Endoglucanase Activity of Novel *Aspergillus sydowii* Isolate WIU-01 and Application for Hydrophyte Degradation

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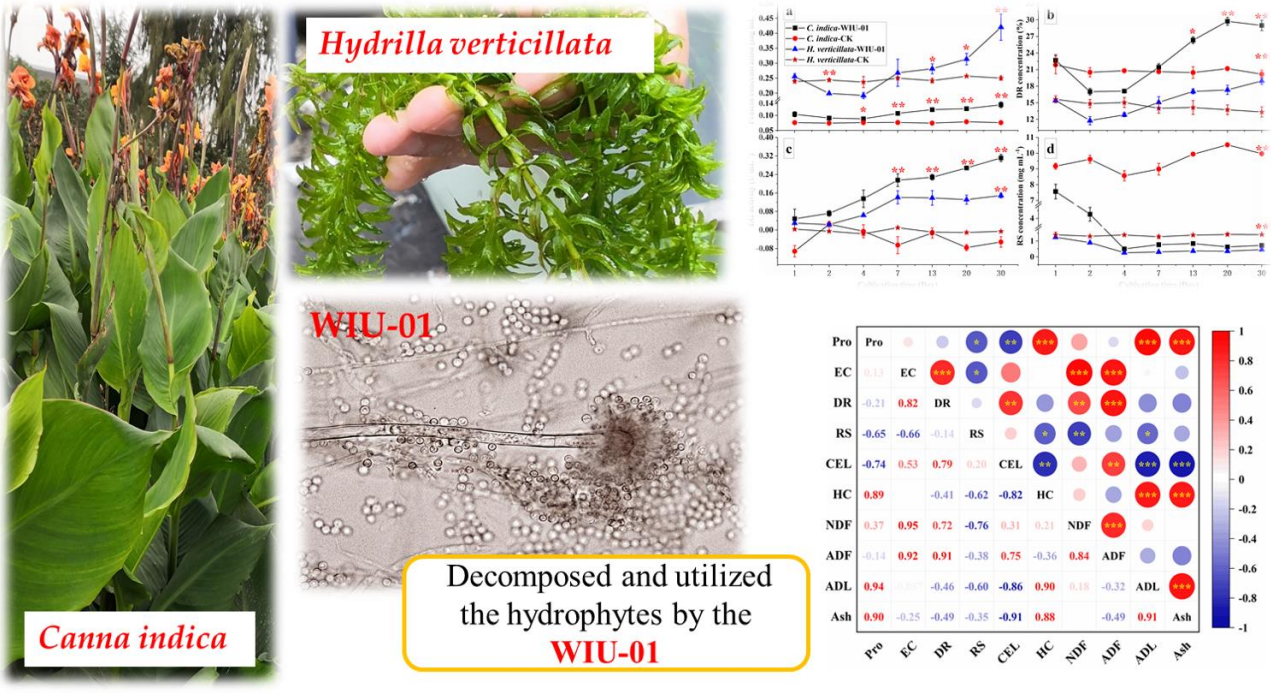


ABSTRACT:

Many eutrophic lakes contain rapidly growing hydrophytes. Overgrown biomass is usually mechanically harvested and thrown away, leading to resource waste and secondary environmental pollution. Microbial degradation is an economically and environmentally friendly approach for managing hydrophytic waste, fuelling the search for efficient biomass degraders. Here, we present isolation and characterization of *Aspergillus sydowii* WIU-01, a novel cellulolytic fungus. Strain WIU-01 was isolated from air. The degradation rate (29.75 vs. 21.95%) and endoglucanase activity (0.31 vs. 0.16 U mL^{-1}) of the fungus were higher in *Canna indica* (emergent plant) medium than in *Hydrilla verticillata* (submerged plant) medium, accordingly. Further, fungal endoglucanase activity was significantly positively correlated with the degradation rate, neutral detergent fiber content, and acid detergent fiber content of hydrophyte powder. Fungal biomass was significantly negatively correlated with reducing sugar and cellulose content of hydrophyte medium, but was significantly positively correlated with hemicellulose, acid detergent lignin, and ash content of the medium. Collectively, these observations indicate that *A. sydowii* decomposes emergent and submerged plant mass without acid–base sample pretreatment, albeit its endoglucanase activity is relatively low. This highlights the role of cellulolytic microorganisms in the natural environment and the notion that the environment can be a source of cellulolytic microorganisms for potential environmentally friendly applications.

Keywords: Cellulolytic fungus; endoglucanase; *Canna indica*; *Hydrilla verticillata*; fiber fraction

1. Introduction

Hydrophytes are important biological resources in the aquatic ecosystem, as they play central role in the energy-flow of aquatic material and ecosystem stability (Van Echelpoel and Goethals, 2018). However, increase in organic nutrient levels (e.g., from pollution, abusive discharge, or atmospheric deposition) or introduction of alien hydrophytes into the environment (e.g., water lily) can cause hydrophyte overgrowth (Zhao *et al.*, 2012). The overgrowth usually leads to the accumulation of

considerable biomass in some eutrophic lakes. Generally, aquatic plant overgrowth negatively impacts the ecosystem, e.g., by creating hypoxic conditions, crowding the water body, decreasing the light penetrance, and reducing the biodiversity (Geary and Kim, 2001). Further, the decaying hydrophyte residue may act as a secondary pollutant that is detrimental to nutrient elements, such as releasing the nitrogen and phosphorus, in the water body (Welsch and Yavitt, 2003). Consequently, the overgrown hydrophytes have to be effectively managed and utilized.

At present, hydrophytes are mainly used for the production of biological fertilizers, fuels, etc. (Kaur *et al.*, 2014). As the biomass is a recalcitrant network of lignocellulose containing cellulose (CEL), hemicellulose (HC), and lignin, lignocellulose hydrolysis is the rate-limiting step of biomass utilization (Amezcua-Allieri *et al.*, 2017). Consequently, the biomass have to be pretreated to disrupt lignocellulosic structure to enhance accessibility of CEL and other fiber fractions. CEL is a carbohydrate polymer composed of repetitive fibrils of β-D-glucopyranose linked by β-1,4-glycosidic bonds (Sajab *et al.*, 2019). Various physical, chemical, biological, and physicochemical methods are used for biomass pretreatment. The non-biological methods have serious limitations, such as high energy consumption, and generation of by-products or chemical waste. By contrast, biological pretreatment methods that utilize microbes for lignocellulose degradation are environmentally friendly and low-cost methods.

Synergistic activity of multiple enzymes greatly improves the depolymerization of CEL. Endoglucanases (EG) preferentially cleave the β-glucosidic bond, randomly hydrolyzed the amorphous regions of fibrils. Some microorganisms (bacteria, fungi, or yeasts) produce EG that convert CEL to soluble oligosaccharides (Mohapatra *et al.*, 2018b). Filamentous fungi, such as *Aspergillus* spp., play a vital role in CEL degradation, as they produce an array of enzymes that decompose plant cell wall polysaccharides (CEL, HC, and pectin) (Kowalczyk *et al.*, 2017). *Aspergillus* is an ancient organism with strong adaptive capacity, and is widely distributed in the biosphere (Jurjevic *et al.*, 2012). In fact, many *Aspergillus* species have been studied from the perspective of plant biomass degradation, and possess good cellulase and β-glucosidase activity (Benoit *et al.*, 2015, Makela *et*

68 *al.*, 2018, Raulo *et al.*, 2016). However, in many cases, acid–base pretreatment of the plant biomass
69 is required before microbial degradation and utilization, and this increases the processing cost.
70 Accordingly, identification of new microbes that efficiently decompose and utilize plants without
71 any pretreatment and in the natural environment draws considerable interest.

72 Some CEL decomposing microorganisms have been identified over the years. As early as in
73 1987, Bhat and Maheshwari (1987) studied the activity of components of the extracellular cellulase
74 system of the thermophilic fungus isolated from the soil. Hernández *et al.* (2001) isolated *Strep-*
75 *tomyces* sp. UAH 47 from lignocellulosic residues. Yoon and Kim (2005) showed that cellulases
76 produced by the brown rot basidiomycete *Fomitopsis palustris* degrade crystalline CEL to soluble
77 sugars. Further, Herve *et al.* (2014) reported on the white-rot fungus *Phanerochaete chrysosporium*
78 screened from the beech forest soil for wood decomposition.

79 The above microbes have been isolated from an environment that contains CEL or decaying
80 plants. However, we focused that the harvested plant leaves would be decayed and decomposed in
81 the air environment. These microbes using CEL as a carbon source should survive dispersion in air.
82 Therefore, it is also important to characterize air-borne CEL-decomposing microorganisms. As a
83 proof of concept, we here isolated strains with crystalline CEL-degrading activity from air to prove
84 that the presence of microbes in the air could cause the decomposition of plants. Moreover, we also
85 characterized the hydrophyte-degrading ability of the most promising isolate to assess its future
86 potential applications.

87 **2. Materials and Methods**

88 *2.1. Isolation of CEL-degrading Microorganisms from Air*

89 Air-borne microbes with strong survival capacity were isolated in a primary screening medium, with
90 sodium carboxymethyl CEL (CMC-Na) as the only carbon source. The medium also contained
91 $(NH_4)_2SO_4$, K_2HPO_4, $MgSO_4 \cdot 7H_2O$, peptone, yeast extract, and agar (2%, w/v). The medium was
92 autoclaved (121 °C for 30 min), and poured into a sterile culture dish (10-cm in diameter). Next, an
93 uncovered culture dish containing the medium was placed on the workbench, and incubated at 30 °C

for 5 d. All reagents used in the current study were purchased from Shanghai Aladin Biochemical Technology Co. LTD.

2.2. Purification and Further Screening

After a 5-d static cultivation, several distinct colonies with different morphological features were observed growing on the plate. The microbes were purified by passaging on CMC-Na medium until uniform colony morphology was obtained. Single-colony diameter was measured, and the plates were stained with a solution of Congo red (1 mL mg^{-1}) and de-stained with sodium chloride (1 mol L^{-1}) to compare the cellulose degrading capacity of the isolates (Teather and Wood, 1982). The isolate degradation capacity was compared by calculating the ratio of the transparent halo around the colony to the colony diameter. A fungal isolate (strain WIU-01) with pronounced cellulose degrading ability was selected for detailed characterization.

2.3. Isolate Identification

The fungal Internal Transcribed Spacer (ITS) rDNA gene was sequenced after PCR amplification using the ITS1 forward primer (5′-TCCGTAGGTGAACCTGCGG-3′) and the ITS4 reverse primer (5′-TCCTCCGCTTATTGATATGC-3′) (Schoch *et al.*, 2012) at Shanghai SANGON Biotech Co., Ltd. The obtained sequence was then compared with the sequences deposited at (https://blast.ncbi.nlm.nih.gov/Blast.cgi) using the Basic Local Alignment Search Tool. Strain WIU-01 was identified based on the degree of sequence homology (Embley and Stackebrandt, 1994). The phylogenetic tree was constructed by using MEGA v. 7 and the neighbor-joining method (bootstrap analysis with 500 replicates) (Tamura *et al.*, 2004). ClustalW program was used for multiple-sequence alignment and the maximum composite likelihood method was used to compute evolutionary distances.

2.4. EG activity and Reducing Sugar (RS) Content Determinations

Logarithmic-phase fungal culture was refreshed by dilution (1%, v/v) in 50 mL of fresh medium. EG activity was monitored in CMC-Na medium containing 1% (w/v) CMC-Na (prior to the experiment, CMC-Na was dissolved in acetate–sodium acetate buffer) as the substrate, as described by Ghose

120 (1987), with slight modification. The experiment was conducted for 14 d, with shaking (180 rpm), at
121 30 °C.

122 For the EG activity assay, the culture was sampled every 24 h. At each time point, 2 mL of
123 culture were withdrawn and centrifuged at 4000 rpm for 20 min. Then, 0.5 mL of the supernatant was
124 mixed with 1.5 mL of substrate in a 25-mL volumetric flask, and incubated at 50 °C for 30 min. Next,
125 1.5 mL of 3,5-dinitrosalicylic acid was added to stop the reaction (Miller, 1959). The samples were
126 then heated in a boiling water bath for 5 min to allow color development. After cooling to room
127 temperature, the volume was adjusted to the 25-mL mark with deionized water. Finally, sample ab-
128 sorbance was determined at 540 nm using an UV-vis spectrophotometer (UV-1800, Shimadzu,
129 Kyoto, Japan). The amount of RS released was calculated using a curve constructed with glucose as a
130 standard. The glucose standard curve was prepared as described elsewhere Ghose (1987). Inactivated
131 sample (supernatant placed in a boiling water bath for 10 min) was used as the blank control. One unit
132 (U) of EG activity was defined as the amount of enzyme required for the release of 1 μmol of glucose
133 per min under the above conditions. The RS content was determined by DNS method.

134 *2.5. Fungal Decomposition of Submerged and Emergent Plant Material*

135 The emergent plant *Canna indica* L. and submerged plant *Hydrilla verticillata* (L. f.) Royle were
136 chosen for the hydrolysis assays as they are widely distributed in the tropical to temperate regions of
137 Eurasia (Biswabijayinee *et al.*, 2008, Cook, 1985).

138 Mature and healthy leaves and stems of *C. indica* and *H. verticillata* were used. The plant mate-
139 rial was washed several times in water, heated at 100 °C for 30 min, and dried at 80 °C. It was then
140 crushed and sieved through a 60-size mesh. Next, 1.00 g of plant material was weighed (starting
141 weight, W1) and mixed with 30 mL of mineral salt medium, according to Mandels and Weber (1969),
142 with slight modification. Specifically, the solution contained $K_2HPO_4 \cdot 3H_2O$, $(NH_4)_2SO_4$,
143 $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, $FeSO_4 \cdot 7H_2O$, $ZnCl_2$, and $CoCl_2 \cdot 6H_2O$. The mixtures were then sterilized
144 at 121 °C for 30 min. One milliliter of logarithmically growing fugal culture (prepared as described in
145 section 2.4) was used to inoculate the solutions, in triplicate, and cultivated with shaking (180 rpm) at

30 °C. The culture medium without the fungal inoculum was used as the control. The cultures were sampled on days 1, 2, 4, 7, 13, 20, and 30. At each sampling point, 2 mL of culture were centrifuged (4000 rpm for 20 min), and EG activity and RS were determined in the supernatants, as described in section 2.4. The pellets were washed three times with ultrapure water, dried at 100 °C, and then weighed (final weight, W2). The degradation rate of plant material was calculated using the following formula:

$$\text{Degradation rate (\%)} = [(W1 - W2)/W1] \times 100,$$

W1 is the starting weight of the plant material in the mineral salt medium. W2 is the final weight of plant material after 30 d of incubation in the presence or absence of strain WIU-01, as described above.

Protein content in the samples was determined by using Bradford assay, with bovine serum albumin as the standard (Bradford, 1976).

2.6. Fiber Analysis of Plant Material

CEL, HC, neutral detergent fibrils (NDF), acid detergent lignin (ADL), acid detergent fibrils (ADF), and ash content in the *C. indica* and *H. verticillata* material were determined by using Van Soest method (Van Soest *et al.*, 1991).

2.7. Statistical Analysis

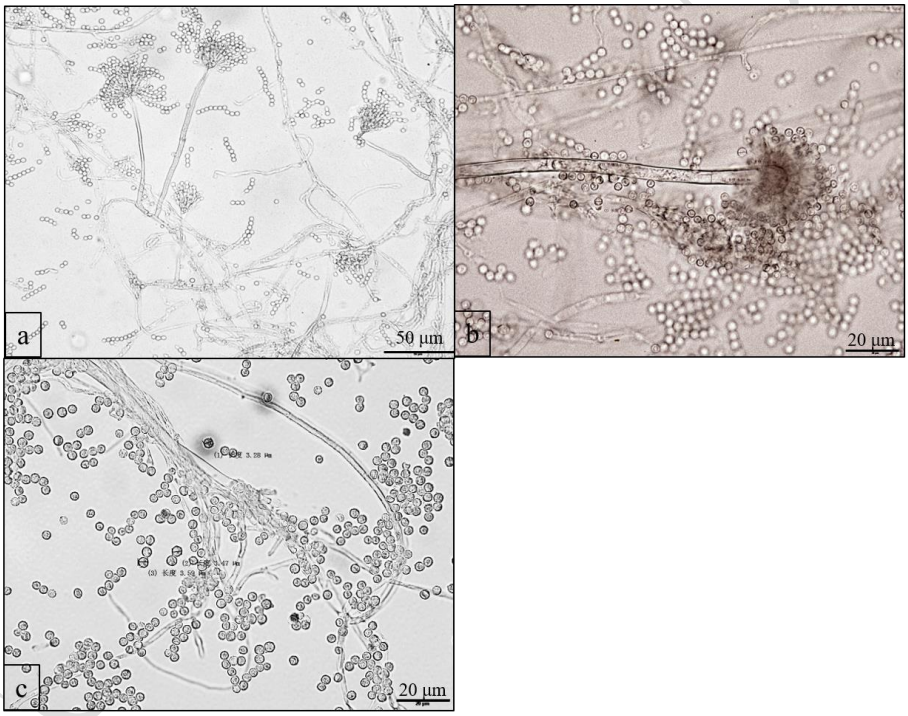
Preliminary data analysis was performed using Excel 2016 (Microsoft Office, Microsoft). The figures and histograms were prepared using Origin Pro 2021 Beta3 (Origin Lab Corporation). Phylogenetic analyses were performed using MEGA v. 7. SPSS 22 (SPSS Statistics, IBM) was used for statistical analysis of independent samples (*t*-test). Correlation analysis was performed based on Pearson correlation. The significance threshold was set at $p < 0.05$ ($^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

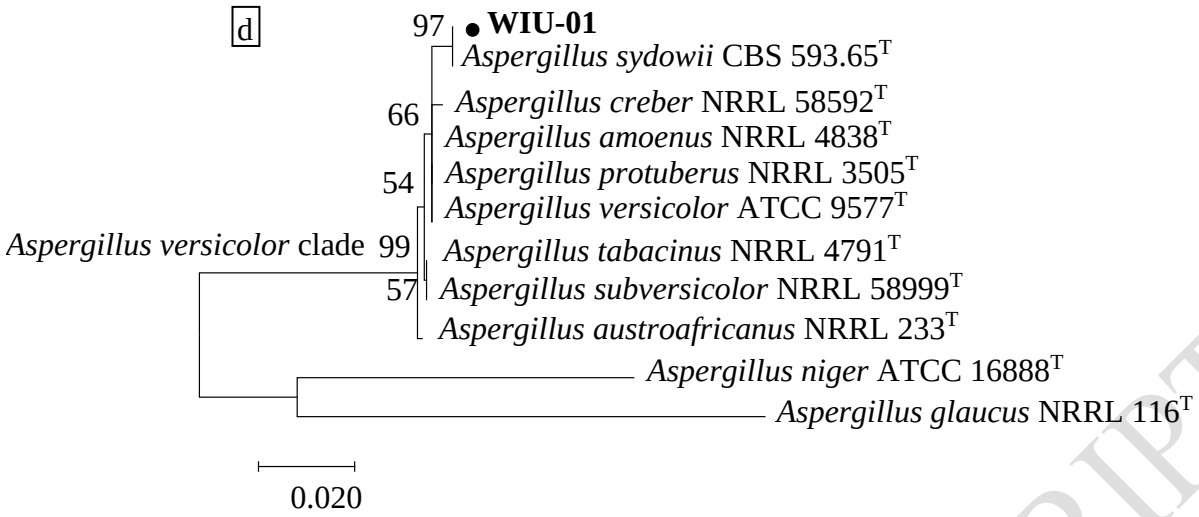
3. Results and Discussion

3.1. Morphological Characterization and Genetic Identification of the Isolated Fungus

In the current study, we aimed to isolate air-borne cellulolytic microbes. Based on the colony characteristics, microscopic morphological evaluation, and molecular and phylogenic analysis, we

identified strain WIU-01 as a cellulolytic fungus. According to the microscopic evaluation (Figure 1a–c), the fungus grew on CMC-Na agar medium, forming an intricate network of extended hyphae. It formed fluffy and white colonies on CMC-Na agar medium at the early cultivation stage, which then became green, and finally, dark green. The conidia grew on the hyphae, with smooth stipes formed. The conidial heads were loose and radiated. The conidiophores were spherical, approximately 3.5 μm in diameter, light green, and with small surface spines observed under 10 × 100 magnification. Based on the ITS rDNA sequence and phylogenic analysis, the fungus WIU-01 was identified as *Aspergillus sydowii* (Figure 1d) (Jurjevic *et al.*, 2012).





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184 **Figure 1.** The morphology and phylogenic analysis of the cellulolytic fungal isolate WIU-01. (**a**)
185 Representative fungal hypha viewed under 10 × 40 magnification. (**b**) Representative hypha viewed
186 under 10 × 100 magnification. (**c**) Conidiophores viewed under 10 × 100 magnification. (**d**) Phylo-
187 genic analysis of the ITS rDNA gene sequence of the WIU-01 strain. The strain was identified as
188 *Aspergillus sydowii*. The scale bar represents 0.2 substitutions per site, and the numbers at the nodes
189 are bootstrap values (%).

190 Fungi are a large and diverse group of organisms. Filamentous fungi are preferentially used in
191 commercial applications because they produce more enzymes than bacteria (Mrudula and
192 Murugammal, 2011). Filamentous fungi from the genus *Aspergillus* are widely distributed in the
193 biosphere and have a strong adaptive capacity (de Vries and Visser, 2001). Qaisar *et al.* (2014) have
194 enriched an *A. versicolor* strain from paper reprocessing areas and sugarcane field, and showed its
195 ability to degrade crystalline cellulose. Recent studies have shown that some *A. sydowii* strains hy-
196 drolyze CEL. For example, Ghareib *et al.* (1992) have studied the effect of alkali pretreatment on
197 degradation of some cellulosic material (such as pods of bean, rice plant straw, wheat bran) by *A.*
198 *sydowii*. Nair *et al.* (2008) isolated and purified xylanase from culture filtrate of *A. sydowii* with the
199 wheat bran and birch wood as the carbon source. We here successfully isolated another *A. sydowii*

200 strain, which utilizes CMC-Na as a single carbon source. We next proceeded to evaluate its EG and
201 plant-degrading abilities in detail.
202
203 *3.2. EG Activity and RS Generation by Isolate WIU-01*
204 We monitored its EG activity and RS levels in a medium containing 1% (w/v) CMC-Na to better
205 characterize the crystalline CEL degrading activity of isolate WIU-01. RS content is indicative of the
206 cellulolytic fungal capacity to utilize and decompose crystalline CEL and amorphous polysaccha-
207 rides, with enzymes, such as EG, attacking the amorphous zone of CEL chains and releasing oligo-
208 saccharides of various lengths (Kaur *et al.*, 2014). The experiment was performed over a 14-d period
209 (Figure 2). Overall, the EG activity was low during the first 4 d of growth and then slowly increased
210 over the remaining cultivation time. The EG activity reached a maximum (0.027 U mL^{-1}) at the end of
211 the incubation period (day 14). The RS concentration trend was similarly to that of EG activity.
212 Specifically, the RS concentration was low in the first 3 d of cultivation, before slowly rising on day
213 4, and reaching the highest value (0.41 mg mL^{-1}) at the end of the incubation period.
214

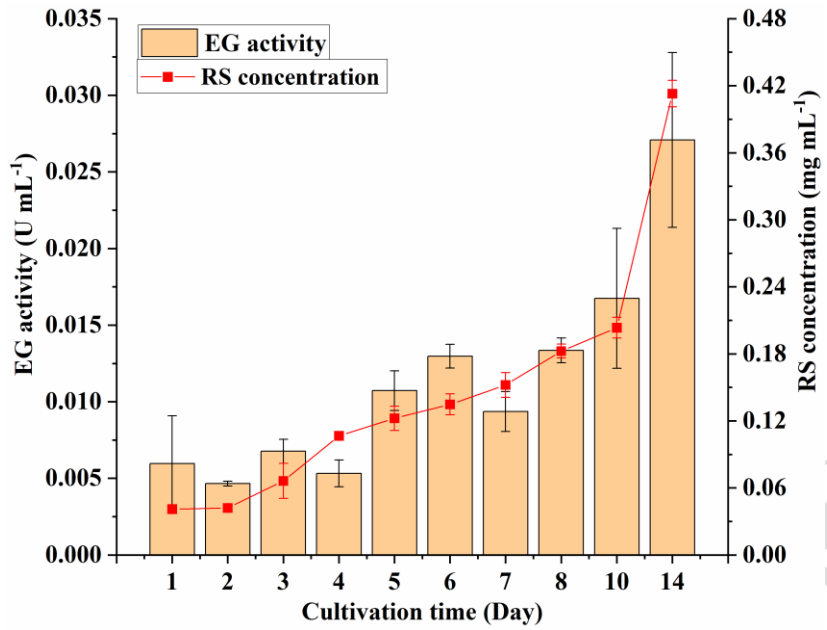

Figure 2. Endoglucanase (EG) activity of isolate WIU-01 and reducing sugar (RS) concentration during incubation in a medium containing 1% (w/v) CMC-Na. The experiment was performed in triplicate and the data are presented as the mean ± SD.

Fungi play a vital role in biomass degradation in nature but their EG activities vary widely. For instance, Mohapatra *et al.* (2018a) isolated an *Aspergillus fumigatus* strain from a soil-enriched decomposed lignocellulosic waste, and reported EG activity of 0.287 U mL^{-1} at 30 °C. Yamada *et al.* (2014) studied a wild-type *Aspergillus oryzae* strain and reported EG activity of 0.0014 U mL^{-1}. In that study, EG activity of the cellulase-expressing strains was 0.0299 U mL^{-1}. Further, Facchini *et al.* (2011) reported EG activity of an *Aspergillus japonicus* strain isolated from farming regions of São Paulo State (Brazil) as 0.55 U mL^{-1}, which was higher than that of isolate WIU-01 reported in the current study. The EG activity of yet another *A. versicolor* strain, screened from soil samples from paper reprocessing areas and sugarcane field, was high, at approximately 200 U mL^{-1} in 1% CMC-Na culture (Qaisar *et al.*, 2014). These discrepancies may be associated with individual strain characteristics and different testing conditions.

3.3. Degradation of Emergent Plant and Submerged Plant Material by Isolate WIU-01

We next tested the ability of the strain isolate WIU-01 to degrade the emergent plant and submerged plant materials. In the absence of the fungus, protein concentration in a medium supplemented with *H. verticillata* powder (approximately 0.24 mg mL^{-1}) was significantly higher than that in a medium supplemented with *C. indica* powder (approximately 0.075 mg mL^{-1}) (Figure 3a). Conversely, protein content in both media inoculated with isolate WIU-01 showed a decreasing trend in the first 4 d, which was significantly lower than that in the medium without the fungus. Upon prolonged cultivation with the fungus, the protein content increased to 0.14 and 0.42 mg mL^{-1} in the *C. indica* culture and in the *H. verticillata* culture, respectively.

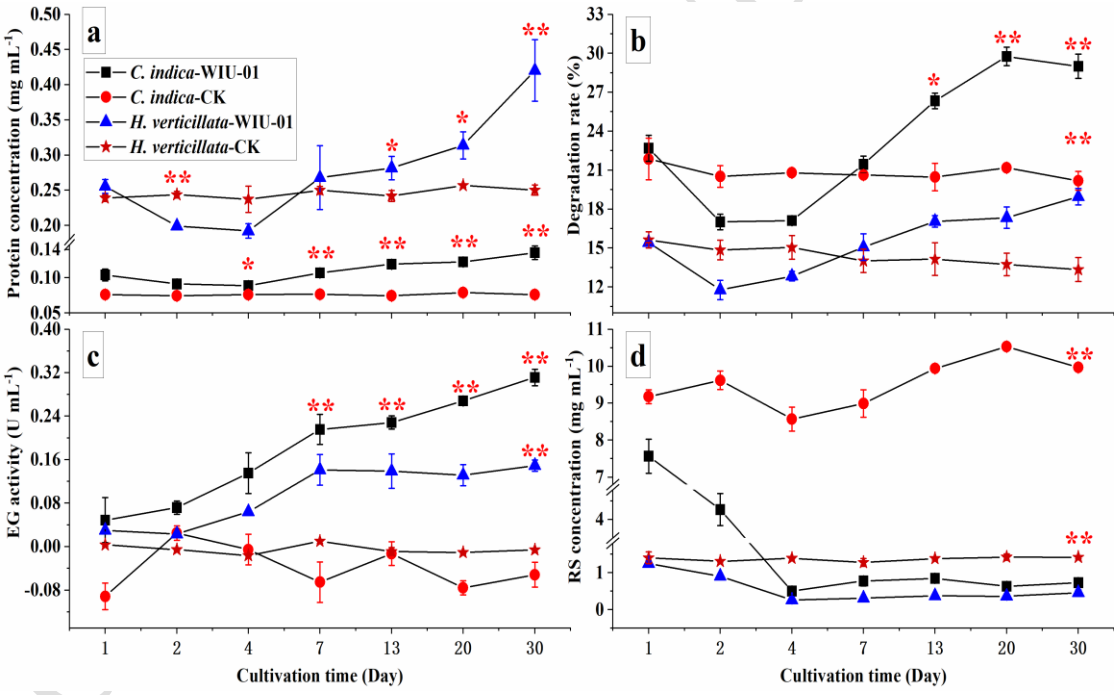

Figure 3. Changes in protein concentration, substrate degradation rate, endoglucanase (EG) activity, and reducing sugar (RS) concentration in media containing *C. indica* and *H. verticillata* powders, in the presence and absence of strain WIU-01. The cultures contained *C. indica* and *H. verticillata* plant material and were conducted at 30 °C. (**a**) Protein concentration in the culture medium. (**b**) The degradation rate of plant material. (**c**) EG activity of strain WIU-01. (**d**) RS concentration in the culture medium. Please note y-axis breaks in panels (**a**) and (**d**). The experiment was performed in

triplicate, and the data are presented as the mean ± SD (*$p < 0.05$, **$p < 0.01$, independent *t*-test, no-fungus control vs. fungus treatment).

Isolate WIU-01 hydrolyzed both plant materials (Figure 3b). The degradation rates of *C. indica* and *H. verticillata* materials in the absence of the fungus were approximately 20% and 14%, respectively, throughout the incubation period (30 d). The plant material is poorly soluble in cold water, but some polysaccharides and oligomers can become partially soluble in hot water during the autoclaving process (Zhang *et al.*, 2020). This may be one of the reasons for the apparent degradation of the plant material in the medium without the fungus. Upon the addition of the fungus, the degradation rate of *C. indica* did not significantly change in the first 4 d of culture, but exhibited a marked increasing trend from day 7, reaching a maximum of 29.75% on day 20. The degradation of *H. verticillata* powder was relatively slower, but showed a similar trend, i.e., it first declined (days 1–2) and then increased (18.95%). These findings indicate that isolate WIU-01 degrades and utilizes *C. indica* more efficiently that *H. verticillata*.

EG activity of isolate WIU-01 in the plant material-supplemented media gradually increased during the experiment (Figure 3c). Specifically, it increased from 0.049 to 0.31 U mL^{-1} with prolonged incubation in the *C. indica* culture, and it increased from 0.03 to 0.15 U mL^{-1} in the *H. verticillata* culture. The EG activity detected in the emergent and submerged plant media was thus significantly higher than that in the CMC-Na culture medium. This was consistent with the findings of Sun *et al.* (2008), who reported a higher enzyme activity of cellulolytic organisms exposed to complex substrates, such as wheat bran, than those grown in the presence of microcrystalline CEL substrate. Su *et al.* (2018) reported that EG activity was closely associated with the CEL degradation rate, which they attributed to the fact that enzymes break down the heterogeneous compounds to small components. The degradation rate of *C. indica* and *H. verticillata* exhibited an obviously increasing trend after day 4. Similarly, the EG activity rapidly increased phase day 2.

RS concentrations in the two plants material-supplemented media in the absence of the fungus [i.e., generated during the sterilization stage of medium preparation (121 °C, 30 min)] were relatively stable, at approximately 9.54 and 1.37 mg mL^{-1} in the *C. indica* and *H. verticillata* media, respectively (Figure 3d). RS levels in the former were significantly higher than those in the latter. In the presence of the fungus, RS concentrations in the two media rapidly declined early in the cultivation (0–4 d), and then remained constant and low.

Detailed analysis of protein concentration, degradation rate, EG activity, and RS content revealed an interesting phenomenon: except for the EG activity, the values measured in the presence of isolate WIU-01 markedly dropped during the early adaptive stage of cultivation (days 0–4). This was followed by a rapid degradation phase between days 4 and 20, accompanied by a continuous increase in protein concentration and EG activity. However, the RS content remained low. This might be explained by the fungus initially adapting to the hydrophyte medium, and then rapidly proliferating and utilizing RS produced during medium sterilization. Low RS levels in the medium led to isolate WIU-01 breaking down and subsequently utilizing the fibril components of *C. indica* and *H. verticillata*. RS remained low during the remainder of cultivation, while the degradation rate and EG activity continued to increase. This might be because RS produced by isolate WIU-01 decomposing CEL only sufficed to maintain biomass growth, with no RS accumulation in the medium, or because microbes degraded and utilized CEL without the production of soluble RS (Gao *et al.*, 2001).

The observed EG activity with *C. indica* and *H. verticillata* substrates was different. That could be explained by the different composition of the two substrates and was consistent with a reported differential EG activity levels in *Aspergillus* spp. incubated with different lignocellulosic substrates (Prajapati *et al.*, 2020). In addition, the observed low EG activity during the late cultivation stage might be associated with the reduction of nutrient levels in the medium (Karim *et al.*, 2015), as well as pH changes and accumulation of inhibitory metabolites (Abdullah *et al.*, 2015). Further, enzyme binding may limit CEL accessibility to cellulase during the subsequent saccharification step (Qin *et al.*, 2016). By contrast, some enzymes are adsorbed onto the CEL substrate, reducing the detectable

cellulase activity in the medium filtrate (Zheng *et al.*, 2020). Finally, formation of phenolic groups during the degradation process can hamper cellulolytic enzyme reaching CEL chains, thus influencing the measurable enzymatic activity (Mohapatra *et al.*, 2017).

3.4. Fiber Fraction Analysis of Plant Material and Effect of Fungal Activity

The plant cell wall mainly comprises CEL, HC, and lignin, which may inhibit the decomposition and utilization of plant biomass (Sánchez, 2009). Since we observed that isolate WIU-01 degraded *C. indica* and *H. verticillata* powder with different efficiency, we then analyzed the fiber fractions of the two plant materials, in the presence and absence of the fungus. *H. verticillata* powder contained significantly more HC, ADL, and ash than *C. indica* powder (Figure 4).

The analysis revealed that the *C. indica* and *H. verticillata* substrates had more CEL, NDF, and ADF than ADL, HC, and ash (Figure 4). In addition, CEL, NDF, and ADF levels in *C. indica* medium were significantly increased after the fungal treatment. By contrast, the fungal treatment significantly reduced the ash content in the medium. The fungal treatment significantly increased the NDF, ADF, and ash content in the *H. verticillata* medium. We observed no significant effect of the fungus on the ADL and HC content in the two media.

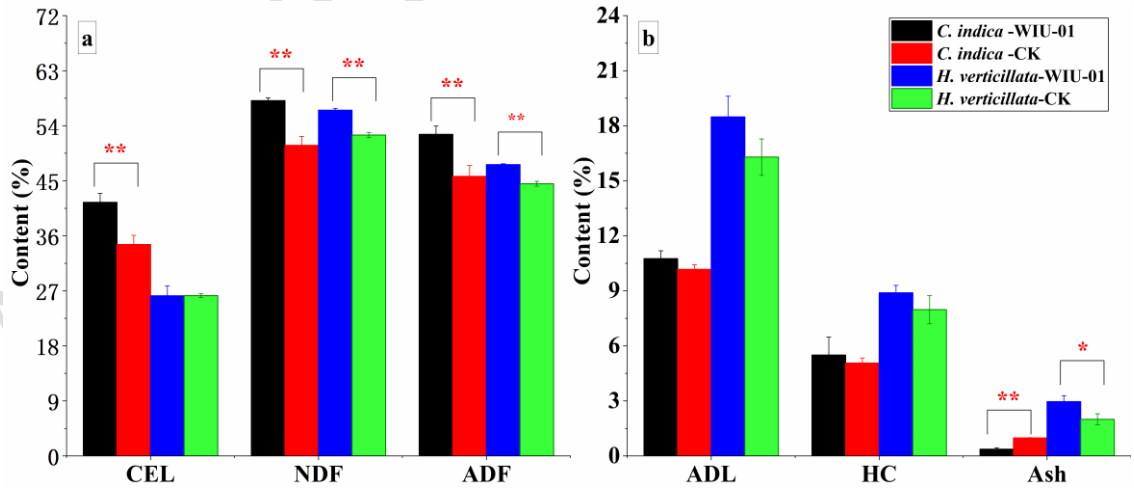

Figure 4. Fiber fractions in the *C. indica* (**a**) and *H. verticillata* (**b**) residual powders. Cellulose (CEL), hemicellulose (HC), neutral detergent fibrils (NDF), acid detergent lignin (ADL), acid detergent fibrils (ADF), and ash levels were determined, with and without fungal treatment, after 30 d at

30 °C. The experiment was performed in triplicate, and the data are presented as the mean + SD (*p < 0.05, **p < 0.01, independent t-test, no-fungus control vs. fungus treatment).

We next performed a correlation analysis to investigate the factors that affected the WIU-01 EG activity in the media tested. We analyzed the relationship between EG activity, and the degradation rate and fibril components in the two plants (Figure 5). The analysis revealed a significant positive relationship between EG activity and degradation rate (r = 0.823**), NDF (r = 0.954**), and ADF (r = 0.916**), with no significant correlation with the other factors examined. Similarly, degradation rate was significantly positively correlated with NDF (r = 0.715**), ADF (r = 0.912**), and CEL (r = 0.787**).

Lignin is found in all vascular plants and is a highly complex aromatic polymer, with a three-dimensional structure formed by benzene-propane units that are linked via ether and carbon–carbon bonds. On the other hand, HC is a branched polymer that is embedded in CEL elementary fibrils, with different sugars linked via glycosidic bonds (Jia *et al.*, 2017). Generally, the main components of fibrils are determined using the Van Soest method (Van Soest *et al.*, 1991), and their content is then calculated based on the dissolution degree of fibril components in a detergent solution. NDF represents the material with the protein, fat, and other extracts removed, mainly composed of CEL, HC, lignin, and ash. ADF mainly contains CEL, lignin, and ash (Wang *et al.*, 2019). Fatica *et al.* (2019) reported that CEL (ADF minus ADL) and lignin (ADL) contents have different effects on anaerobic ecosystems. Our analysis revealed a significant increase in CEL, NDF, and ADF in the *C. indica* fibrils and *H. verticillata* fibrils after enzymatic hydrolysis by the fungus.

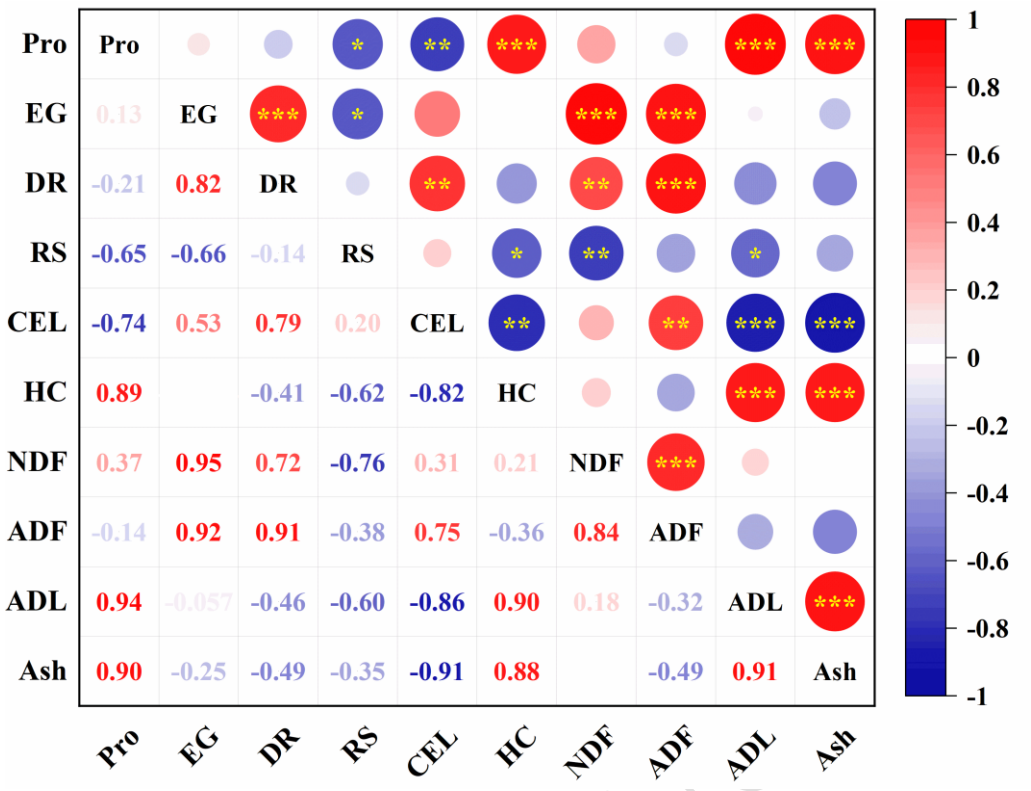

Figure 5. Correlation analysis of protein (Pro), degradation rate (DR), and fiber fractions (defined as in Figure 4 legend) in *C. indica* and *H. verticillata* residual powders with endoglucanase (EG) activity of isolate WIU-01. Pearson's correlation coefficient (r) is shown. The magnitude of correlation is illustrated by the numbers (left) and circles (right), with the color scale indicated (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

4. Conclusions

We here successfully isolated and identified a cellulolytic *Aspergillus sydowii* isolate, strain WIU-01, from the air. The fungus exhibited a high activity with powdered material from the emergent plant *C. indica* (degradation rate of 29.75% and EG activity of 0.31 U mL^{-1}) indicating the possibility of its application in biomass decomposition and utilization. However, it showed a low activity with powdered material from the submerged plant *H. verticillata* (degradation rate of 21.95% and EG activity of 0.16 U mL^{-1}). The CMCase activity significantly impacted the degradation rate. Further, correlation analysis indicated that CEL, HC, ADL, and ash in the hydrophyte residue significantly affected fungal biomass growth. Overall, these findings indicate that some air-borne fungi potentially utilize

CEL, and may play a role in large-scale macrophyte degradation without the need for acid–base pretreatment. However, there are some limitations to the immediate use of the novel *A. sydowii* isolate, e.g., its EG activity is relatively low and the fungus may produce some toxic secondary metabolites. Further studies could focus on, e.g., extracting the EG gene encoded by strain WIU-01 and generation of recombinant proteins for specific applications. Nonetheless, the current study underpins the cellulolytic potential of naturally occurring microorganisms and the need for their continued characterization.

Funding: This research was funded by the Strategic Priority Research Program of the Chinese Academy of Sciences, grant number XDA23040401, and the State Key Laboratory of Freshwater Ecology and Biotechnology, grant number 2019FBZ03.

Acknowledgments: The authors would like to thank Liping Zhang for organizing the sampling survey, and our colleagues from State Key Laboratory of Freshwater Ecology and Biotechnology for collecting and identifying hydrophytes.

Data Availability: All data related to this study are presented in this article.

Conflicts of Interest: The authors declare no conflict of interest.

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