 72 h, grown in BF and SmF systems.

DISCUSSION

Microbial cellulolytic enzyme production under both SmF and SSF systems have been assessed in recent studies (Renge et al., 2012). *A. niger* can be successfully cultured under both SmF and SSF systems for cellulase production. Cellulase production by SSF system was found to be 3- to 14-fold higher as compared to that by SmF. Factors that affect the production of microbial enzymes include inoculum size, carbon and nitrogen sources, as well as physicochemical conditions such as temperature, pH, and incubation period (Shivanna and Venkateswaran, 2014; Reddy et al., 2015).

Furthermore, cellulase activity of *A. flavus* AT-2 cultured under the SSF system was 42.69%, which was higher as compared to that obtained under the SmF system. It was proposed that optimal conditions for maximum cellulase production included a fungal inoculum dose of 5%, an incubation period of 5 days, a temperature of 30°C, a pH of 4.8, and usage of rice straw as the substrate (Dutt and Kumar, 2014). However, the proposed optimal conditions differed between studies due to different sources of isolates (water, soil, decaying plants) and inherent production capacity of the isolates. Among the different types of fungi, *A. flavus* was favored for cellulase production (Gomathi et al., 2012; Utharalakshmi et al., 2014).

A. terreus (used for oil palm composting) is capable of producing high levels of the three main classes of cellulase (CMCase, β-glucosidase, and FPase) under the SSF system using agricultural wastes as substrates. Yeast extract was found to be the preferred nitrogen source, with a pH of 5.5, and a temperature of 28°C. Treatment with chemicals such as Tween 80, magnesium sulfate, and calcium chloride led to a two-fold increase in enzyme production (Shahriarinour et al., 2011). A few reports confirmed that *A. terreus* can carry out high cellulase

production under short incubation periods using agricultural wastes (Gao et al., 2008; Jahromi et al., 2011).

Our transcriptional analysis suggested that fermentation systems can have an effect on the expression of enzyme-encoding genes, and increased the level of transcription was achieved using the BF system than using the SmF system. The *Aspergillus* genus has been characterized as a cellulolytic enzyme producer, and multiple genes encoding cellulase, endoxylanase, and β-xylosidase have been identified in its isolates (Aro et al., 2005). Several cellulase genes and their expression patterns have been identified through genomic sequencing. Fungal cellulase gene expression and secretion are tightly controlled at the transcriptional level (Stricker et al., 2008). Factors such as isolate efficiency, richness of nutrient broth, pH, oxygen concentration, culture conditions, and fermentation systems play a role in fungi gene expression (Aro et al., 2005; Ward et al., 2006).

In this study, we found that isolate efficiency and fermentation systems affect gene expression. In contrast to the SmF system, in a BF system, fungal species adhere to the substrate. This is a metabolically active process involving various signaling pathways and gene interactions (Osherov and May, 2001). This may explain the higher enzyme activities observed in the BF system, as transcriptional activities are upregulated by specific proteins in BF cultures. The SmF system may be governed by a different set of regulatory mechanisms, and lacks the upregulation in protein activities (Villena et al., 2009). It is possible that SmF cultures work as a homogeneous system, while the BS cultures show heterogeneity in terms of regulatory mechanisms involved in the transcription of cellulolytic enzyme genes (Viniegra-González et al., 2003).

mRNA stability is another important factor for the regulation of gene expression. A balance between mRNA synthesis and degradation is not only important for gene expression, but is also vital for cell adaptation (Caddick et al., 2006). Differential EG and βG gene expression in *A. terreus* under liquid and solid cultures is regulated by carbon sources and culture conditions (Nazir et al., 2010). The role of cell adhesion in *A. niger* is an important factor for biofilm development. Therefore, the particular morphogenetic and physiological behaviors of *A. niger* need to be further investigated to establish factors that regulate surface-adhesion fermentation system (Villena et al., 2009).

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Al-Sheikh H, Watson AJ, Lacey GA, Punt PJ, et al. (2004). Endoplasmic reticulum stress leads to the selective transcriptional downregulation of the glucoamylase gene in *Aspergillus niger*. *Mol. Microbiol.* 53: 1731-1742. http://dx.doi.org/10.1111/j.1365-2958.2004.04236.x

Aro N, Pakula T and Penttilä M (2005). Transcriptional regulation of plant cell wall degradation by filamentous fungi. *FEMS Microbiol. Rev.* 29: 719-739. http://dx.doi.org/10.1016/j.femsre.2004.11.006

Bak JS (2015). Lignocellulose depolymerization occurs via an environmentally adapted metabolic cascades in the wood-rotting basidiomycete *Phanerochaete chrysosporium. MicrobiologyOpen* 4: 151-166. http://dx.doi.org/10.1002/mbo3.228

Caddick MX, Jones MG, van Tonder JM, Le Cordier H, et al. (2006). Opposing signals differentially regulate transcript stability in *Aspergillus nidulans*. *Mol. Microbiol.* 62: 509-519. http://dx.doi.org/10.1111/j.1365-2958.2006.05383.x

Dutt D and Kumar A (2014). Optimization of cellulase production under solid-state fermentation by *Aspergillus flavus* (AT-2) and *Aspergillus niger* (AT-3) and its impact on stickies and ink particle size of sorted office paper. *Cellul. Chem. Technol.* 48: 285-298.

Gao J, Weng H, Zhu D, Yuan M, et al. (2008). Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. *Bioresour. Technol.* 99: 7623-7629. http://dx.doi.org/10.1016/j.biortech.2008.02.005

Gehlot P, Purohit DK and Singh SK (2011). Molecular diagnostics of human pathogenic *Aspergillus* species. *Indian J. Biotechnol.* 10: 207-211.

Ghigo JM (2003). Are there biofilm-specific physiological pathways beyond a reasonable doubt? *Res. Microbiol.* 154: 1-8. http://dx.doi.org/10.1016/S0923-2508(02)00012-8

Gomathi D, Muthulakshmi C, Guru KD, Ravikumar G, et al. (2012). Submerged fermentation of wheat bran by *Aspergillus flavus* for production and characterization of carboxy methyl cellulase. *Asian Pac. J. Trop. Biomed.* •••: S67-S73. http://dx.doi.org/10.1016/S2221-1691(12)60132-4

Gutiérrez-Correa M and Villena GK (2003). Surface adhesion fermentation: a new fermentation category. *Rev. Peru. Biol.* 10: 113-124.

Higgins KL, Arnold AE, Miadlikowska J, Sarvate SD, et al. (2007). Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. *Mol. Phylogenet. Evol.* 42: 543-555. http://dx.doi.org/10.1016/j.ympev.2006.07.012

Jahromi MF, Liang JB, Rosfarizan M, Goh YM, et al. (2011). Efficiency of rice straw lignocelluloses degradability by *Aspergillus terreus* ATCC 74135 in solid state fermentation. *Afr. J. Biotechnol.* 10: 4428-4435.

Kuhad RC, Gupta R and Singh A (2011). Microbial cellulases and their industrial applications. *Enzyme Res.* 2011: 280696. http://dx.doi.org/10.4061/2011/280696

Mahmood N, Mariyah A, Ishafaq A, Shahida A, et al. (2014). Molecular expression profile of different cellulolytic enzyme genes in *Aspergillus niger* in response to UV radiation and chemical mutagenesis. *Cellul. Chem. Technol.* 48: 529-533.

Mahmoud MA, Ali HM, El-Aziz ARM, Al-Othman MR, et al. (2014). Molecular characterization of aflatoxigenic and non-aflatoxigenic *Aspergillus flavus* isolates collected from corn grains. *Genet. Mol. Res.* 13: 9352-9370. http://dx.doi.org/10.4238/2014.November.11.2

Marui J, Kitamoto N, Kato M, Kobayashi T, et al. (2002). Transcriptional activator, AoXlnR, mediates cellulose-inductive expression of the xylanolytic and cellulolytic genes in *Aspergillus oryzae. FEBS Lett.* 528: 279-282. http://dx.doi.org/10.1016/S0014-5793(02)03328-8

Nazir A, Soni R, Saini HS, Kaur A, et al. (2010). Profiling differential expression of cellulases and metabolite footprints in *Aspergillus terreus*. *Appl. Biochem. Biotechnol.* 162: 538-547. http://dx.doi.org/10.1007/s12010-009-8775-9

Ogawa M, Kobayashi T and Koyama Y (2013). ManR, a transcriptional regulator of the b-mannan utilization system, controls the cellulose utilization system in *Aspergillus oryzae. Biosci. Biotechnol. Biochem.* 77: 426-429. http://dx.doi.org/10.1271/bbb.120795

Osherov N and May GS (2001). The molecular mechanisms of conidial germination. *FEMS Microbiol. Lett.* 199: 153-160. http://dx.doi.org/10.1111/j.1574-6968.2001.tb10667.x

Osuna Y, Sandoval J, Saade H, López RG, et al. (2015). Immobilization of *Aspergillus niger* lipase on chitosan-coated magnetic nanoparticles using two covalent-binding methods. *Bioprocess Biosyst. Eng.* 38: 1437-1445. http://dx.doi.org/10.1007/s00449-015-1385-8

Parameswaran B, Palkhiwala P, Gaikaiwari R, Nampoothiri K, et al. (2013). Industrial enzymes-present status & future perspective for India. *J. Sci. Ind. Res.* 72: 271-286.

Rabinovich ML, Melnik MS and Bolobova AV (2002). Dedicated to the memory of I.V. Berezin and R.V. Feniksova Microbial cellulases. *Appl. Biochem. Microbiol.* 38: 305-322. http://dx.doi.org/10.1023/A:1016264219885

Raper KB and Fennell DI (1965). *Aspergillus* section *Flavi* In: The Genus of *Aspergillus* (Raper KB and Fennell DI, eds.). Williams & Wilkins Company, Baltimore, [538].

Reddy GPK, Narasimha G, Kumar KD, Ramanjaneyulu G, et al. (2015). Cellulase production by *Aspergillus niger* on different natural lignocellulosic substrates. *Int. J. Curr. Microbiol. Appl. Sci.* 4: 835-845.

Renge V, Khedkar SV and Nandurkar NR (2012). Enzyme synthesis by fermentation method: a review. *Sci. Rev. Chem.*

Commun. 2: 585-590.

Saitou N and Nei M (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4: 406-425.

Shahriarinour M, Wahab MNA, Mohamad R, Mustafa S, et al. (2011). Effect of medium composition and cultural condition on cellulase production by *Aspergillus terreus*. *Afr. J. Biotechnol.* 10: 7459-7467.

Shivanna GB and Venkateswaran G (2014). Phytase production by *Aspergillus niger* CFR 335 and *Aspergillus ficuum* SGA 01 through submerged and solid-state fermentation. *Sci. World J.* 2014: 392615. http://dx.doi.org/10.1155/2014/392615

Sørensen A, Lübeck M, Lübeck PS and Ahring BK (2013). Fungal Beta-glucosidases: a bottleneck in industrial use of lignocellulosic materials. *Biomolecules* 3: 612-631. http://dx.doi.org/10.3390/biom3030612

Sridevi A, Narasimha G, Ramanjaneyulu G, Dileepkumar K, et al. (2015) Saccharification of pretreated sawdust by *Aspergillus niger* cellulase. *3 Biotech.* 5: 883-892.

Stricker AR, Mach RL and de Graaff LH (2008). Regulation of transcription of cellulases- and hemicellulases-encoding genes in *Aspergillus niger* and *Hypocrea jecorina* (*Trichoderma reesei*). *Appl. Microbiol. Biotechnol.* 78: 211-220. http://dx.doi.org/10.1007/s00253-007-1322-0

Todaka N, Inoue T, Saita K, Ohkuma M, et al. (2010). Phylogenetic analysis of cellulolytic enzyme genes from representative lineages of termites and a related cockroach. *PLoS One* 5: e8636. http://dx.doi.org/10.1371/journal.pone.0008636

Utharalakshmi N, Kumar AG and Narendrakumar G (2014). Optimization of cellulase producing *Aspergillus flavus* SB4 by solid state fermentation using rice bran. *J. Pure Appl. Microbiol.* 8: 713-719.

Villena GK, Fujikawa T, Tsuyumu S and Gutiérrez-Correa M (2009). Differential gene expression of some lignocellulolytic enzymes in *Aspergillus niger* biofilms. *Rev. Peru. Biol.* 15: 97-102.

Villena GK and Gutiérrez-Correa M (2006). Production of cellulase by *Aspergillus niger* biofilms developed on polyester cloth. *Lett. Appl. Microbiol.* 43: 262-268. http://dx.doi.org/10.1111/j.1472-765X.2006.01960.x

Viniegra-González G, Favela-Torres E, Aguilar CN, Rómero-Gomeza S, et al. (2003). Advantages of fungal enzyme production in solid state over liquid fermentation systems. *Biochem. Eng. J.* 13: 157-167. http://dx.doi.org/10.1016/S1369-703X(02)00128-6

Ward OP, Qin WM, Dhanjoon J, Ye J, et al. (2006). Physiology and biotechnology of *Aspergillus*. *Adv. Appl. Microbiol.* 58: 1-75. http://dx.doi.org/10.1016/S0065-2164(05)58001-8

Watanabe H and Tokuda G (2001). Animal cellulases. *Cell. Mol. Life Sci.* 58: 1167-1178. http://dx.doi.org/10.1007/PL00000931

Zhou M, Huang ZQ, Zhou B, Luo YL, et al. (2015). Construction and expression of two-copy engineered yeast of feruloyl esterase. *Electron. J. Biotechnol.* 18: 338-342. http://dx.doi.org/10.1016/j.ejbt.2015.06.004