Agr. Nat. Resour. 57 (2023) 523–532

AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Transcriptional changes in the xylanolytic filamentous fungus *Aspergillus tubingensis* NBRC 31125 grown under repressive conditions

Ririn Krisnawati[a,†], Muhammad Nur Cahyanto[a,†], Sardjono Sardjono[a,†], Dian Anggraini Suroto[a,†], Jaka Widada[b,†,*]

[a] *Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*

[b] *Department of Agricultural Microbiology, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*

Article Info

Article history:
Received 22 December 2022
Revised 26 June 2023
Accepted 30 June 2023
Available online 30 June 2023

Keywords:
Aspergillus tubingensis,
β-xylosidase,
Carbon catabolite repression,
Endo-xylanase,
RNA sequencing

Abstract

Importance of the work: The effect of glucose addition to *Aspergillus tubingensis* NBRC 31125 grown in xylan medium contributes to understanding the mechanism of carbon catabolite repression related to endoxylanase and *β*-xylosidase production.

Objectives: To investigate and describe changes in endoxylanase production, *β*-xylosidase production and gene transcription in *A. tubingensis* NBRC 31125 after glucose addition.

Materials & Methods: *A. tubingensis* NBRC 31125 was grown in xylan medium and xylan medium supplemented with glucose. The crude xylan medium and xylan medium supplemented with glucose were measured for endoxylanase and *β*-xylosidase production. The RNA of mycelia was extracted and sequenced using the next-generation RNA sequencing Illumina NextSeq 550 platform, followed by different expression genes analysis.

Results: Xylan medium supplemented with 5% glucose completely repressed endoxylanase and *β*-xylosidase production. The more glucose added, the more the production of both enzymes decreased. Transcriptome analysis identified 2,242 downregulated and 2,105 upregulated genes supporting enzyme production. The addition of glucose resulted in the downregulation of 10 genes that were involved in the breakdown of xylan. As activators, the transcription factors XlnR and AraR induced more expression in the xylan medium than in the xylan medium supplemented with glucose. However, repressor CreA and other regulation factors (CreB, CreC and CreD) produced various responses.

Main finding: The downregulated genes in the xylan supplemented with glucose medium showed carbon catabolite repression, consistent with glucose regulating endoxylanase and *β*-xylosidase production in *A. tubingensis* NBRC 31125.

† Equal contribution.

* Corresponding author.
E-mail address: jwidada@ugm.ac.id (J. Widada)

https://doi.org/10.34044/j.anres.2023.57.3.16

Introduction

Xylanase is an enzyme complex comprising endoxylanase and *β*-xylosidase as its backbone degrading enzymes (Beg et al., 2001). In the food industry, these enzymes are used to produce xylose or xylooligosaccharide, to clarify wine and juice and to improve bread quality (Polizeli et al., 2005; Butt et al., 2008; Dhiman et al., 2008; Kumar et al., 2017). *Aspergillus tubingensis* NBRC 31125 can produce high levels of endoxylanase and *β*-xylosidase (Akiba et al., 1996; Shimokawa et al., 2012). This fungus has the potential to produce xylooligosaccharide or xylose for industrial food use (Adhyaru et al., 2016; Intasit et al., 2022).

The carbon source influences enzyme production in fungi, as a suitable carbon source will induce enzyme production, while other carbon sources can inhibit enzyme production (Metreveli et al., 2021; Wang et al., 2020; Zhang et al., 2020). One obstacle to fungal xylanase production is carbon catabolite repression (CCR) (Rojas-Rejón et al., 2011), which represses xylanase production when a preferred sugar is available in the medium as a carbon source (Deutscher, 2008). Among many sugars, glucose induces CCR when the fungus is inoculated in mixtures of glucose and other substrates (Prathumpai et al., 2004; Pinaga et al., 2006; Katz et al., 2008). In addition, the glucose concentration affects the enzyme repression level and hydrolysis yield (Mardawati et al., 2014).

CCR regulation starts at the transcriptional level and in *Aspergillus,* it is controlled by CreA (Cre = catabolite responsive elements) (Katz et al., 2008; Ichinose et al., 2014), while enzyme induction is controlled by XlnR (a (hemi)-cellulolytic regulator) (de Vries et al., 1999). The former substantially represses the gene-encoding-enzymes required for xylanase production in a favoured carbon source medium (Ichinose et al., 2018). On glucose sensing, CreA directly represses xylanases by binding to the consensus DNA sequence 5′-SYGGRG-3′ in its promoter regions (Cubero and Scazzocchio, 1994). In addition, CreA regulates XlnR (Tamayo et al., 2008). Thus, CreA indirectly represses all XlnR-regulated genes. There are other factors related to the CCR, including *CreB, CreC, CreD, HulA, ApyA, RcoA, SsnF, SchA* and Glc7-Reg1. The CreB-CreC deubiquitination (DUB) complex may remove ubiquitin molecules from the protein to activate CreA. CreD may oppose CreB-CreC DUB. CreD-HulA ubiquitination ligase complex may ubiquitinate CreA to make it proteasome-targetable (Ries et al., 2016; Adnan et al., 2018).

Since the expression of glycoside hydrolase genes in filamentous fungi is suppressed by CCR, it is of great scientific and industrial importance to study the regulatory mechanism of CCR. The current study investigated transcriptome analysis through next-generation RNA sequencing to understand the CCR transcription dynamic of *Aspergillus*.

Next-generation RNA sequencing provides highly sensitive reads for genes responding to substrates at the genome level (Wang et al., 2009). However, other fungal transcriptomic studies focused on developing cocktail enzymes or acquiring information about the effects of different substrates on gene expression (Delmas et al., 2012; Pullan et al., 2014; Daly et al., 2017). The transcriptional regulation of hydrolysis enzymes in *Aspergillus niger* and *Aspergillus fumigatus* has been studied on various carbon sources (Jørgensen et al., 2009; De Souza et al., 2011; Borin et al., 2017; de Gouvêa et al., 2018). Many studies have used lignocellulose materials, such as agricultural wastes, in their transcriptome analyses (Häkkinen et al., 2012; Miao et al., 2015; Brown et al., 2016; Steindorff et al., 2021). CCR in *Aspergillus* has been studied using many species, such as *Aspergillus nidulan* or *Aspergillus oryzae*. However, currently, there are no available molecular reports or studies on the influence on enzyme production of *Aspergillus tubingensis* CCR in a xylan and glucose medium. The current study aimed to address this shortfall by investigating and describing the differences in transcriptional response and enzyme activities of *A. tubingensis* with xylan and glucose. Xylan was used instead of lignocellulose material as the sole carbon source to investigate the specific catabolite repression of xylanase. It seems that this was the first transcriptome comparison study exploring the molecular basis of microbial responses to two carbon sources using *A. tubingensis* inoculated in a mixture of xylan and glucose. Detailed knowledge related to genes expressed by *A. tubingensis* inoculated on xylan medium and xylan medium supplemented with glucose could improve understanding of its mechanisms in enzyme production and carbon catabolite repression. Furthermore, the data could be compared to transcriptome analysis of other *Aspergillus* species or fungi under repressive conditions to find prominent CCR patterns in fungi. In addition, the data could provide valuable information for exploiting genes encoding xylan degradation enzymes through genetic engineering that improved its hydrolytic potential.

Materials and Methods

Microorganism

A. tubingensis NBRC 31125 was obtained from the Food and Nutrition Culture Collection of Universitas Gadjah Mada (Yogyakarta, Indonesia). The strain was grown in potato dextrose agar medium for 7 d at 30°C before being used.

Enzyme production

A. tubingensis NBRC 31125 spores were inoculated in Mandels mineral salts solution (Mandels and Reese, 1956; Chand et al., 2005). The medium consisted of 2.0 g/L monopotassium phosphate, 1.4g/L ammonium sulfate, 1.0 g/L peptone, 0.4 g/L calcium chloride, 0.3 g/L magnesium sulfate, 0.3g/L urea, 0.2 g/L Tween 80, 20.0 mg/L cobalt chloride, 5.0 mg/L iron sulfate, 1.6 mg/L manganese sulfate and 1.4 mg/L zinc sulfate. A sample (10 g/L) of xylan (Sisco; India) and 10–50 g/L glucose were used as carbon sources. A sample (1 mL) of a solution containing 1×10^7 spores/mL was inoculated in 25 mL of liquid medium containing xylan or glucose and then incubated for 7 d at 30°C. The supernatant was passed through Whatman's filter paper No. 1 for enzyme analysis, while the mycelia were collected and dried at 100°C until constant weight to measure biomass. The glucose concentration was measured using a glucose assay kit (Diasys; Germany) based on the manufacturer's instructions. Glucose determination was based on enzymatic oxidation using glucose oxidase. The colorimetric indicator was quinoneimine, which is produced by hydrogen peroxide under the catalytic activity of peroxidase from 4-aminoantipyrine and phenol (Barham and Trinder, 1972). Each treatment was performed in duplicate. Data were presented as the arithmetic mean ± SE.

Analysis of endoxylanase and β-xylosidase activities

Endoxylanase was quantified using a Xyl6x kit (Megazyme; Ireland) performed according to the manufacturer's protocol. One unit of endoxylanase activity was defined as the amount of enzyme required to release 1 µmol of 4-nitrophenol from the substrate in 1 min under the assay conditions. The β-xylosidase activity was quantified according to Kundu and Ray (2013). Here, 500 µL of the crude enzyme was added to 0.5 mL of 1 mM p-nitrophenyl β-D-xylopyranoside (pNPX; Megazyme; Ireland) in 0.05 M citrate buffer (pH 4.0). The solution was vortexed and incubated at 55°C for 15 min using a water bath. Next, the solution was added to 2 mL of 1M sodium carbonate to stop the reaction. Then, the solution was measured using a spectrophotometer (Genesys 10S UV-VIS; Thermo Scientific; USA) at 405 nm. p-Nitrophenol (Sigma-Aldrich; USA) was used as the standard solution. One-unit of β-xylosidase activity was defined as the amount of enzyme required to release 1 µmol of p-nitrophenol from the pNPX substrate in 1 min under the assay conditions (Kundu and Ray, 2013).

RNA extraction and purification

The RNA was extracted from the strain that was inoculated in the liquid medium containing xylan only and in xylan supplemented with 5% glucose following a incubation for 5 d at 30°C (Midorikawa et al., 2018). The mycelia were harvested using filtration by passing through Whatman's filter paper No. 1 and then grinding into powder using liquid nitrogen. A Direct-Zol RNA Miniprep Plus R2073 kit (Megazyme; USA) was used to extract RNA according to the manufacturer's protocol. A NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies; USA) and an Agilent 2100 Bioanalyzer (Aligent RNA 6000 Nano Kit; Agilent; USA) were used to measure the RNA concentration and quality. Strands specific to the cDNA library were constructed using the Illumina Stranded mRNA Prep Kit (Illumina; USA).

Next-generation RNA sequencing and data analysis

The cDNA library was paired-end sequenced using the Illumina Nextseq 550 platform (Illumina; USA). The qualities of cleaned reads were assessed using the FastQC software (v.0.11.9; (Andrews, 2010)). Adapters and low-quality reads in the raw data were removed. Transcripts were quantified and pseudo-mapped using the Kallisto v.0.461 software (Bray et al., 2016) with *A. tubingensis* WU-2223L as the reference genome (Yoshioka et al., 2020). The raw sequence data were submitted to the US National Centre for Biotechnology Information Sequence Read Archive database with accession numbers SRR15412365 and SRR15412366 for *A. tubingensis* NBRC 31125 inoculated in xylan medium supplemented with glucose and xylan medium only, respectively (Krisnawati et al., 2022a). Gene expression was calculated as transcripts per million mapped reads (TPM). Differential gene expression was assessed using the edgeR software (v.3.34.0; Robinson et al., 2009). Genes with a fold change ≥1 and $p < 0.05$ were considered differentially expressed. The data were visualised using the ClustVis website 2.0 (https://bio.tools/clustvis#!).

Results

Endoxylanase and β-xylosidase production in xylan medium with and without supplementation of glucose

Endoxylanase and *β*-xylosidase production were quantified 7 d after incubation in liquid medium containing xylan and glucose to investigate the effect of glucose on enzyme production. The endoxylanase and *β*-xylosidase activities of *A. tubingensis* NBRC 31125 inoculated in xylan medium showed similar production patterns (Figs. 1A and 1B, respectively). Enzyme production increased with increasing incubation time and reached optimum levels after 5 d with 20.82 U/mL endoxylanase and 2.38 U/mL *β*-xylosidase.

The enzyme activities of endoxylanase and *β*-xylosidase were lower in the xylan medium supplemented with any concentration of glucose compared to the xylan medium (Figs 1A and 1B). Their inhibition by glucose started at a 1% concentration. Increasing the glucose concentration decreased the production of endoxylanase and *β*-xylosidase. On day 4, glucose was depleted in the xylan medium supplemented with 1% glucose, while on day 5, glucose was depleted in the xylan medium supplemented with 3% glucose. On day 5, the glucose concentration in the xylan medium supplemented with 5% glucose remained approximately 50 mM. After the glucose concentration fell below 50 mg/mL (Fig. 1C), endoxylanase and *β*-xylosidase production was detected. The results showed that the catabolite repression effect was glucose concentration-dependent. Based on the CCR observed during the optimum activity of endoxylanase and *β*-xylosidase, xylan supplemented with 5% glucose and 5 d of incubation were selected as the conditions for transcriptomic analysis of the response to carbon sources.

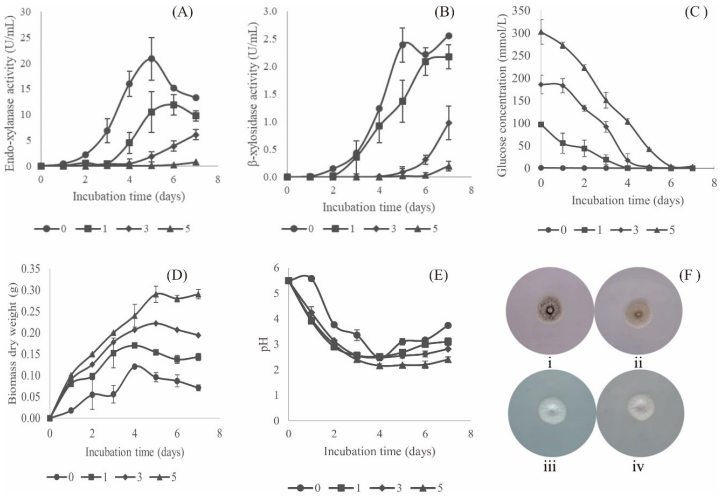

Fig. 1 Responses to *Aspergillus tubingensis* NBRC 31125 grown on xylan medium and xylan medium supplemented with glucose: (A) Endo-xylanase production; (B) *β*-xylosidase production; (C) glucose consumption; (D) dry biomass weight; (E) medium pH, where error bar indicates ± SD and morphology of fungi grown on xylan medium (Fi), xylan supplemented with 1% glucose (Fii), 3% glucose (Fiii), and 5% glucose (Fiv) at 30°C after incubation for 7 d.

However, the dry biomass weight was higher with the xylan supplemented with glucose compared to xylan alone, increasing as a function of the glucose concentration. The dry biomass weight changed slightly after 5 d of incubation (Fig. 1D). The pH of the medium decreased with increasing glucose concentration (Fig. 1E) and fermentation time. In addition, the presence of glucose also affected sporulation. *A. tubingensis* NBRC 31125 has black spores, with their black color decreasing with increasing glucose concentration (Fig. 1F).

Transcriptome of A. tubingensis NBRC 31125 grown in xylan medium and xylan supplemented with glucose

Next-generation RNA sequencing was performed using a paired-end sequencing protocol on the Illumina NextSeq 550 platform. The fragment length of each sample was 35–149 bp and the total guanine/cytosine (GC) percentage was 51.5–52%. The quality of raw data produced was measured using FastQC. The high-quality sequences comprised 85.5%, based on a FastQC threshold >30 after trimming off the adapter. Total reads with incubation on the xylan medium and the xylan supplemented with 5% glucose were 32,017,750 and 34,061,614 reads, respectively.

The cDNA library-generated gene expression sequence data were successfully aligned to 11,545 gene models in the reference *A. tubingensis* WU-2223L genome (GCF_013340325.1). Incubation on the xylan medium and the xylan supplementation with 5% glucose identified 10,922 and 10,797 expressed genes, respectively. The relative abundance of expressed genes was calculated by aligning the sample and gene reading counts to the genome reference. The mapped read counts for each sample were compared to identify differentially expressed genes in cultures grown on the xylan medium and the xylan supplemented with 5% glucose as the carbon source following a incubation for 5 d at equivalent time points. In total, 2,242 downregulated and 2,105 upregulated genes were identified

in the presence of glucose (Fig. 2). The top 133 *A. tubingensis* NBRC 31125 gene expression profiles were represented in a global heatmap comparing growth on the xylan medium only with the xylan supplemented with 5% glucose (Fig. 3). Genes related to xylan degradation showed higher levels on xylan alone compared to xylan supplemented with 5% glucose. In addition, the expression of genes related to glucose degradation was higher on xylan supplemented with 5% glucose.

The expression of 10 genes associated with xylan degradation was higher than for the other genes on the xylan medium (Table 1). All genes related to xylan degradation showed lower expression on the xylan supplemented with 5% glucose, except feruloyl esterase. Among these genes, *xyn* and *xynB* (encoding endoxylanase) had the highest transcription counts of 6,419.99 TPM and 2,562.85 TPM, respectively. Endoxylanase had the highest activity (Fig. 1). The genes *abn* encoding arabinan endo-1,5-*α*-L-arabinosidase and *axeA* encoding acetyl xylan esterase also showed higher transcription.

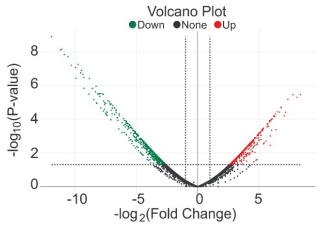

Fig. 2 Volcano plot of differential gene expression of *A. tubingensis* NBRC 31125 grown on xylan and xylan medium supplemented with 5% glucose after incubation for 5 d, where red dots indicate upregulated genes, green dots indicate downregulated genes and black dots are not different.

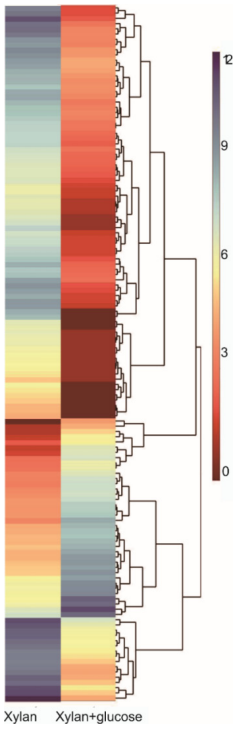

Fig. 3 Heat map of hierarchical clustering of gene transcript modulation patterns observed in *A. tubingensis* NBRC 31125 following growth on xylan medium supplemented with 5% glucose compared to xylan medium after incubation for 5 d, where blue indicates higher expression and red indicates lower expression.

Table 1 List of genes related to xylan degradation, xylose utilization, and hemicellulose and cellulose degradation, with significantly different expressions after growing on xylan medium compared to xylan supplemented with 5% glucose after incubation for 5 d

Description		Gene name	Differential expression		
			Xylan (TPM)	Xylan + glucose (TPM)	log2FC
Xylan accessory enzyme	Acetylxylan esterase	axeA	699.70	0.17	-11.10
	Feruloyl esterase	faeB	0.29	7.84	5.67
	α-glucuronidase	aguA	34.20	0.13	-7.15
	Arabinoxylan arabinofuranohydrolase	axhA	559.86	0.95	-8.30
	Arabinan endo-1,5-*α*-L-arabinosidase	abnA	1087.52	9.95	-5.87
	α-L-arabinofuranosidase	abfA	15.82	0.17	-5.62
Xylan backbone degradation enzyme	*β*-xylosidase	xyl4	132.79	0.53	-7.07
	α-xylosidase	xylS	72.96	0.06	-9.38
	4- *β* -D-xylan xylanohydrolase	xynB	2562.85	0.35	-11.93
	Endo-1,4-*β*-xylanase	xynA	805.57	0.93	-8.86
	Endo-1,4-*β*-xylanase	xyn	6419.99	3.93	-9.77
Hemicellulase and cellulase	*α*-galactosidase	galA	37.98	0.07	-8.18
	α-glucuronidase	aguA	34.20	0.13	-7.15
	Xyloglucanase	cel74A	7.14	0.01	-8.49
	Putative *β*-glucosidase	bglX	22.90	0.22	-5.84
	β-glucosidase	bglX	66.68	0.15	-7.93
	1,4-*β*-D-glucan cellobiohydrolase	cbh1	258.58	4.04	-5.11
Xylose degradation enzyme	Xylitol dehydrogenase	xdhA	24.97	235.82	4.14
	Xylulose reductase	cdxr	50.59	404.08	3.90
	L-xylulose reductase	cdxr	49.69	308.10	3.53
	D-xylulose kinase	xkiaA	97.71	38.33	-
	Xylose isomerase	xylA	0.63	0.18	-
	Putative D-xylulose reductase	cdxr	72.41	14.49	-1.42
	D-xylose reductase	xyrA	497.82	6.62	-5.33

In addition to inducing the transcription of genes related to xylan degradation, xylan also induced other hemicellulose and cellulose genes (Table 1). The gene associated with hemicellulose and cellulase showed increased transcription on the xylan compared to the xylan supplemented with 5% glucose. Moreover, it showed downregulation on the xylan supplemented with glucose, with a TPM < 4.04. The genes *bglX* encoding *β*-glucosidase and *cbh1* encoding 1,4-*β*-D-glucan cellobiohydrolase showed the highest transcription on the xylan, with TPMs of 258.58 and 66.68, respectively.

Transcription and regulator factors regarding CCR of *A. tubingensis* NBRC 31125 on the xylan with and without glucose supplementation are shown in Fig. 4. The transcription of the activator genes *XlnR* and *AraR* was higher on the xylan alone than on the xylan supplemented with 5% glucose. However, the transcription of regulator genes varied. While transcription of *CreA* and *CreB* was higher on the xylan than on the xylan and glucose, transcription of *CreD* was lower on the xylan than on the xylan and glucose. Notably, the transcription of *CreC* was unchanged.

Discussion

Glucose induced CCR in *A. tubingensis* NBRC 31125 since it is more readily metabolized than xylan, enabling the strain to grow faster. Glucose supported fungal growth better than xylan (Fig. 1), as indicated by the increasing biomass weight and final decrease due to cell lysis at the end of fermentation. The higher the glucose concentration, the better the fungi grew. However, increased fungal growth was not accompanied by increased enzyme production. In this study, xylan was consumed after glucose levels started to diminish, delaying the production of endoxylanase and *β*-xylosidase. Glucose at all concentrations inhibited endoxylanase and *β*-xylosidase production. Endoxylanase and *β*-xylosidase production began after the glucose had been depleted, consistent with other studies. For example, glucose also inhibited xylanase, pectinase, CMCase and Fpase production by *Aspergillus tamarii* (Midorikawa et al., 2018) and *β*-xylosidase production by *Aspergillus versicolor* (Andrade et al., 2004). In many fungi, such as *A. oryzae, A. tamarii, Penicillium citrinum, Trichoderma asperellum* and *Trichoderma virens*, glucose at 3% concentration completely repressed endoxylanase and *β*-xylosidase (Krisnawati et al., 2022b). A transcriptomic analysis was performed to support these results, with the transcriptome of *A. tubingensis* NBRC 31125 grown on the xylan alone and on the xylan supplemented with 5% glucose the samples compared at the same stage of batch fermentation. Compared to fungi incubated on the xylan alone, those incubated on xylan supplemented with 5% glucose had lower xylanase gene expression. This finding showed that CCR in *A. tubingensis* NBRC 31125 was due to the presence of glucose in the culture medium, which was consistent with Daly et al. (2019), who analyzed the transcriptomes *of Dichomitus squalens* and reported the presence of glucose in avicel or xylan medium that repressed the expression of 7% of the genes (Daly et al., 2019). The presence of 1–2% glucose in a lignocellulose medium also inhibited several genes related to lignocellulose gene expression in the *T. reseei* strains QM6a and Rut-C30 (Ma et al., 2016; Ries et al., 2013). Midorikawa et al. (2018) examined the sugarcane bagasse and glucose transcriptome, finding that *A. tamarii* showed higher transcription of many genes from different Carbohydrate Active EnZyme (CAZy) classifications in response to sugar bagasse as a carbon source but lower transcription with a glucose medium. Li et al. (2014) examined glucose, xylose and arabinose and found they were bottlenecks in cellulose production using lignocellulosic material. In addition, Grimmler et al. (2010) showed repression of xylanase and endoglucanase gene expression in *Clostridium acetobutylicum*.

The current study also detected the expression of various genes encoding enzymes with distinct functions required to degrade xylan. The complex xylanase system includes endoxylanase (Enzyme Commision; EC 3.2.1.8), *β*-xylosidase (EC 3.2.1.37), acetylxylan esterase (EC 3.1.1.6), feruloyl esterase (EC 3.1.1.73), *α*-glucuronidase (EC 3.2.1.39), arabinoxylan arabinofuranohydrolase (EC 3.2.1.55), arabinan endo-1,5-*α*-L-arabinosidase (EC 3.2.1.55) and *α*-L-arabinofuranosidase (EC 3.2.1.55) (Goswami and Rawat, 2015; Uday et al., 2016). The expression of xylan backbone-degrading enzymes was greater than the expression of xylan side-chain-degrading enzymes

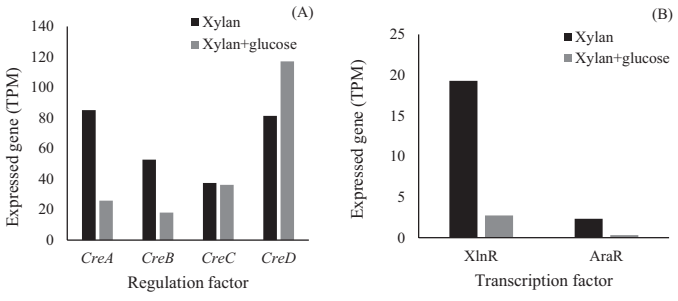

Fig. 4 Expression of regulator factor (A) and transcription factor (B) of *A. tubingensis* NBRC 31125 grown on xylan medium and xylan medium supplemented with 5% glucose after incubation for 5 d.

in strains grown on the xylan alone. The higher expression of many xylanase genes on the xylan alone showed that they were induced by the xylan. This observation was supported by the high transcription of the activator *xlnR,* known as a xylanase activator (Fig. 4) that regulates endoxylanase and *β*-xylosidase production (Mach-Aigner et al., 2010; Raulo et al., 2016).

Additionally, the transcription of arabinosefuranosidase (*abf*) and arabinoreductase (*axhA*), which function in xylanase degradation, was higher on the xylan alone. Their transcription is reportedly under the control of XlnR and AraR. Furthermore, XlnR plays a role in D-xylose reductase (*xyrA)* regulation, similar to the report by Hasper et al. (2000). Increased expression of xylose utilization pathway members, such as xylose reductase (*xyrA)*, D-xylulose kinase (*xkiaA*), D-xylose reductase (*xyrA*) and xylose isomerase (*xylA*), was observed 5 d after inoculation when the primary carbon source available was pentose sugar. In addition, the higher transcription of the xylose reductase *xyrA* and other genes related to the xylose utilization pathway (Table 1) on the xylan alone than for the xylan supplemented with 5% glucose showed that xylose was already being degraded from the medium. However, the lower transcription on the xylan supplemented with 5% glucose suggested that the fungi still used glucose as a carbon source.

Inhibition of endoxylanase and *β*-xylosidase production by glucose (Fig. 1) indicated CCR in *A. tubingensis* NBRC 31125. Repression was affected by CreA, which is widely recognized as the repressor protein in *Aspergillus* that is responsible for CCR, including xylanase repression (Delmas et al., 2012). Another study showed that CreB affected CCR (Ichinose et al., 2018). However, the current study found that the transcription of *CreA* and *CreB* was higher on the xylan alone than on the xylan supplemented with 5% glucose. The presence of high *CreA* and *CreB* expression did not decrease the production of endoxylanase and *β*-xylosidase. CreA must enter the nucleus to bind the endoxylanase and *β*-xylosidase promoters to inhibit their transcription. CreA can translocate from the cytoplasm to the nucleus and vice versa. The presence of CreA in the nucleus and cytoplasm suggests that posttranslational regulation of CreA activity is required. Phosphorylation has been found to affect CreA activity (Ribeiro et al., 2019; Assis et al., 2021), with phosphorylated CreA leading to its translocation from the nucleus to the cytoplasm. Because of the absence of CreA in the nucleus, xylanase transcription was not inhibited (Adnan et al., 2018). However, the current study could not determine the phosphorylation status and *CreA* transcription also needs to be quantified based on various glucose concentrations and time conditions.

CreC is a protein with a WD-40 domain that functions in protein-protein interactions. To activate CreA, the CreB-CreC deubiquitination (DUB) complex may remove ubiquitin molecules from the protein. In the current study, there was no difference between *CreC* transcription in the xylan medium and in the xylan supplemented with glucose. Notably, other research has indicated that CreC is involved in the regulation and cellular localization of CreA. CreC mutation rendered CreA undetectable by Western blotting. CreA of *A. nidulans* was not present in the nucleus when glucose was present (Ries et al., 2016). In addition, CreB and CreC disruption significantly reduced the amount of CreA in *A. oryzae* in the presence of glucose (Tanaka et al., 2018). *CreD* transcription was elevated in the xylan medium supplemented with glucose compared to the xylan medium alone. CreD and HulA form a complex that ubiquitinates CreA. In the presence of both the CreD dephosphorylation mutation and the *creB* disruption, *A. oryzae* can produced more amylolytic enzymes (Tanaka et al., 2017). Additional transcriptomic or proteomic investigation under a variety of conditions is required to determine the effect of *CreC* and *CreD* transcription on CCR.

In conclusion, glucose inhibited endoxylanase and *β*-xylosidase production in *A. tubingensis* NBRC 31125. Transcriptional analysis of *A. tubingesis* NBRC 31125 using next-generation RNA sequencing showed different gene expression patterns related to the xylan metabolism and its regulation when incubated on xylan alone compared to on xylan supplemented with 5% glucose. CCR in *A. tubingensis* NBRC 31125 was evidenced by the downregulation of genes in the presence of xylan and glucose. These findings supported that endoxylanase and *β*-xylosidase production in *A. tubingensis* NBRC 31125 is regulated by glucose. Further investigation should grow fungi in various glucose concentrations for various times.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This study was supported by: the Ministry of Research, Technology/National Research; and the Innovation Agency of Republic Indonesia (RISTEK-BRIN) through research grants of a PMDSU Batch III Scholarship (6/AMD/E1/KP.PTNBH/2020 and 3193/UN1.DITLIT/DIT-LIT/PT/2020).

References

Adhyaru, D.N., Bhatt, N.S., Modi, H.A., Divecha, J. 2016. Insight on xylanase from *Aspergillus tubingensis* FDHN1: Production, high yielding recovery optimization through statistical approach and application. Biocatal. Agric. Biotechnol. 6: 51–57. doi.org/10.1016/j.bcab.2016.01.014

Adnan, M., Zheng, W., Islam, W., Arif, M., Abubakar, Y.S., Wang, Z., Lu, G. 2018. Carbon catabolite repression in filamentous Fungi. Int. J. Mol. Sci. 19: 48. doi.org/10.3390/ijms19010048

Akiba, S., Takei, A., Hagihara, H., Ota, T., Kodama, H., Kimura, Y. 1996. Proteinase-resistant cellulase, micro-organism producing the same and process for producing the same. European Patent. EP0458162 B1, filed May 13, 1991, and issued January 24, 1996.

Andrade, S.d.V., Polizeli, M.d.L.T.d.M., Terenzi, H.F., Jorge, J.A. 2004. Effect of carbon source on the biochemical properties of β-xylosidases produced by *Aspergillus versicolor*. Process Biochem. 39: 1931–1938. doi.org/10.1016/j.procbio.2003.09.024

Andrews, S. 2010. FastQC: A quality control tool for high throughput sequence data. Cambridge, UK. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/, 30 June 2021.

Assis, L.J. De, Silva, P., Bayram, O., Dowling, P., Kniemeyer, O., Krüger, T., Brakhage, A.A., Chen, Y., Dong, L., Tan, K., Wong, K.H., Ries, L.N.A., Goldmanb, G.H. 2021. Carbon catabolite repression in filamentous fungi is regulated by phosphorylation of the transcription factor CreA. mBio. 12: 3146–3166. doi.org/10 .1128/mBio.03146-20

Barham, D., Trinder, P. 1972. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst 97: 142–145. doi.org/10.1039/an9729700142

Beg, Q.K., Kapoor, M., Mahajan, L., Hoondal, G.S. 2001. Microbial xylanases and their industrial applications: A review. Appl. Microbiol. Biotechnol. 56: 326–338. doi.org/10.1007/s002530100704

Borin, G.P., Sanchez, C.C., de Santana, E.S., et al. 2017. Comparative transcriptome analysis reveals different strategies for degradation of steam-exploded sugarcane bagasse by *Aspergillus niger* and *Trichoderma reesei*. BMC Genomics 18: 501. doi.org/10.1186/s12864-017-3857-5

Bray, N.L., Pimentel, H., Melsted, P., Pachter, L. 2016. Near-optimal probabilistic RNA-seq quantification. Nat. Biotechnol. 34: 525–527. doi.org/10.1038/nbt.3519

Brown, N.A., Ries, L.N.A., Reis, T.F., Rajendran, R., Corrêa Dos Santos, R.A., Ramage, G., Riaño-Pachón, D.M., Goldman, G.H. 2016. RNAseq reveals hydrophobins that are involved in the adaptation of *Aspergillus nidulans* to lignocellulose. Biotechnol. Biofuels 9: 145. doi.org/10.1186/s13068-016-0558-2

Butt, M.S., Tahir-Nadeem, M., Ahmad, Z., Sultan, M.T. 2008. Xylanases and their applications in baking industry. Food Technol. Biotechnol. 46: 22–31.

Chand, P., Aruna, A., Maqsood, A.M., Rao, L.V. 2005. Novel mutation method for increased cellulase production. J. Appl. Microbiol. 98: 318–323. doi.org/10.1111/j.1365-2672.2004.02453.x

Cubero, B., Scazzocchio, C. 1994. Two different, adjacent and divergent zinc finger binding sites are necessary for CREA-mediated carbon catabolite repression in the proline gene cluster of *Aspergillus nidulans*. EMBO J. 13: 407–415. doi.org/10.1002/j.1460-2075.1994.tb06275.x

Daly, P., Munster, J.M. Van, Kokolski, M., et al. 2017. Transcriptomic responses of mixed cultures of ascomycete fungi to lignocellulose using dual RNA-seq reveal inter-species antagonism and limited beneficial effects on CAZyme expression. Fungal Genet. Biol. 102: 4–21. doi.org/10.1016/j.fgb.2016.04.005

Daly, P., Peng, M., Di Falco, et al. 2019. Glucose - mediated repression of plant biomass utilization in the white - rot fungus *Dichomitus squalens*. Appl. Environ. Microbiol. 85: e01828-19. doi.org/10.1128/AEM.01828-19

de Gouvêa, P.F., Bernardi, A.V., Gerolamo, L.E., de Souza Santos, E., Riaño-Pachón, D.M., Uyemura, S.A., Dinamarco, T.M. 2018. Transcriptome and secretome analysis of *Aspergillus fumigatus* in the presence of sugarcane bagasse. BMC Genomics 19: 232. doi.org/10.1186/s12864-018-4627-8

De Souza, W.R., De Gouvea, P.F., Savoldi, M., et al. 2011. Transcriptome analysis of *Aspergillus niger* grown on sugarcane bagasse. Biotechnol. Biofuels 4: 40. doi.org/10.1186/1754-6834-4-40

Delmas, S., Pullan, S.T., Gaddipati, S., et al. 2012. Uncovering the genome-wide transcriptional responses of the filamentous fungus *Aspergillus niger* to lignocellulose using RNA sequencing. PLoS Genet. 8: e1002875. doi.org/10.1371/journal.pgen.1002875

Deutscher, J. 2008. The mechanisms of carbon catabolite repression in bacteria. Curr. Opin. Microbiol. 11: 87–93. doi.org/10.1016/j.mib.2008.02.007

de Vries, R.P., Visser, J., de Graaff, L.H. 1999. CreA modulates the XlnR-induced expression on xylose of *Aspergillus niger* genes involved in xylan degradation. Res. Microbiol. 150: 281–285. doi.org/10.1016/S0923-2508(99)80053-9

Dhiman, S.S., Sharma, J., Battan, B. 2008. Industrial applications and future prospects of microbial xylanases: A review. BioResources 3: 1377–1402.

Goswami, G.K., Rawat, S. 2015. Microbial xylanase and their applications–A review. Int. J. Curr. Res. Acad. Rev. 3: 436–450.

Grimmler, C., Held, C., Liebl, W., Ehrenreich, A. 2010. Transcriptional analysis of catabolite repression in *Clostridium acetobutylicum* growing on mixtures of D-glucose and D-xylose. J. Biotechnol. 150: 315–323. doi.org/10.1016/j.jbiotec.2010.09.938

Häkkinen, M., Arvas, M., Oja, M., Aro, N., Penttilä, M., Saloheimo, M., Pakula, T.M. 2012. Re-annotation of the CAZy genes of *Trichoderma reesei* and transcription in the presence of lignocellulosic substrates. Microb. Cell Fact. 11: 134. doi.org/10.1186/1475-2859-11-134

Hasper, A.A., Visser, J., De Graaff, L.H. 2000. The *Aspergillus niger* transcriptional activator XlnR, which is involved in the degradation of the polysaccharides xylan and cellulose, also regulates D -xylose reductase gene expression. Mol. Microbiol. 36: 193–200. doi.org/10.1046/j.1365-2958.2000.01843.x

Ichinose, S., Tanaka, M., Shintani, T., Gomi, K. 2014. Improved α-amylase production by *Aspergillus oryzae* after a double deletion of genes involved in carbon catabolite repression. Appl. Microbiol. Biotechnol. 89: 335–343. doi.org/10.1007/s00253-013-5353-4

Ichinose, S., Tanaka, M., Shintani, T., Gomi, K., 2018. Increased production of biomass-degrading enzymes by double deletion of *creA* and *creB* genes involved in carbon catabolite repression in *Aspergillus oryzae*. J. Biosci. Bioeng. 125: 141–147. doi.org/10.1016/j.jbiosc.2017.08.019

Intasit, R., Cheirsilp, B., Suyotha, W., Boonsawang, P. 2022. Purification and characterization of a highly-stable fungal xylanase from *Aspergillus tubingensis* cultivated on palm wastes through combined solid-state and submerged fermentation. Prep. Biochem. Biotechnol. 52: 311–317. doi.org/10.1080/10826068.2021.1941105

Jørgensen, T.R., Goosen, T., van den Hondel, C.A., Ram, A.F., Iversen, J.J. 2009. Transcriptomic comparison of *Aspergillus niger* growing on two different sugars reveals coordinated regulation of the secretory pathway. BMC Genomics 10: 44. doi.org/10.1186/1471-2164-10-44

Katz, M.E., Bernardo, S.M., Cheetham, B.F. 2008. The interaction of induction, repression and starvation in the regulation of extracellular proteases in *Aspergillus nidulans*: Evidence for a role for CreA in the response to carbon starvation. Curr. Genet. 54: 47–55. doi.org/10.1007/s00294-008-0198-6

Krisnawati, R., Nur, M., Sardjono, S., Anggaraini, D., Widada, J. 2022a. RNA-seq data of *Aspergillus tubingensis* NBRC 31125 in carbon catabolite repressor related to xylanase production. Data Brief 45: 108700. doi.org/10.1016/j.dib.2022.108700

Krisnawati, R., Sardjono, Widada, J., Suroto, D.A., Cahyanto, M.N. 2022b. Effect of glucose on endo-xylanase and β-xylosidase production by fungi isolated in Indonesia. J. Pure Appl. Microbiol. 16: 226–234.

Kumar, D., Kumar, S.S., Kumar, J., Kumar, O., Mishra, S.V., Kumar, R., Malyan, S.K. 2017. Xylanases and their industrial applications: A review. Biochem. Cell. Arch. 17: 353–360.

Kundu, A., Ray, R.R. 2013. Production of intracellular β-xylosidase from the submerged fermentation of citrus wastes by *Penicillium janthinellum* MTCC 10889. 3 Biotech 3: 241–246. doi.org/10.1007/s13205-012-0091-3

Li, J., Lin, L., Li, H., Tian, C., Ma, Y. 2014. Transcriptional comparison of the filamentous fungus *Neurospora crassa* growing on three major monosaccharides D-glucose, D-xylose and L-arabionose. Biotechnol. Biofuels 7: 31. doi.org/10.1186/1754-6834-7-31

Ma, L., Chen, L., Zhang, L., Zou, G., Liu, R., Jiang, Y., Zhou, Z. 2016. RNA sequencing reveals Xyr1 as a transcription factor regulating gene expression beyond carbohydrate metabolism. Biomed Res. Int. 2016: 4841756. doi.org/10.1155/2016/4841756

Mach-Aigner, A.R., Pucher, M.E., Mach, R.L. 2010. D-xylose as a repressor or inducer of xylanase expression in *Hypocrea jecorina* (*Trichoderma reesei*). Appl. Environ. Microbiol. 76: 1770–1776. doi.org/10.1128/AEM.02746-09

Mandels, M., Reese, E.T. 1956. Induction of cellulase in *Trichoderma viride* as influenced by carbon sources and metals. J. Bacteriol. 73: 269–278. doi.org/10.1128/jb.73.2.269-278.1957

Mardawati, E., Werner, A., Bley, T., Mtap, K., Setiadi, T. 2014. The enzymatic hydrolysis of oil palm empty fruit bunches to xylose. J. Japan Inst. Energy 93: 973–978. doi.org/10.3775/jie.93.973

Metreveli, E., Khardziani, T., Elisashvili, V. 2021. The carbon source controls the secretion and yield of polysaccharide-hydrolyzing enzymes of basidiomycetes. Biomolecules 11: 1341. doi.org/10.3390/biom11091341

Miao, Y., Liu, D., Li, G., Li, P., Xu, Y., Shen, Q., Zhang, R. 2015. Genome-wide transcriptomic analysis of a superior biomass-degrading strain of *Aspergillus fumigatus* revealed active lignocellulose-degrading genes. BMC Genomics 16: 459. doi.org/10.1186/s12864-015-1658-2

Midorikawa, G.E.O., Correa, C.L., Noronha, E.F., et al. 2018. Analysis of the transcriptome in *Aspergillus tamarii* during enzymatic degradation of sugarcane bagasse. Front. Bioeng. Biotechnol. 6: 123. doi.org/10.3389/fbioe.2018.00123

Pinaga, F., Fernandez-Espinar, M.T., Vallas, S., Raman, D. 2006. Xylanase production in *Aspergillus nidulans* : induction and carbon catabolite repression. FEMS Microbiol. Lett. 115: 319–323. doi.org/10.1111/j.1574-6968.1994.tb06657.x

Polizeli, M.L.T.M., Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A., Amorim, D.S. 2005. Xylanases from fungi: Properties and industrial applications. Appl. Microbiol. Biotechnol. 67: 577–591. doi.org/10.1007/s00253-005-1904-7

Prathumpai, W., McIntyre, M., Nielsen, J. 2004. The effect of CreA in glucose and xylose catabolism in *Aspergillus nidulans*. Appl. Microbiol. Biotechnol. 63: 748–753. doi.org/10.1007/s00253-003-1409-1

Pullan, S.T., Daly, P., Delmas, S., et al. 2014. RNA-sequencing reveals the complexities of the transcriptional response to lignocellulosic biofuel substrates in *Aspergillus niger*. Fungal Biol. Biotechnol. 1: 3. doi.org/10.1186/s40694-014-0003-x

Raulo, R., Kokolski, M., Archer, D.B. 2016. The roles of the zinc finger transcription factors XlnR, ClrA and ClrB in the breakdown of lignocellulose by *Aspergillus niger*. AMB Express 6: 5. doi.org/10.1186/s13568-016-0177-0

Ribeiro, L.F.C., Chelius, C., Boppidi, K.R., et al. 2019. Comprehensive analysis of *Aspergillus nidulans* Pka phosphorylome identifies a novel mode of crea regulation. mBio. 10: 2825–2843. doi.org/10.1128/mbio.02825-18

Ries, L., Pullan, S.T., Delmas, S., Malla, S., Blythe, M.J., Archer, D.B. 2013. Genome-wide transcriptional response of *Trichoderma reesei* to lignocellulose using RNA sequencing and comparison with *Aspergillus niger*. BMC Genomics 14: 541. doi.org/10.1186/1471-2164-14-541

Ries, L.N.A., Beattie, S.R., Espeso, E.A., Cramer, R.A., Goldman, G.H. 2016. Diverse regulation of the CreA carbon catabolite repressor in *Aspergillus nidulans*. Genetics 203: 335–352. doi.org/10.1534/genetics.116.187872

Robinson, M.D., McCarthy, D.J., Smyth, G.K. 2009. edgeR: A bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139–140. doi.org/10.1093/bioinformatics/btp616

Rojas-Rejón, O.A., Poggi-Varaldo, H.M., Ramos-Valdivia, A.C., Martínez-Jiménez, A., Cristiani-Urbina, E., Martínez, M.d.l.T., Ponce-Noyola, T. 2011. Production of cellulases and xylanases under catabolic repression conditions from mutant PR-22 of *Cellulomonas flavigena*. J. Ind. Microbiol. Biotechnol. 38: 257–264. doi.org/10.1007/s10295-010-0821-7

Shimokawa, T., Shibuya, H., Ishihara, M., et al. 2012. Screening of lignocellulolytic enzyme producers: Enzyme system from *Aspergillus tubingensis* for hydrolysis of sugi pulp. Bulletin of FFPRI 11: 57–63.

Steindorff, A.S., Serra, L.A., Formighieri, E.F., de Faria, F.P., Pocas-Foncesa, M.J., de Almeida, J.R.M. 2021. Insights into the lignocellulose-degrading enzyme system of *Humicola grisea* var. *thermoidea* based on genome and transcriptome analysis. Am. Soc. Microbiol. 9: e01088-21. doi.org/10.1128/Spectrum.01088-21

Tamayo, E.N., Villanueva, A., Hasper, A.A., de Graaff, L.H., Ramón, D., Orejas, M. 2008. CreA mediates repression of the regulatory gene xlnR which controls the production of xylanolytic enzymes in *Aspergillus nidulans*. Fungal Genet. Biol. 45: 984–993. doi.org/10.1016/j.fgb.2008.03.002

Tanaka, M., Hiramoto, T., Tada, H., Shintani, T., Gomi, K. 2017. Improved α-amylase production by dephosphorylation mutation of CreD, an arrestin-like protein required for glucose-induced endocytosis of maltose permease and carbon catabolite derepression in *Aspergillus oryzae*. Appl. Environ. Microbiol. 83: e00592-17. doi.org/10.1128/AEM.00592-17

Tanaka, M., Ichinose, S., Shintani, T., Gomi, K. 2018. Nuclear export-dependent degradation of the carbon catabolite repressor CreA is regulated by a region located near the C-terminus in *Aspergillus oryzae*. Mol. Microbiol. 110: 176–190. doi.org/10.1111/mmi.14072

Uday, U.S.P., Choudhury, P., Bandyopadhyay, T.K., Bhunia, B. 2016. Classification, mode of action and production strategy of xylanase and its application for biofuel production from water hyacinth. Int. J. Biol. Macromol. 82: 1041–1054. doi.org/10.1016/j.ijbiomac.2015.10.086

Wang, B.T., Hu, S., Yu, X.Y., Jin, L., Zhu, Y.J., Jin, F.J. 2020. Studies of cellulose and starch utilization and the regulatory mechanisms of related enzymes in Fungi. Polymers 12: 530. doi.org/10.3390/polym12030530

Wang, Z., Gerstein, M., Snyder, M. 2009. RNA-Seq: A revolutionary tool for transcriptomics. Nat. Rev. Genet. 10: 57–63. doi.org/10.1038/nrg2484.RNA-Seq

Yoshioka, I., Takahashi, H., Kusuya, Y., Yaguchi, T., Kirimuraa, K. 2020. Draft genome sequence of *Aspergillus tubingensis* WU-2223L, a citric acid-producing filamentous fungus belonging to *Aspergillus* section Nigri. Microbiol. Resour. Announc. 9: e00702-20. doi.org/https://doi.org/ 10.1128/MRA.00702-20

Zhang, T., Liu, H., Lv, B., Li, C. 2020. Regulating strategies for producing carbohydrate active enzymes by filamentous fungal cell factories. Front. Bioeng. Biotechnol. 8: 691. doi.org/10.3389/fbioe.2020.00691