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A Novel Exo-Glucanase Explored from a *Meyerozyma* sp. Fungal Strain

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Abstract

Isolating cellulase-secreting microbes followed-by screening their cellulolytic activities has been an essential approach to discover novel and potential cellulases for cellulolytic industrial applications. This study was aimed to explore competitive exoglucanases by screening avicelase activities for 92 fungal strains isolated from environmental airborne-fungal-spore samples. Results showed that an isolated fungal strain numbered 58 exhibited the best avicelase activity of 0.209 U/mL when cultured for six days at pH 5.0 - 5.3 and 25˚C - 27˚C, and was lately identified as a yeast strain of *Meyerozyma* sp. (96% ITS fragment similar with *Meyerozyma caribbica*, HG970748). Based on amino acid sequences revealed from LC/MS/MS, the target exoglucanase was identical to 1,4-beta-D-glucan cellobiohydrolases and was named *Mc*-CBHI which had optimal avicelase reaction conditions of pH 5 and 70˚C and could remain fairly stable after 4-hr incubation at acid conditions (pH 3 - 5) or wide temperature ranges (30˚C - 80˚C). Additionally, the *Mc*-CBHI (~70 kDa and ~3.6% of crude enzyme) had specific FPase and avicelase activities of 0.179 U/mg and 0.126 U/mg, respectively (which were about 40% - 50% activities of a commercial cellulase Accellerase-1000). These results demonstrated that the newly-found *Mc*-CBHI could become one of potential exoglucanase resources for related cellulolytic industrial applications.

Keywords

Airborne-Fungal-Spore, Exoglucanase, 1,4-Beta-D-Glucan Cellobiohydrolases, CBHI, *Meyerozyma* sp.

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1. Introduction

Cellulose of ligno-biomass is one of the most abundant natural biopolymers for many industrial products (e.g., paper, rayon, filter membrane etc.) [1], and has been considered as a major raw material for renewable biofuel production as well [2] [3]. Enzymatic hydrolysis of polymeric cellulose to monomeric sugar is a crucial step prior to sugar fermentation for renewable bioethanol production owing to slow reaction rate for complicate hydrolysis procedure coordinately-conducted by three types of cellulases, *i.e.*, exo-glucanase (CBH) (EC 3.2.1.91 and 0.74), endo-glucanase (EG) (EC 3.2.1.4), and beta-glucosidase (BGL) (EC 3.2.1.21) [4]. In order to enhance bioethanol production rate and reduce overall manufacture cost, scientific and engineering communities have continually explored novel or modified cellulases with better hydrolyzing activity and cheaper production cost although some promising cellulases are already commercially available up to now.

Some of recent strategies for improving cost, quality, and/or quantity of cellulases have been focused on increasing enzyme stability and/or functionality (by optimizing hydrolyzing conditions or modifying enzyme component and/or structure) or expediting enzyme production titer (by optimizing fermentation conditions, mutating cellulase-secreting microbial strains, or manipulating hyper-expressing system) [5] [6]. Besides, isolation of cellulase-secreting microbial strains followed-by screening their cellulases is still an essential approach to discover novel and potential cellulases [7]-[9]. Several novel and potential cellulases have been continually discovered from some fungal strains (e.g., *Schizochytrium aggregatum* and *Chaetomella raphigera*) [10]-[12] other than the most common cellulase producers such as *Aspergillus* sp., *Penicillium* sp., and *Trichoderma* sp. suggesting some highly-active cellulases might stay undiscovered in diverse fungal consortium.

Fungi are ubiquitous in diverse natural environments or even in residential home. Because of their unique feature to utilize polymeric cellulose in ligno-material as carbon and energy sources for their growth, most of potential cellulases have been explored by screening cellulosic activity for crude enzymes produced from fungal strains gathered and/or isolated from diverse lignocellulose-rich samples such as soil, compost, saw dust, straw dust, decaying wood, intestinal of insects, or feces of ruminants [7] [13]-[16]. Most of fungal strains isolated from such samples were mainly belonged to the common cellulase-producing fungi of *Ascomycota* phylum such as *Aspergillus* sp., *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp., or *Trichoderma* sp. [7] [16]. Additionally, because most of fungi reproduce by forming spores, large amount of airborne fungal spores produced by microfungi grown on wood or plant debris could present in outdoor air throughout the year and most of them could be allergenic [17]; whereas, some of them may germinate potential cellulase-secreting fungal strains as well. Nevertheless, fungal spores in air still have seldom been screened for cellulosic activities.

Besides, it has been suggested that hydrolyses activity for mixtures of different types of cellulases was higher than the sum of activities for each component acting alone, indicating the components were acting on cellulose synergistically [18] [19]. For synergistic formulation of cellulases conducted in Taiwan, availability of indigenous exo-glucanase (or cellobiohydrolases, CBH) has been relatively less than endo-glucanase (EG) and beta-glucosidase (BGL). Searching novel and potential CBH for local or even global applications has been essential for bioethanol industrial. The research goal of this study was to screen and identify novel/potential exo-glucanase secreted by fungal strains isolated from environmental aerosol samples. Optimal reacting conditions and specific activities of newly-found exo-glucanase were also determined to evaluate its compatibility.

2. Materials and Methods

2.1. Sample Collection and Fungal Strain Isolation

Fungal spores were collected from several locations of central Taiwan area during July-December of 2011 by pumping air into 0.005% Triton-X100 buffer (at a flow rate of 300 L/min for 10 minutes) with a Coriolis® μ Air Sampler (Bertin Technologies, France). Serially diluted air samples were spread on potato dextrose agar plate (PDA, Difco, USA) and incubated at 25˚C for fungal strain germination and isolation. Isolated fungal strains (numbered from #1 to #92 in this study) were stored at −20˚C for follow-up screening for potential cellulases.

Each of the isolated fungal strains or a benchmark strain of *Trichoderma reesei* from culture stocks were individually recovered by culturing on PDA plates at pH ~ 5.6 and room temperature ~ 27˚C; and then, a ~5 mm^3 7-day-old mycelia block of each strain was re-cultured at pH ~ 5.3 and ~27˚C on modified MR plate (Mandel-Reese medium without peptone, MR-P) with 2% α-cellulose (each liter had 1.4 g $(NH_4)_2SO_4$, 0.8 mL of 6 M urea, 2 g KH_2PO_4, 0.34 g $CaCl_2$, 0.3 g $MgSO_4{\cdot}7H_2O$, 0.00005 g $FeSO_4{\cdot}7H_2O$, 0.000016 g $MnSO_4{\cdot}7H_2O$, 0.000014 g $ZnSO_4{\cdot}7H_2O$, and 0.00002 g $CoCl_2{\cdot}6H_2O$, and 20 g agar). Consequently, 6 of ~5 mm^3 7-day-old

mycelia blocks from the MR-P plate were transferred into 100-mL MR-P liquid medium (duplicate n = 2 for environmental isolates and n = 6 for *T. reesei*) and cultured for another 7 days by shaking at 150-rpm to harvest crude enzyme (secretome) from each of isolated fungal strains and the benchmark strain *T. reesei* for following cellulolytic activity tests and enzyme characteristics assessments.

2.2. Screening Potential Cellulases Based on Activity Tests

The crude enzymes (or secretomes) produced from fungal strains were tested for cellulolytic activities using pNPG (5mM), CMC (1%, w/v), and avicel (1%, w/v) as substrates in order to screen potential cellulases. Additionally, FPase activity was extra conducted for potential exoglucanase using a piece of 3 mg filter paper as substrate (6mm-diameter circle shape cut by paper puncher) in order to compare with other previous studies. The enzymatic *p*NPGase activity was determined based on amount of *p*-nitrophenol (*p*NP) released from 5 mM *p*NPG after 5-min reaction at 55˚C and pH 5 by measuring absorbance at a wavelength of 405 nm in a microtiter plate reader (SpectraMax M5e, Molecular Devices, USA). Additionally, the CMCase, avicelase, and FPase activities were assessed by measuring reducing sugar concentration at a wavelength of 550 nm using the di-nitrosalicylic acid (DNS) method after reacting at 55˚C and pH 5 for 10 minutes (for CMCase) or 60 minutes (for avicelase and FPase) in 50 mM sodium acetate buffer. All the cellulolytic activities were represented as U/mL (μmol of product, *i.e.*, *p*NP or reducing sugar, produced within 1 minute per mL of enzyme).

2.3. Optimal Cultivation Conditions

Optimal cultivation conditions for the potential fungal strains (*i.e.*, strains #58, #60, and #71) (original sample names: 13-5-2-2, 13-6-2A, and THU4) were investigated by culturing the fungal strains at two temperature levels of 25˚C or 30˚C (using thermal-control water bath) and three pH values of 5, 6, or 7; and, followed by avicelase activity assessments for the fungal secretomes at six temperature levels (*i.e.*, 30˚C, 40˚C, 50˚C, 60˚C, 70˚C, and 80˚C) and eight pH values (*i.e.*, pH 2, 3, 4, 5, 6, 7, 8, and 9). The avicelase activities were represented as relative activity (%) by comparing each activity with the highest activity measured throughout the assessments and used to determine the optimal cultivation conditions and the optimal avicelase reaction conditions.

In addition, pH stability and thermostability for the most potential crude enzyme were also resolved by incubating the fungal secretome at eight different pH levels (pH 2, 3, 4, 5, 6, 7, 8, and 9) or six temperature levels (30˚C, 40˚C, 50˚C, 60˚C, 70˚C, and 80˚C) for four hours and followed by avicelase activity test under optimal reaction conditions. All measured avicelase activities were also converted to relative activity (%) by deviding each activity with the highest activity measured throughout the assessments for assessing enzyme stability.

2.4. Identification of Potential Cellulase-Secreting Fungal Strain

Genomic DNA of the potential fugal strain #58 was purified from 6-day cultured fungal mycelia using a commercial extraction kit (Genomic DNA Extraction Kit, Fast ID, Genetic ID NA, IA, USA). Identification of the fungal strain #58 was conducted by comparing gene sequences of internal transcribed spacer (ITS) region that was PCR-amplified using ITS1 forward primer (TCC GTA GGT GAA CCT GCG G) and ITS4 reverse primer (TCC TCC GCT TAT TGA TAT GC). Each 25-μl PCR reaction contained 12.5 μl of Master Mix (EmeraldAmp® MAX HS PCR Master Mix, TaKaRa, Shiga, JP), 1 μl of each 10 μM forward and reverser primers (final concentrations were 400 nM), 8.5 μl of PCR-grade water, and 2 μl of the fungal DNA extract. A PCR reaction were run in a thermal cycler (MyCycler™, Bio-Rad, Hercules, CS, USA) based on the following program: 5 min at 94˚C; followed by 35 cycles at 94˚C for 1 min, 50˚C for 1 min, 72˚C for 1 min; and finally 10 min at 72˚C.

PCR amplicon was purified from agarose gel and sent for sequencing at MissionBio Co (Taipei, TW). Sequencing result was compared with NCBI database to determine fugal genus and species. The ITS fragments of the fungal strain #58, the first matched fungal species, and some closely related fungal strains were aligned by ClustalX method [20] and phylogenetic trees were constructed by neighbor-joining method and displayed with TreeView program [21].

2.5. PAGE for Target Exo-Glucanase Identification and Quantification

Crude enzyme concentration was determined based on Bio-Rad Protein Assay using BSA (bovine serum albu-

min) as calibration standards (Bio-Rad, USA). For better visualization on PAGE (polyacrylamide gel electrophoresis), the crude enzyme was further concentrated by lyophilisation for 4 hours and followed by dialyses for 3 hours to eliminate salts and metals in the MR culture medium using 3500 MWCO SnakeSkin™ Dialysis Tubing (Thermo Fisher Scientific, Life Technologies, Waltham, MA, USA).

Native PAGE and SDS (sodium dodecylsulfate) PAGE were performed based on the protocols suggested by Hoefer's Protein Electrophoresis Applications Guide [22]. Briefly, the concentrated crude enzyme was directly loaded in a 10% native-PAGE or first denatured by heating for 3 minutes at 100˚C in a 2× protein treatment buffer (containing 0.125 M Tris-HCl, 4% SDS, 20% glycerol, 0.2 M DTT, and 0.02% bromophenol blue) and then loaded in a 10% SDS-PAGE gels to conduct electrophoresis (90 V for 30 minutes and then 140 V for 90 minutes). Followed by electrophoresis, the gel was stained with Coomassie blue R250 to view the native-PAGE or SDS-PAGE images. For zymographic assessment, the native-PAGE gel was further merged in a MUC solution (pH 5) and incubated at 70˚C and 20 rpm for 30 minutes and subsequently observed under UV light. Exoglucanase activity was visible as clear light bands against a black background.

The bands of target exoglucanase observed on the SDS-PAGE were cut and purified from gel and analyzed for amino acid sequences at a Proteomics Core Laboratory (located in the Agricultural Biotechnology Center of Academia Sinica, Taipei, Taiwan) using Applied Biosystems Procise® Protein Sequencer model 494 (Applied Biosystems, USA). The sequenced amino acid fragments were compared with sequences from the NCBI protein database using a program of Protein BLAST.

Additionally, proportion of the target exoglucanase within the crude enzyme produced from the fungal strain #58 was estimated by using another 10% SDS-PAGE with different amounts of BSA as standards (*i.e.*, 0.5, 1, 2, 3, 5, and 10 μg BSA). A calibration curve was constructed based on correlations between protein concentrations and image intensities of protein on the PAGE; and, was used to calculate the quantity of the target exoglucanase. Specific FPase and avicelase activities were determined afterward according to the exoglucanase concentration evaluated from the above-mentioned approach.

3. Results and Discussion

3.1. Fungal Strains Culturing and Cellulosic Activities Screening

During 7-day cultivation for the fungal strains isolated from environments together with the benchmark strain *T. reesei*, avicelase activities of the fungal-secreted crude enzymes (for indicating exo-glucanase titers) increased gradually and reached highest levels mostly around 6 days (**Figure 1**). CMCase and pNPGase activities also reached highest levels after 5 - 7 days cultivation (time course activity profile data not showed). Among the 92

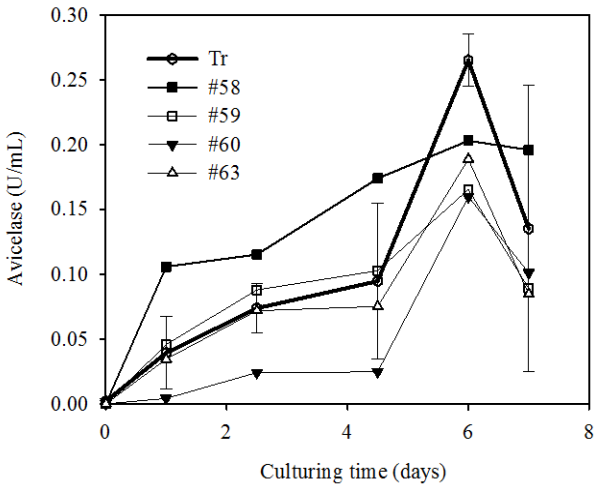

Figure 1. Time-course profiles of avicelase activities for *T. reesei* (n = 6) and fungal strains #58, #59, #60, and #63 (n = 2) during 7-day cultivation at pH ~ 5.3 and room temperature ~ 27˚C.

environmental isolates, most of them had highest avicelase activities less than 0.1 U/mL while only some fungal strain such as #58, #59, #60, and #63 showed more significant avicelase activities (>0.1 U/mL) as illustrated in **Figure 1**. The avicelase activities for fungal strain #58 increased more significantly (up to about 0.106 U/mL) than other fungal strains only after about 1 day cultivation and reached a highest level of 0.209 U/mL at 6th-day making it one of the top candidates for finding novel potential exoglucanase.

The highest avicelase, CMCase, and pNPGase activities for each fungal strain during 7-day cultivation were selected for further comparisons to screen potential cellulases (**Figure 2**). Most of the fungal strains had notable CMCase (endo-glucanase) activities and few of them even had higher CMCase activity than that for *T. reesei* (**Figure 2(b)**). Besides, few fungal strains had pNPGase (beta-glucosidase) activities (**Figure 2(c)**). In addition, six out of 92 fungal strains had avicelase activities higher than 0.1 U/mL (*i.e.*, strains # 26, #58, #59, #60, #63, and #71) as *T. reesei* had an avicelase activity of 0.27 ± 0.02 U/mL (**Figure 2(a)**). Beside the results from tube activity assay mentioned above, MUC zymogram was also used to preliminarily screen exoglucanase activity for these six strains. MUC zymogram results showed only strains #58, #60, and #71 had significant MUCase activities (images showed in **Figure 2(a)**). Therefore, the fungal strain #58, #60, and #71 were selected for further assessing their optimal culturing conditions and optimal avicelase reaction conditions.

3.2. Optimal Culturing Conditions, Enzyme Reaction Conditions, and Enzyme Stability

Fungal strain #58 grew at a pH of 5 and a temperature of 25˚C exhibited better avicelase activity than other culturing conditions (**Figure 3**). Higher pH values at 6 or 7 and higher temperature at 30˚C resulted in less avicelase activity. In addition, optimal enzyme reaction conditions for secretome of the fungal strain #58 (produced at pH 5 and 25˚C) were in a pH range of 4 - 6 and a temperature range of 60˚C - 80˚C (**Figure 3(a)**). Furthermore,

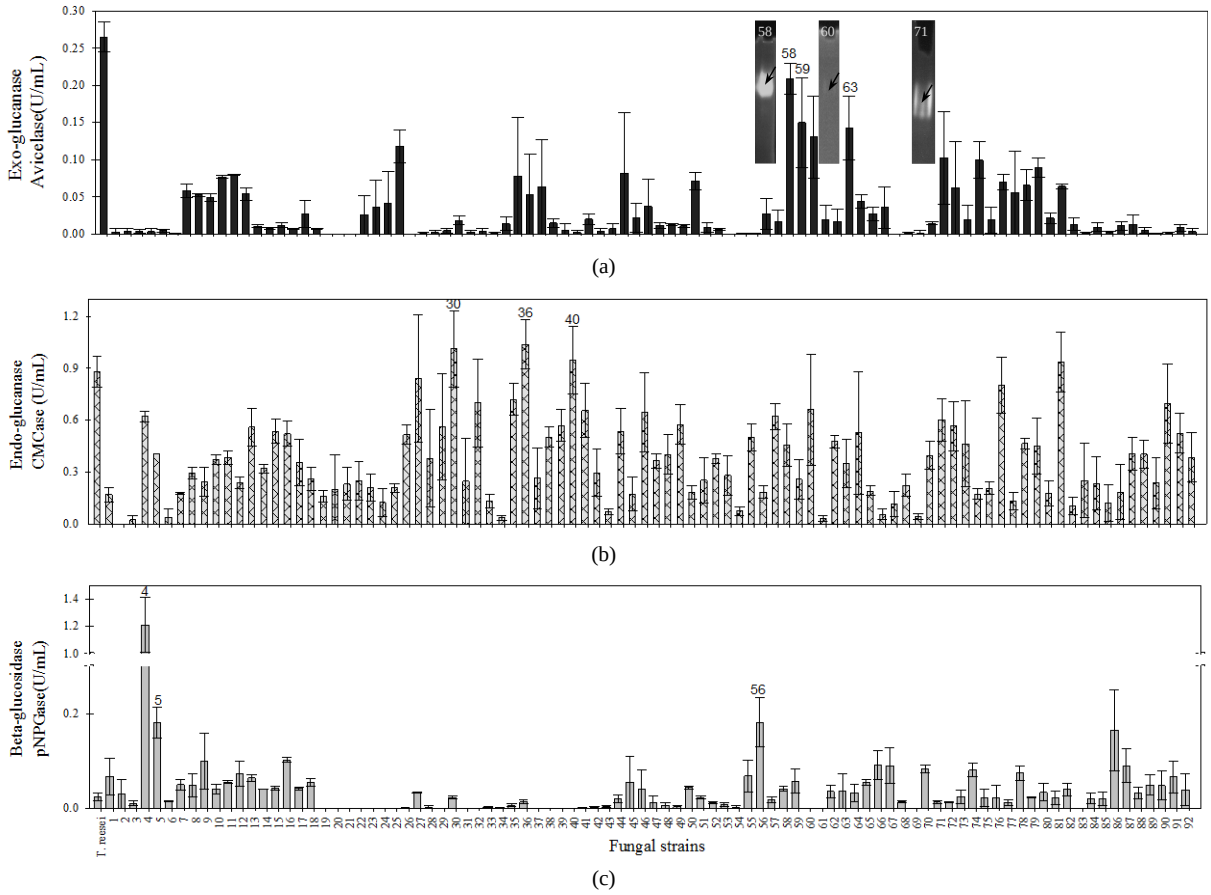

Figure 2. Avicelase (a); CMCase (b); and pNPGase (c) activities for *T. reesei* and 92 fungal strains from environments. Top three strain numbers were indicated.

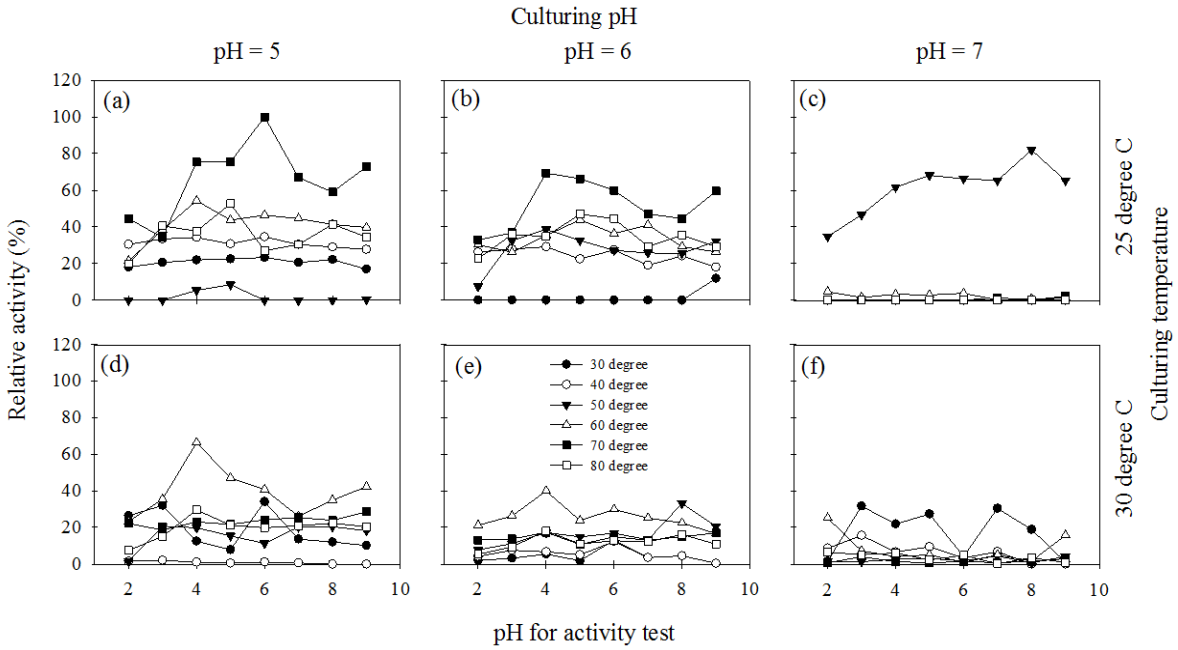

Figure 3. Optimal growth conditions of fungal strains #58 were determined based on assessments of relative activity (%) of crude enzymes produced at three pH levels of 5 (a) (d); 6 (b) (e); or 7 (c) (f) and two incubation temperatures of 25 (a) (b) (c) or 30˚C (d) (e) (f). Avicelase activity tests were conducted under 8 different pH levels (*i.e.*, 2, 3, 4, 5, 6, 7, 8, or 9) and 6 different reaction temperatures (*i.e.*, 30˚C, 40˚C, 50˚C, 60˚C, 70˚C, or 80˚C).

the exo-glucanase activity for the fungal strain #58 remained more stable at acid conditions (pH 3 - 5) than at base conditions (**Figure 4(a)**); and, could stay rather stable over a wide temperature range even at high temperature of 70˚C - 80˚C (**Figure 4(b)**). By considering enzyme stability, optimal avicelase reaction conditions were lastly determined as a pH of 5 and a temperature of 70˚C for the secretome of strain #58.

During 7-day cultivations for the 92 isolated fungal strains, the culturing pH was around 5.3 for the MR-P medium and incubation temperature was round 27˚C for room temperature of the laboratory with air conditioner. These culturing conditions were close to those culture conditions determined for the fungal strain #58 (pH 5 and 25˚C); and, preliminarily screening results indicated the strain #58 was the most competitive candidate for exploring potential exo-glucanase. The avicelase activity gained during screening (0.209 U/mL) was even higher than that at pH 5 and 25˚C (0.145 U/mL) for optimal culturing conditions assessment. Furthermore, the screening conditions might not suitable for other fungal strains as the strains #60 and #71 had better avicelase activities when cultured at 25˚C and at pH 7 and 6, respectively (data not showed). Nevertheless, the highest avicelase activities for strain #60 (relative activity 50.3% under optimal culturing and enzyme reaction conditions) and #71 (94.2%) were still lower than that for the stain #58 (100%). Hence, only the results for the strain #58 were showed in the **Figure 3**. When the fungal strain #58 was cultured at 30˚C, the avicelase activities of harvested secretomes were typically less than 60% of the highest avicelase activities observed at 25˚C and pH5 suggesting lower temperature around 25-27˚C and pH about 5-5.3 were more suitable for exo-glucanase production from the strain #58 than at 30˚C and pH 6-7.

Interestingly, as the fungal strain #58 was cultured at 30˚C, optimal avicelase reaction conditions of the crude enzyme appeared to be shifted from pH 6 and 70˚C (**Figure 3(a)** and **Figure 3(b)**) to pH 4 and 60˚C (**Figure 3(d)** and **Figure 3(e)**). Besides, when it was cultured at pH 7, the optimal enzyme reaction conditions (*i.e.*, pH 7 - 8 and 30˚C - 50˚C, **Figure 3(c)** and **Figure 3(f)**) were different from those for the secretomes harvested at pH 5 and 6 (*i.e.*, pH 4 - 6 and 60˚C - 70˚C, **Figure 3(a)**, **Figure 3(b)**, **Figure 3(d)**, and **Figure 3(e)**). The results indicated that various types of enzymes (e.g., isozymes) with diverse characteristics might produce from the strain #58 when it was cultured under different pH and temperature conditions. A previous study had suggested that productions of lignolytic enzymes from a basidiomycetous fungal strain were dissimilar under different culture conditions [23]; and, another report had indicated that *Aspergillus fumigates* Z5 produced different proteins after

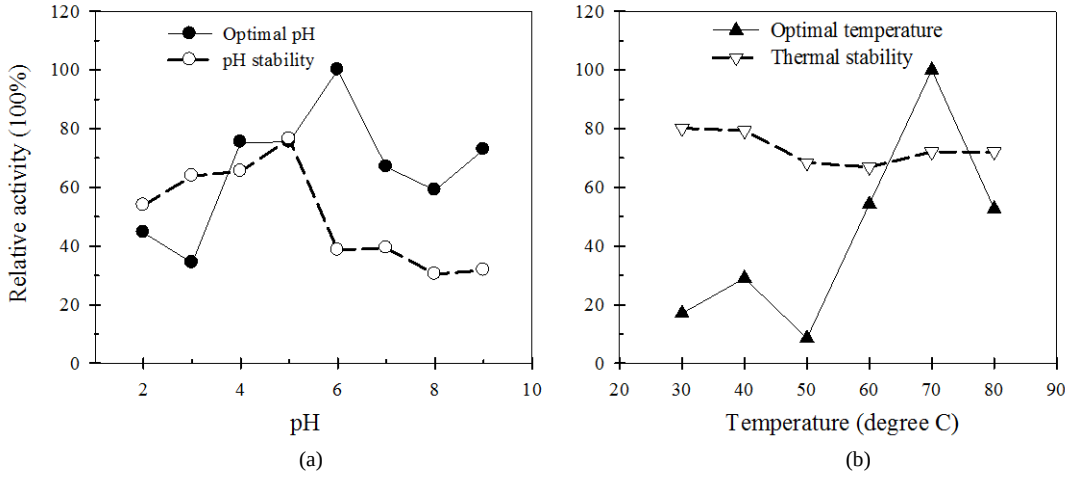

Figure 4. (a) Optimal reaction pH and temperature and (b) pH and thermal stabilities of the crude enzymes for fungal strain #58.

cultivation with various carbon sources (*i.e.*, glucose, avicel, and rice straw) [24]. It was surmised some cellulolytic enzymes might not be produced or might just be produced at very low level under inappropriate culturing conditions.

Given that some other common cellulase-secreting fungi (e.g., *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., and *Trichoderma* sp.) typically having optimal culturing pH level of about 5 and optimal temperature of around 28˚C - 30˚C, the fungal strain #58 isolated in this study seemed more suitable to be cultured under low pH of 5 as well but at lower temperature of 25˚C - 27˚C. Besides, crude enzyme harvested from the strain #58 had better avicelase activities when reacted at pH 5 that was similar to most of other exo-glucanases (**Table 1**); but, had an optimal reaction temperature of 70˚C which was much higher than typical reaction temperature of 50˚C - 60˚C for other exo-glucanases. In addition, the strain #58 secretome remained stable at high temperatures of 60˚C - 80˚C for at least four hours which was seldom found for many of general cellulases from fungi. Some of these unique features inferred that the fungal strain #58 might different from the common cellulase-secreting fungi and some new exo-glucanases might be able to be found from this potential fungal strain.

3.3. Fungal Strain and Exo-Glucanase Identification

The internal transcribed spacer (ITS) region of the fungal strain #58 was PCR amplified and further sequenced for fungal type identification. Sequencing result of the ITS fragment indicated that the fungal strain #58 was closely matched with a yeast *Meyerozyma caribbica* (96% similarity, NCBI accession number HG970748) in the *Saccharomycetes* class of *Ascomycota* phylum and formed a noticeable group together with *Candia* sp. and *Pichia* sp. in a phylogenetic tree (**Figure 5**).

The *Meyerozyma* sp. had been identified in yeast population of olive mill wastes [25] and had been found capable of improving maize productivity and minimizing requisite chemical fertilization [26]. Besides, a yeast strain of *Meyerozyma caribbica* MG20W had been isolated from plant rhizosphere soil and its complete genome had recently been sequenced [27]. These previous studies well supported that *Meyerozyma* sp. could ubiquitously present in some environments and might be able to secrete glycosidase, hydrolase, or specific cellulase.

Currently, a beta-1,3 exo-glucanase had been reported for the yeast *Meyerozyma caribbica* [28] which was belong to the glycosyl hydrolase (GH) 5 family (EC 3.2.1.58). But, no beta-1,4 exo-glucanase has been discovered from this yeast up to the best of our understanding. The avicelase activity observed for the secretomes produced from the fungal strain #58 isolated in this study indicated this *Meyerozyma* sp. yeast might capable of secreting beta-1,4 exo-glucanase.

The target exoglucanase of the *Meyerozyma* sp. strain #58 had a molecular size of about 70 kDa based on the SDS-PAGE analyses for the enzyme purified from bands observed and cut from the native-PAGE (**Figure 6(a)** and **Figure 6(c)**). Besides, the target exoglucanase showed notable activity on the MUC zymogram like a commercial enzyme C1.5 L did (**Figure 6(b)**). It appeared that specific activity of C1.5L was higher than that of the

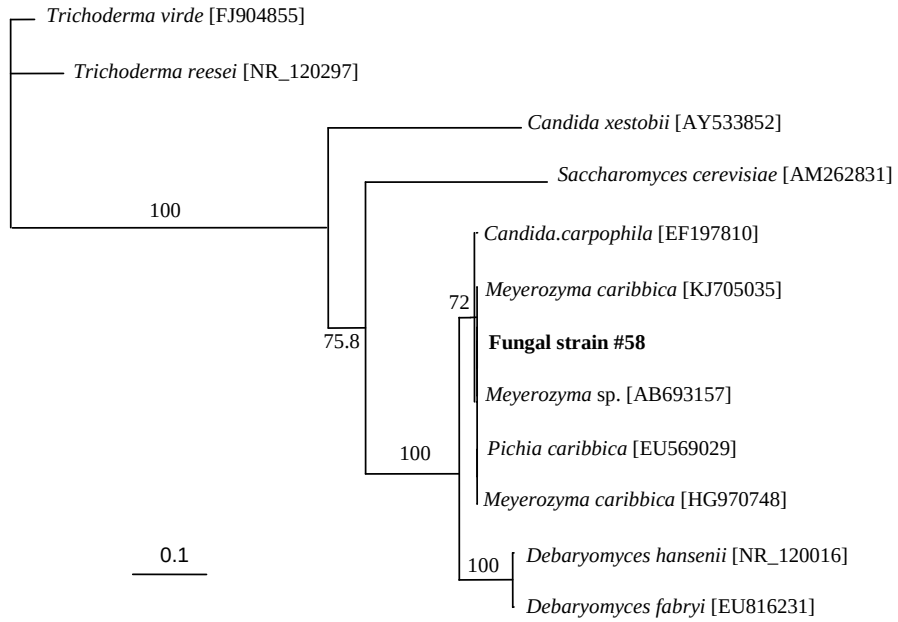

Figure 5. A phylogenetic tree showed fungal strain #58 belongs to the cluster of *Meyerozyma* sp.

Table 1. Comparisons of optimal reaction pH and temperature for activity tests of exo-glucanases for the exo-glucanases.

Fungi	Optimal pH	Optimal temp (˚C)	Reference
Aspergillus sp.	5.0	70	[31]
	4.8	-	[7]
Penicillium brefeldianum	4.2	50	[31]
Penicillium decumbens	4.0	50	[32]
Trichoderma reesei	5.0	55	[31]
	4.0 - 5.0	55	[33]
	5.0	65	[34]
Fungal strain #58	**5.0**	**70**	**This study**

strain #58 while C1.5L required smaller amount of enzyme than the strain #58 to exhibit strong image intensity like observed on the native-PAGE and MUC-zymogram (**Figure 6(a)** and **Figure 6(b)**).

The target exoglucanase of the *Meyerozyma* sp. strain #58 was further extracted from the SDS-PAGE and subsequently analyzed for the amino acid sequences using LC/MS/MS. Two amino acid fragments from sequencing results (*i.e.*, YSGTCDPDGCDFNPYR and AGAKYGTGYCDSQCPR) were identical with 1,4-beta-D-glucan cellobiohydrolases (CBHI) from *Aspergillus aculeatus* (NCBI accession number O59843) [29]. Two amino acid fragments revealed from LC/MS/MS demonstrated the target *Mc*-CBHI was beta-1,4 exo-glucanase belonged to glycosyl hydrolase family 7 (GH7). The two fragments were also extensively found in some cellobiohydrolases of other strains including *Aspergillus parasiticus* SU-1 (KJK66765.1), *Acytostelium subglobosum* LB1 (GAM28417.1), *Chaetomium senegalense* (CDF76452), *Lophodermium* sp. ZY-2014 (AIJ50313.1), *Penicillium roqueforti* FM164 (CDM37977.1), or *Rhizopus stolonifer* var. reflexus (AHI87690.1) etc. within the NCBI database. These results demonstrated that the target exoglucanase of the *Meyerozyma* sp. strain #58 belonged to beta-1,4 exo-glucanase (or 1,4-beta-D-glucan cellobiohydrolases, CBHI) (EC 3.2.1.91) and was lately named *Mc*-CBHI.

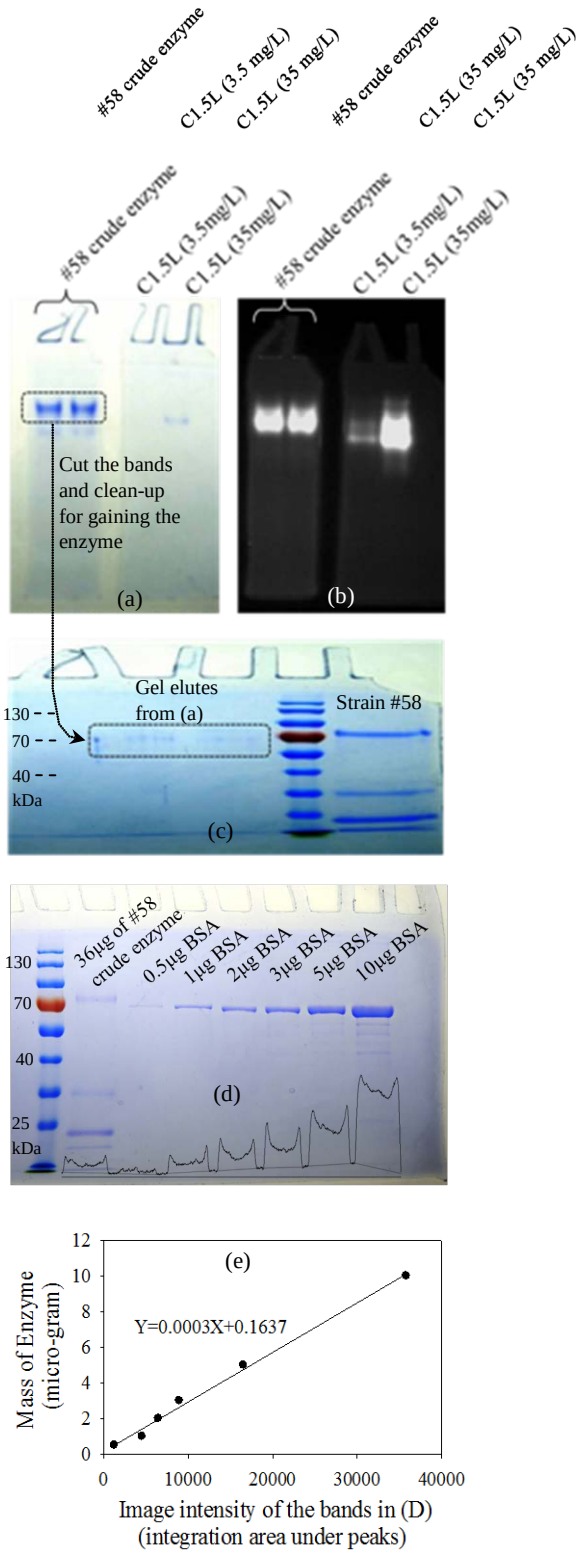

Figure 6. Native PAGE (a), MUC zymogram (b); SDS PAGE (c) showed target exo-glucanase *Mc*-CBHI had a size of around 70 kDa and had significant exo-glucanase activity (using MUC as substrate). Proportion of *Mc*-CBHI in the crude enzyme mixture produced from fungal strain #58 was estimated using BSA as standards (d) and (e).

3.4. Specific FPase and Avicelase Activities of *Mc*-CBHI

The proportion of the *Mc*-CBHI within the crude enzyme produced from the strain #58 was further determined by comparing band intensity of the *Mc*-CBHI on the SDS-PAGE with those for known amount of BSA. Image intensities for different amount of BSA were well correlated with the quantity of BSA (**Figure 6(e)**) and the target exoglucanase observed on the SDS-PAGE was estimated to be about 1.3 μg (or 1.66 mg/mL) which was approximately 3.6% of total crude enzyme (*i.e.*, 36 μg on SDS-PAGE or 45.93 mg/mL) harvested from the *Meyerozyma* sp. strain #58.

The specific FPase and avicelase activities of the *Mc*-CBHI became 0.179 U/mg and 0.126 U/mg calculated from the volumetric activity of 0.376 U/mL and 0.209 U/mL (**Table 2**); which were close to those for *T. reesei* CDU-11 (*i.e.*, 0.25 ± 0.05 and 0.11 ± 0.03 U/mg, respectively) and were about 40% - 50% of a commercial cellulase Accellerase-1000 (*i.e.*, 0.44 ± 0.01 and 0.25 ± 0.01 U/mg) [30]. These results indicated that of the newly-found *Mc*-CBHI was potentially competitive. If enzyme quality and quantity could be further improved by optimizing fermentation conditions or enzyme purification, the *Mc*-CBHI could be considered as a potential exoglucanase resource for related cellulolytic industrials.

4. Conclusion

In summary, a novel exoglucanase named *Mc*-CBHI was explored from a *Meyerozyma* sp. fungal strain #58 (96% identical with a yeast strain of *Meyerozyma caribbica* based on ITS fragment comparison) which was isolated

Table 2. Comparisons of the exo-glucanase activity using FPase and avicelase for some various fungal strains from previous reports and this study.

Fungi	FPase (U/mL)	Avicelase (U/mL)	Reference
Aspergilllus niger	0.123 (0.006 - 0.330)	-	[7]
Aspergilllus flavus	0.137 (0.117 - 0.163)	-	
Aspergillus tereus	0.164 (0.069 - 0.300)	-	
Aspergillus nidulans	0.188 (0.009 - 0.300)	-	
Cladosporium sp.	0.052	-	
Ahernaria sp.	0.056 (0 - 0.205)	-	
Fusarium sp.	0.023 (0 - 0.059)	-	
Neocallimastix frontalis	0.067 U/mg	0.132 U/mg	[35]
	-	0.32	[33]
Penicillium janthinellum	0.360 (0.24 - 0.55)	-	[36]
Penicillium sp.	0.078 (0 - 0.139)	-	[7]
	0.973	-	[37]
Rhizopus sp.	0.103 (0 - 0.222)	-	[7]
Trichoderma harziamum	0.034	-	
Trichoderma longibrachiatum	0.059	-	
Trichoderma reesei	0.25 ± 0.05 U/mg	0.11 ± 0.03 U/mg	[30]
	-	0.39 ± 0.004	[34]
Trichoderma viride	0.63 (0.28 - 1.00)	-	[36]
	-	0.016 U/mg	[38]
Fungal strain #58 (*Meyerozyma* sp.)	**0.376** **0.179** U/mg	**0.209** **0.126** U/mg	**This study**

from environmental airborne-fungal-spore samples. The *Mc*-CBHI could remain fairly stable after 4-hr incubation at acid condition (pH 3 - 5) and wide temperature range even at high temperature (70˚C - 80˚C). Additionally, it had specific FPase and avicelase activities of 0.179 U/mg and 0.126 U/mg, respectively, which were about 40% - 50% of a commercial cellulase Accellerase-1000 (0.44 ± 0.01 and 0.25 ± 0.01 U/mg). These results demonstrated that the newly-found *Mc*-CBHI from *Meyerozyma* sp. had high stability and specific activity and could be a potential exoglucanase resource.

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