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Effect of glucose on endoglucanase and β-glucosidase production by some indigenous Indonesian fungi

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Abstract The production of polysaccharide-degrading enzymes in most fungi is affected by carbon catabolite repression. The effect of glucose on the production of endoglucanase and β-glucosidase was examined by some indigenous Indonesian fungi. After screening for cellulase production using the clear zone method in solid medium, 11 indigenous fungi isolated from various sources in Indonesia were grown in liquid medium containing carboxymethyl cellulose as a carbon source and supplemented with glucose at various concentrations (0, 1, 3, 5%) for 4 days. Endoglucanase and β-glucosidase activities in the growth medium were then examined. Strains PK1J2, MLT3J2, MLT4J1, and MLT5J1 produced more endoglucanase and β-glucosidase than the other strains in medium without glucose supplementation. *Trichoderma asperellum* PK1J2 was the highest enzyme producer, giving 0.79 U/mL endoglucanase and 1.49 U/mL β-glucosidase. Except for strains MLT2J2, MLT5J1 and G2J2, endoglucanase and β-glucosidase production by the other strains was partially repressed by 1% glucose. However, endoglucanase and β-glucosidase were hardly produced by all strains when the fungi were grown in medium supplemented with 3% glucose.

Keywords: Repression, Glucose, Endoglucanase, β-glucosidase, Indigenous fungi

Introduction

Cellulases are multi-enzymes consisting of endoglucanase, exoglucanase, and β-glucosidase that synergically degrade cellulose to glucose (Singh *et al.*, 2021). Cellulases are being widely used in food processing, biorefineries, and animal feeds (Juturu and Wu, 2014). In the food industry, cellulases assist in the extraction and clarification of fruit and vegetable juices, improve cloud stability in fruit juice, increase sugar yields in fruit syrup, and

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improve the dough properties of cookies and biscuits (Juturu and Wu, 2014). Glucose obtained from the hydrolysis of cellulose by cellulases is being used as a substrate to produce fermentation products such as ethanol, organic acids, and single-cell proteins (Yoon *et al.*, 2014).

Compared with bacteria and *Actinomycetes*, fungi are more widely being used in cellulose production because of their high productivity (Singhania *et al.*, 2016). Fungi such as *Humicola*, *Trichoderma*, *Penicillium*, and *Aspergillus* are considered highly suitable candidate cellulase producers (Imran *et al.*, 2016). Among the 23 fungal strains, *Aspergillus niger* LPB-334 produced the highest amounts of cellulases when Brewer's spent grain was used as a substrate (Liguori *et al.*, 2021). Screening of nine strains of *Aspergillus*, *Trichoderma*, *Penicillium*, and *Talaromyces* in liquid medium containing 1% Avicel as a sole carbon source showed that *Penicillium oxalicum* AG498 exhibited the highest FPase and β-glucosidase activities while *P. oxalicum* AG496 demonstrated the highest CMCase activity (Arnthong *et al.*, 2020). When 16 strains of *Aspergillus*, *Trichoderma*, *Penicillium*, and *Talaromyces* were grown in liquid medium containing microcrystalline cellulose as a substrate, *Trichoderma harzianum* LZ117 showed the highest cellulase activity (Li *et al.*, 2020). *T. harzianum* also produced the highest FPase and CMCase activities among the different *Trichoderma* strains isolated from various locations in India (Pandey *et al.*, 2015).

The production of polysaccharide-degrading enzymes maybe restricted by carbon catabolite repression. Glucose, as an end-product of cellulose or starch hydrolysis, may repress the production of enzymes. Carbon catabolite repression was observed during cellulase production by *Trichoderma asperellum* T-1. Cellulase production decreased as the glucose concentration increased from 0.5% to 2.0% (Wang *et al.*, 2015). Supplementation of Avicel-containing medium with 0.4% glucose and glycerol led to catabolite repression during cellulase and xylanase production by *Panus lecometei* and *Pseudotrametes gibbosa* (Kobakhidze *et al.*, 2016). Repression was also observed during amylase production by *Penicillium chrysogenum* and *Penicillium griseofulvum* when the medium was supplemented with glucose at concentrations above 10 mg/mL (Ertan *et al.*, 2014). However, some researchers revealed that high concentrations of glucose do not influence amylase production. Glucoamylase and α–amylase activities in *A.niger* 10 were higher in medium containing 60 and 120 g/L glucose than in medium containing only starch (Carrillo-Sancen *et al.*, 2016). Addition of 0.25% glucose increased amylase activity whereas addition of 0.5%–2.0% glucose decreased enzyme activity in *Aspergillus ochraceus* (Nahas and Waldemarin, 2002).

An understanding of glucose repression during enzyme production is essential in efforts to develop an enzyme-producing strain for commercial production. Because glucose exerts different effects on the production of polysaccharide-degrading enzymes, examining the effect of glucose on the production of enzymes is an important undertaking. The objective of this work was to examine the effect of glucose on the production of endoglucanase and β-glucosidase by some indigenous Indonesian fungi.

Materials and methods

Microorganisms

The 11 local isolates used in this study were obtained from the Laboratory of Biotechnology, Faculty of Agricultural Technology, and the Food and Nutrition Culture Collection of Gadjah Mada University. The isolates and their sources are shown in Table 1. The microorganisms were maintained on potato dextrose agar and stored at 4 ℃.

Table 1. Indigenous isolates used in this study

No	Fungi	Strain	Isolate Source
1	*Trichoderma asperellum*	PK1J2	Rotten palm empty fruit bunch, Pekanbaru, Riau
2	*Trichoderma asperellum*	MLT1J1	Rotten coconut coir, Maluku
3	*Trichoderma virens*	MLT2J2	Rotten coconut coir, Maluku
4	*Trichoderma asperellum*	MLT3J2	Rotten coconut petiole, Maluku
5	*Trichoderma virens*	MLT4J1	Rotten wood, Maluku
6	*Trichoderma asperellum*	MLT5J1	Soil in Wotay, Central Maluku
7	*Penicillium citrinum*	G2J2	Rotten palm empty fruit bunch, Garut, West Java
8	*Aspergillus aculeatus*	FIG1	Cacao, Gunung Kidul, Yogyakarta
9	*Aspergillus tamarii*	FNCC 6151	Koji, Bantul, Yogyakarta
10	*Aspergillus oryzae*	KKB4	Koji, Kebumen, Central Java
11	*Rhizopus oryzae*	GAP1	Fermented cassava, Sleman, Yogyakarta

Note: All strains except FNCC 6151 were provided by the Laboratory of Biotechnology, Faculty of Agricultural Technology, Gadjah Mada University. Strain FNCC 6151 was obtained from the Food and Nutrition Culture Collection, Gadjah Mada University.

Media

The medium used in this study contained 2.0 g/L KH_2PO_4, 1.4 g/L $(NH_4)_2SO_4$, 0.4 g/L $CaCl_2 \cdot 2H_2O$, 0.3 g/L $MgSO_4 \cdot 7H_2O$, 0.3 g/L urea, 1.0 g/L

peptone, 0.2 g/LTween 80, 20.0 mg/L $CoCl_2 \cdot 6H_2O$, 1.6 mg/L $MnSO_4 \cdot 7H_2O$, 5.0 mg/L $FeSO_4 \cdot 7H_2O$, 1.4 mg/L $ZnSO_4 \cdot 7H_2O$, and 10 g/L carboxymethyl cellulose. The solid medium contained 4 g/L sorbose, 0.1% Triton-X, and 17.5 g/L agar. The media were sterilized at 121 ℃ for 15 min (Chand *et al.*, 2005).

Identification of fungi

Identification of fungi was based on DNA sequence of ITS gene. In order to get that sequence, genome DNA of the strains was isolated using Quick-DNA™ Fecal/Soil Microbe Miniprep Kit (Zymo Reasearch, California). The ITS gene was amplified using universal primer set ITS1 (forward primer) 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4 (reverse primer) 5'-TCCTCCGCTTATTGATATGC-3'. The reaction mixture for PCR using PCR kit MyTaq™ Red Mix (Meridian Bioscience, United Kingdom). The temperature program was as follow one cycle of 3 min at 95 ℃ for initial denaturation, followed by 30 cycles of 15 sec at 95 ℃ for denaturation, 15 sec at 55 ℃ for annealing, and 30 sec at 72 ℃ for an extension, then 1 cycle of 4 min at 72 ℃ for final extension. The PCR product was sequenced by Bi-directional Sequencing, then the sequence was analyzed by BLAST and compared against the NCBI database. The phylogenetic tree was constructed using Neighbor-Joining (Unrooted Tree).

In addition, the morphology of the isolates was examined under microscope. Seven-day old culture grown on PDA plate was examined its sporangiophore, phialide, sporangium and metulae.

Clear zone assay

Each strain was inoculated on separate agar plates and incubated first at 28 ℃ for 3 d and then at 50 ℃ for 18 h to promote cellulose activity. The plates were subsequently flooded with 0.5% Congo red for 15 min and destained with 1 M NaCl solution (Qaisar *et al.*, 2014). The development of a clear zone surrounding the formed colonies indicates the production of cellulase by the studied strain.

Enzyme production in liquid medium

Detection of endoglucanase and β-glucosidase production was performed in liquid medium. The medium was supplemented with 0%, 1%, 3%, or 5% glucose as a carbon catabolite repressor. The strains were separately inoculated into the medium (10^6 spores/mL) and incubated aerobically at 30 ℃ for 4 d. The broth was then centrifuged at 12,000 rpm at 4 ℃ for 10 min. The

supernatant was analyzed for endoglucanase and β-glucosidase activity, and the pellet was used for dry weight determination.

Endoglucanase assay

Analysis of endoglucanase activity was conducted using the CellG5 method (Megazyme Ltd., Ireland) according to the manufacturer's instructions. The substrate used in the analysis was 4,6-O-(3-ketobutylidine)-4-nitrophenyl-β-D-cellopentaoside, a cellopentaoside which is blocked at the nonreducing end and added with a nitrophenyl moiety at the other end. Endoglucanase activity generates a non-blocked colorimetric oligosaccharide that is rapidly hydrolyzed by the β-glucosidase included in the assay kit. The 4-nitrophenol formed could be measured at 400 nm using spectrophotometer. One unit of endoglucanase was defined as the amount of enzyme required to release 1 µmol of 4-nitrophenol in 1 min.

β-Glucosidase assay

Analysis of β-glucosidaseactivity was conducted using *p*-nitrophenyl-β-D-glucopyranoside (Megazyme Ltd.) as a substrate. The β-Glucosidase activity in the substrate releases *p*-nitrophenol, which could be measured at 430 nm. One unit of β-glucosidase was defined as the amount of enzyme required to release 1 µmol of *p*-nitophenol in 1 min (Zhang *et al.*, 2009).

Quantification of fungal dry weight

Mycelial dry weights were obtained by harvesting the mycelium from 10 mL cultures using filter paper. After washing, the mycelium was dried at 105 ℃ for 24 h and then weighed (Barborakova *et al.*, 2012).

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 software. The results were expressed as mean ± standard deviation. One-way ANOVA was used for statistical analysis, and a posthoc test was conducted using Duncan's test. A p value of <0.01 was considered statistically highly significant.

Results

Identification of fungi

Molecular phylogenetic analysis of ITS genes of the strains was shown in Figure 1. The phylogenetic tree formed two main clades, one main clade consists of *Rhizopus oryzae* GAP1 and the other main clade supported by sub-clades. One sub-clade consists of strains from *Trichoderma* and the other sub-clade consists of *Penicillium* and *Aspergillus*. The sub-clade which consists of *Trichoderma* formed two groups, the one group consist of the strains from *Trichoderma asperellum* and the other group consist of *Trichoderma viride*. The strains of the same spesies were grouped in one cluster.

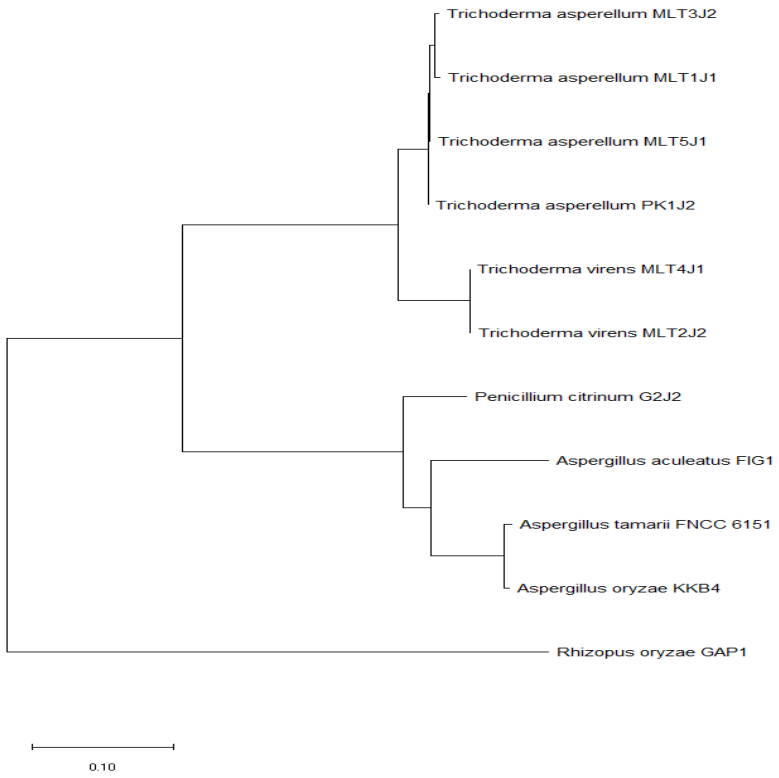

Figure 1. Neightbour-joining tree generated based on ITS sequence of indigenous fungi

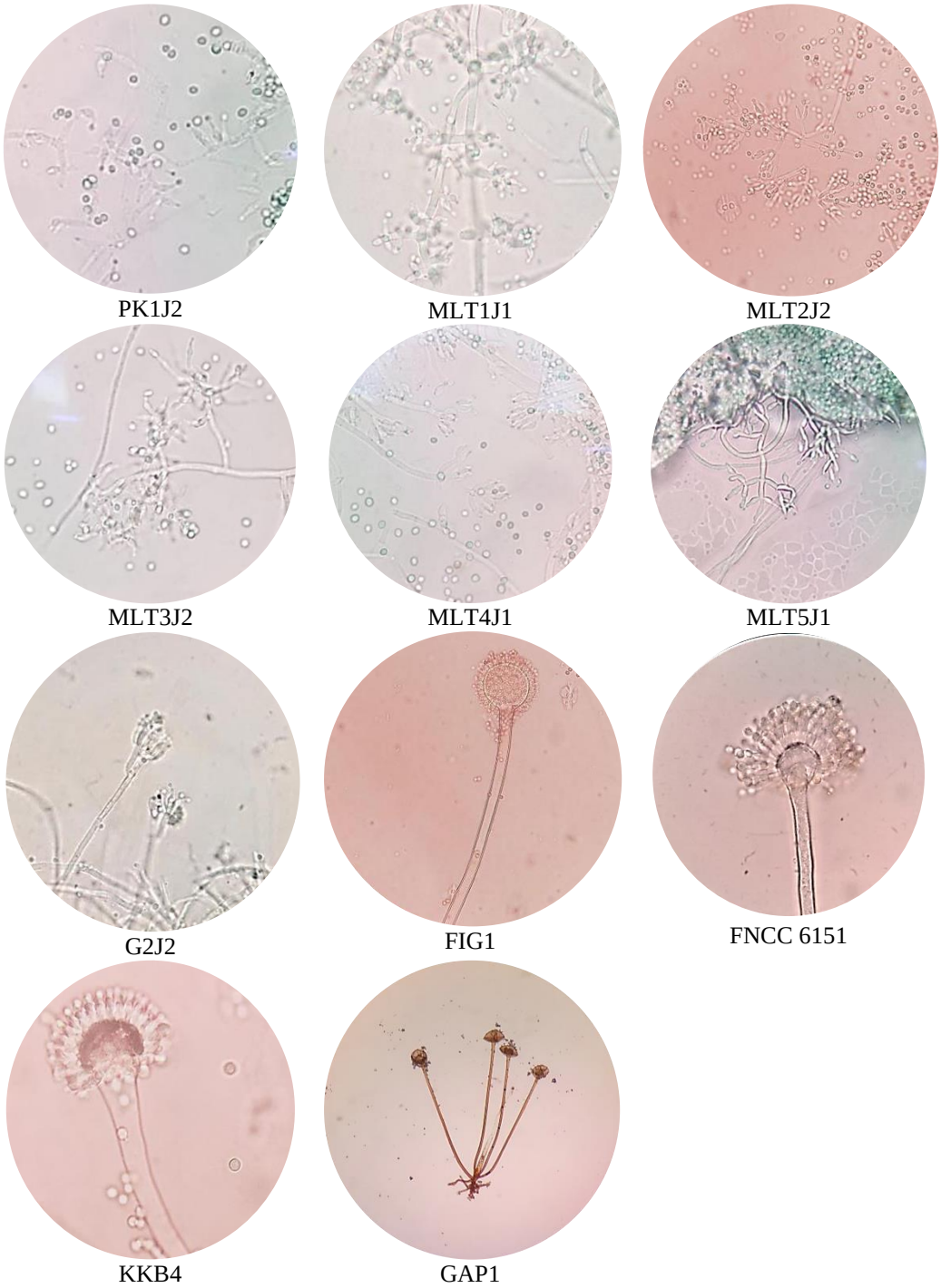

Figure 2. Fungal morphology of indigenous fungi examined

The fungal morphology of the strains is shown in Figure 2. Strain PK1J2, MLT1J1, MLT2J2, MLT3J2, MLT4J1 and MLT5J1 had long conidiophores with short branch. The phialide of these strains was short and the conidia form spherical to ellipsoidal. *Rhizopus oryzae* GAP1 has erect sporangiophores which borne in cluster of four from rizoids with unbranches stipe. The sporangia and columellae were spherical and the sporangiosphores has variable shape from ellipsoidal to broadly fusiform. *Penicillium citrinum* G2J2 produced hyaline hypha and biverticillate conidiophores with divergent structure of metulae and ampuliform phialide subterminally the stipe. The metulae longer than the phialide and the conidia was spherical. Strain KKB4, FIG1 and FNCC 6151 has erect and unbranch conidiophores bearing clavate conidial head composed of globose and catenulate conidia borne on uniseriate phialides develop on subglobose or globose vesicle.

Qualitative cellulase production

The clear zone experiment was performed in this work as an initial assessment of the ability of the selected strains to produce cellulases. The 11 strains showed clear zones surrounding their colonies. The cellulases produced by the strains were qualitatively estimated by the formation of a clear zone surrounding the colonies grown on solid medium. Carboxymethyl cellulose produces a red color when stained with Congo red; however, when this compound is hydrolyzed by cellulases produced by a fungal strain, a clear zone forms around the colonies. Figure 3 shows that all strains examined in this work produced cellulases. Strains PK1J2, G2J2, and F1G1 formed larger clear zones compared with the other strains.

Endoglucanase and β-glucosidase production in liquid medium

All strains examined produced different amounts of endoglucanase and β-glucosidase. Some strains produced considerably more of these enzymes than other strains. *T. asperellum* PK1J2, *T. virens* MLT4J1, *T. asperellum* MLT3J2, and *T. asperellum* MLT5J1, for example, produced 0.79, 0.39, 0.32, and 0.30 U/mL endoglucanase, respectively, whereas the other strains produced only 0.12 U/mL or less of this enzyme. Similarly, these four strains produced considerably more β-glucosidase than the other strains. Strains PK1J2, MLT4J1, MLT3J2, and MLT5J1 produced 1.49, 1.29, 1.28, and 1.22 U/mL β-glucosidase, respectively, while other strains produced not more than 0.32 U/mL β-glucosidase.

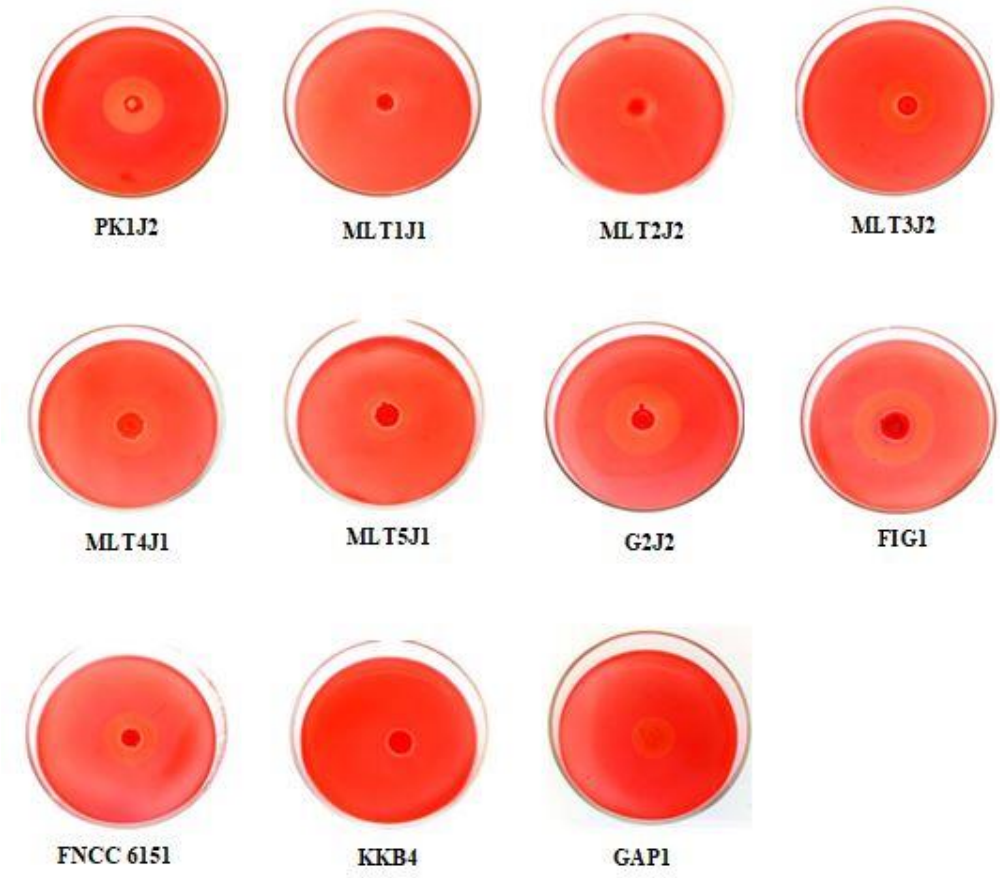

Figure 3. Clear zones formed around the fungal strains studied in this work

Table 2. Cellulase activities of the fungi studied in this work

No	Fungi	Endoglucanase	β-Glucosidase
1	*Trichoderma asperellum* PK1J2	0.79 ± 0.03^{e}	1.49 ± 0.01^{g}
2	*Trichoderma asperellum* MLT1J1	0.00 ± 0.00^{a}	0.01 ± 0.00^{a}
3	*Trichoderma virens* MLT2J2	0.11 ± 0.01^{b}	0.23 ± 0.00^{d}
4	*Trichoderma asperellum*MLT3J2	0.32 ± 0.01^{cd}	1.28 ± 0.03^{f}
5	*Trichoderma virens* MLT4J1	0.39 ± 0.03^{d}	1.29 ± 0.03^{f}
6	*Trichoderma asperellum* MLT5J1	0.30 ± 0.06^{c}	1.22 ± 0.03^{f}
7	*Penicillium citrinum* G2J2	0.12 ± 0.01^{b}	0.27 ± 0.02^{de}
8	*Aspergillus aculeatus* FIG1	0.01 ± 0.00^{a}	0.32 ± 0.02^{e}
9	*Aspergillus tamari* FNCC 6151	0.05 ± 0.00^{ab}	0.08 ± 0.01^{b}
10	*Aspergillus oryzae* KKB4	0.00 ± 0.00^{a}	0.16 ± 0.01^{c}
11	*Rhizopus oryzae* GAP1	0.05 ± 0.01^{ab}	0.34 ± 0.04^{e}

Values indicated represent the mean ± standard deviation of two replicates. Different letters in the plot indicate significant differences at $\alpha \leq 0.01$

Effect of glucose on endoglucanase and β-glucosidase production

Addition of 1% glucose to the liquid medium decreased endoglucanase and β-glucosidase production in all strains examined except MLT2J2, MLT5J1, and G2J2 (Figures 4 and 5). While addition of 1% glucose to the medium decreased the production of endoglucanase and β-glucosidase in most strains to some extent, addition of 3% glucose decreased endoglucanase and β-glucosidase production in all strains examined to nearly negligible levels.

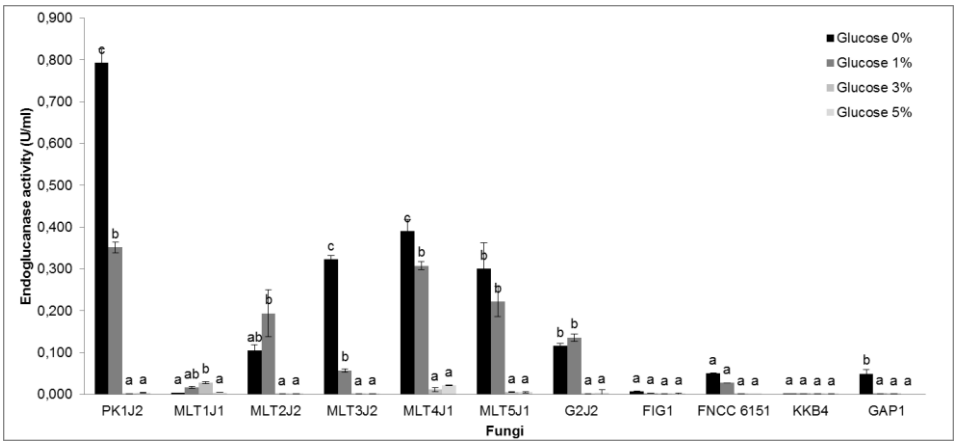

Figure 4. Endoglucanase activity of the fungi grown on carboxymethyl cellulose medium supplemented with 0%, 1%, 3%, or 5% glucose. Different letters in the plot indicate significant differences at $\alpha \leq 0.01$

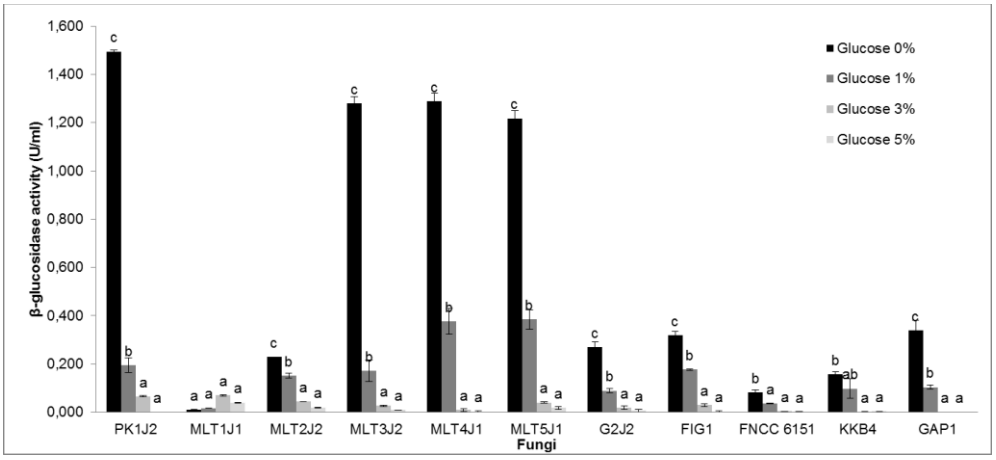

Figure 5. β-Glucosidase activity of the fungi grown on carboxymethyl cellulose medium supplemented with 0%, 1%, 3%, or 5% glucose. Different letters in the plot indicate significant differences at $\alpha \leq 0.01$

Production of fungal biomass

Addition of glucose to the growth medium increased the fungal biomass of all strains. The greater the amount of glucose added to the medium, the larger the biomass formed during fungal growth. The biomass produced by all stains after 4 d ranged from 1.0 g/L to 2.0 g/L in medium without glucose supplementation and from 4.0 g/L to 16.0 g/L in medium supplemented with 5% glucose.

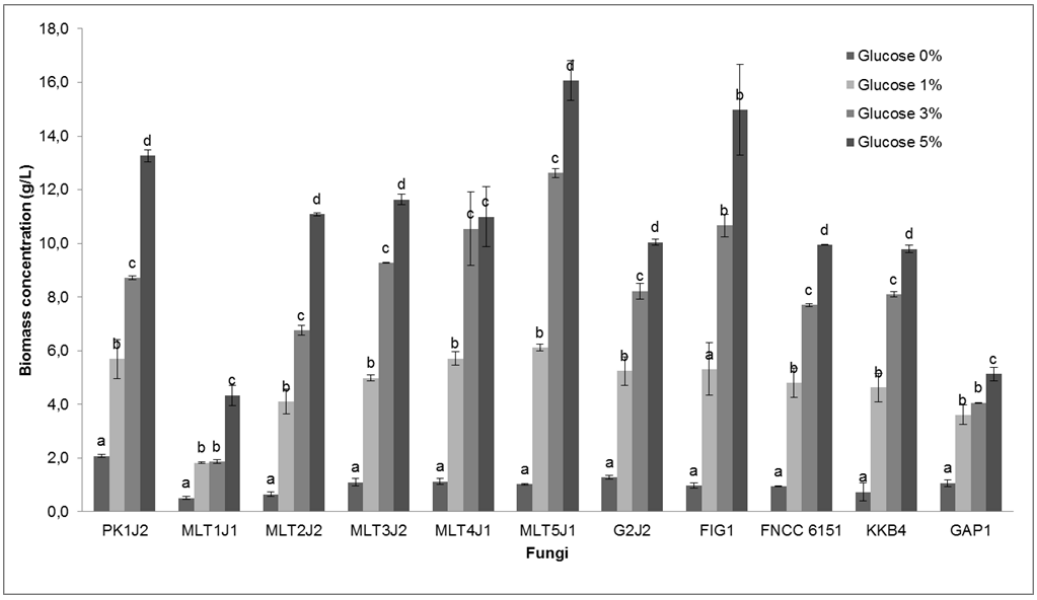

Figure 6. Fungal biomass in carboxymethyl cellulose medium supplemented with 0%, 1%, 3%, or 5% glucose. Different letters in the plot indicatesignificant differences at $\alpha \leq 0.01$

Discussion

Phylogenetic tree showed that *Rhizopus oryzae* GAP1 has a different clade with the other strains, it is due to *Rhizopus* included in Mycoromycota division, whereas the other strains examined in this work included in Ascomycota division. The strains from *Trichoderma* grouped in one clade, whereas *Penicillium citrinum* G2J2 grouped with the strains from *Aspergillus*. *Penicillium* and *Aspergillus* are closely related to compare with *Trichoderma* due to *Penicillium* and *Aspergillus* included in Eurotiales order, whereas *Trichoderma* included in Hypocreales order (Adnan *et al.*, 2019; Borman, 2018). The strains in the same spesies were grouped into one clade. The strains

in the same species which was isolated in the same island has closer relation compared with the strain which was isolated from different island. *Trichoderma asperellum* MLT3J2 is closely related with *T. asperellum* MLT1J1 and MLT5J1 compared with strain PK1J2. Fungal morphology was analysed in this study to confirm the result of molecular identification of the fungi. Based on the characteristic of the fungal morphology, it confirmed that strain PK1J2, MLT1J1, MLT2J2, MLT3J2, MLT4J1 and MLT5J1 included in *Trichoderma*, strain G2J2 was *Penicillium*, strain FNCC 6151, KKB4 and FIG1 included in *Aspergillus*, and strain ROG1 was *Rhizopus* (Pitt and Hocking, 2009; Watanabe, 2002).

The ability of fungal strains to produce cellulases in this work was assessed in solid and liquid media. The size of the clear zones produced by the 11 strains in this study may represent the ability to produce cellulases, although other factors, such as plate thickness and agar medium density, could also affect the size of the clear zone. The clear zone surrounding the colonies was the result of the activity of a single or mixture of cellulases produced by the strain; these cellulases may include endoglucanase, exoglucanase, or β-glucosidase. The production of endoglucanase and β-glucosidase in liquid medium in this work was not necessarily in agreement with the corresponding resulted in solid medium. For example, strain PK1J2, which showed a large clear zone in solid medium, revealed high endoglucanase and β-glucosidase activities in liquid medium. However, strains G2J2 and FIG1 gave large clear zones in solid medium but produced low endoglucanase and β-glucosidase activities in liquid medium. Similarly, strains MLT3J2, MLT4J1, and MLT5J1 showed small clear zones in solid medium but produced high endoglucanase and β-glucosidase activities in liquid medium. Some reports revealed that the size of the clear zone in solid medium did not reflect the amount of cellulase produced in liquid medium. Jha *et al.* (2017) and Liguori *et al.* (2021) showed that some strains producing small clear zones in solid medium gave high cellulase activity in liquid medium whereas other strains giving large clear zones in solid medium exhibited low cellulase activity in liquid medium.

The level of endoglucanase and β-glucosidase production by the fungal strains examined in this study did not differ considerably when compared with those indicated in previous reports. In one report, for instance, *Aspergillus terreus* VBIV, *A. niger* VBVI, *Penicillium* sp. VBVIII, and *A. flavus* VBIX produced 0.05–0.25 U/mL CMCase in liquid medium over 4 days of fermentation (Jha *et al.*, 2017). In another report, the CMCase activity of 12 strains of *Lentinus edodes* and *A. niger* ranged from 0.00 U/mLto 0.17 U/mL after 4 days of fermentation (Liguori *et al.*, 2021). While the CMCase in these reports generally referred to endoglucanase, this enzyme actually includes not

only endoglucanase but also exoglucanase and β-glucosidase to some extent. The endoglucanase determined in the present work was very specific because the substrate used in the enzyme assay was specific for endoglucanase. Seven *Trichoderma* spp. and three *Aspergillus* spp. produced β-glucosidase activities ranging from 0.14 U/mL to 1.75 U/mL (Shahriarinour *et al.*, 2011). *T. harzianum* LZ117 produced 2.91 U/mL β-glucosidase, thereby demonstrating higher β-glucosidase production compared with 11 other strains of *Trichoderma*, fourstrains of *Aspergillus*, sixstrains of *Penicillium*, and two strains of *Talaromyces*; the rest of the fungi produced β-glucosidase activities ranging from 0.00 U/mL to 0.35 U/mL (Li *et al.*, 2020).

Endoglucanase production by all strains examined was repressed by 3% glucose; specifically, endoglucanase production decreased by over 97%. However, endoglucanase production did not considerably decrease when the strains were grown in medium containing 1% glucose. The endoglucanase activity of *T. asperellum* PK1J2 in the medium supplemented with 1% glucose decreased from 0.79 U/mL to 0.35 U/mL; this activity decreased even further to negligible levels when the strain was grown in medium containing 3% glucose. Repression of endoglucanase production by glucose has been reported in previous studies. CMCase production by *T. citrinoviride* and *T. reesei* ATCC 66589 was completely repressed by 0.27% and 2.4% glucose, respectively (Chandra *et al.*, 2009; Ike *et al.*, 2010). CMCase production by *T. asperellum* T-1 was also repressed by over 87% in the presence of 1.5% glucose (Wang *et al.*, 2015). Repression of CMCase production by *Pseudotrametes gibosa* and *Panus lecometei* in the presence of 0.4% glucose has been reported (Kobakhidze *et al.*, 2016).

Production of β-glucosidase by all strains examined was repressed by glucose concentrations at as low as 1%. The β-Glucosidase production by strains PK1J2, MLT3J2, MLT4J1, and MLT5J1, which were among the strongest producers of this enzyme, was repressed by over 68% in the presence of 1% glucose. The level of repression observed increased to over 95% in the presence of 3% glucose, and the strains produced negligible amounts of β-glucosidase when the growth medium was supplemented with 5% glucose. The β-Glucosidase production by *T.citrinoviride* was completely repressed by 0.27% glucose (Chandra *et al.*, 2009). The repression of cellulose production by glucose may be due to a regulatory protein that prevents the transcription of the cellulase gene. The regulatory protein is named Cre1 in *Trichoderma* but called CreA in *Aspergillus* (Amore *et al.*, 2013; Silva-Rocha *et al.*, 2014; Sukumaran *et al.*, 2021). Cre1/CreA is initially present in the cytoplasm as a phosphorylated protein. When high concentrations of glucose are present in the cytoplasm, Cre1/CreA is dephosphorylated and, thus, able to enter the nucleus.

The protein then binds to the promoter of the cellulase gene, which, in turn, prevents the binding of mRNA polymerase to the gene promoter (Adnan *et al.*, 2018; Godbey, 2014).

Strains PK1J2, MLT3J2, MLT4J1, and MLT5J1 produced relatively high amounts of endoglucanase and β-glucosidase and may potentially be used as endoglucanase and β-glucosidase producers. However, carbon catabolite repression should be eliminated in these strains prior to their use in commercial application.

The biomass produced during the growth of all strains examined increased as the initial glucose concentration in the medium increased regardless of whether glucose repression occurred during endoglucanase and β-glucosidase production. Fungal growth results from glucose metabolism by the strain. When endoglucanase and β-glucosidase production was repressed by glucose, the cells grew on the existing (initial) glucose in the medium. The amount of biomass produced may depend on the total amount of carbon source present in the medium. Medium supplemented with glucose provides a larger carbon source compared with medium without glucose supplementation. After the initial glucose in the medium was consumed, production of endoglucanase and β-glucosidase began and carboxymethylcellulose was hydrolyzed to glucose. Previous studies reported that the fungal biomass increases when the medium is supplemented with various amounts of glucose. The fungal growth of *T. asperellum* T-1 and *Aspergillus ocracheus* increased as the glucose concentration increased (Nahas and Waldemarin, 2002; Wang *et al.*, 2015).

Strains PK1J2, MLT4J1, MLT3J2, and MLT5J1 produced more endoglucanase and β-glucosidase than the other strains. *T. asperellum* PK1J2 was the highest producer of endoglucanase and β-glucosidase, giving 0.79 U/mL endoglucanase and 1.49 U/mL β-glucosidase. Except for strains MLT2J2, MLT5J1, and G2J2, endoglucanase and β-glucosidase production by all other strains was partially repressed by 1% glucose. Moreover, all strains are hardly produced endoglucanase and β-glucosidase when grown in medium supplemented with 3% glucose.

Acknowledgments

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