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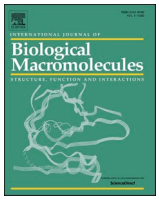

Upgraded cellulose and xylan digestions for synergistic enhancements of biomass enzymatic saccharification and bioethanol conversion using engineered *Trichoderma reesei* strains overproducing mushroom *Le*GH7 enzyme

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ABSTRACT

Crop straws provide enormous lignocellulose resources transformable for sustainable biofuels and valuable bioproducts. However, lignocellulose recalcitrance basically restricts essential biomass enzymatic saccharification at large scale. In this study, the mushroom-derived cellobiohydrolase (*Le*GH7) was introduced into *Trichoderma reesei* (Rut-C30) to generate two desirable strains, namely GH7–5 and GH7–6. Compared to the Rut-C30 strain, both engineered strains exhibited significantly enhanced enzymatic activities, with β-glucosidases, endocellulases, cellobiohydrolases, and xylanase activities increasing by 113 %, 140 %, 241 %, and 196 %, respectively. By performing steam explosion and mild alkali pretreatments with mature straws of five bioenergy crops, diverse lignocellulose substrates were effectively digested by the crude enzymes secreted from the engineered strains, leading to the high-yield hexoses released for bioethanol production. Notably, the *Le*GH7 enzyme purified from engineered strain enabled to act as multiple cellulases and xylanase at higher activities, interpreting how synergistic enhancement of enzymatic saccharification was achieved for distinct lignocellulose substrates in major bioenergy crops. Therefore, this study has identified a novel enzyme that is active for simultaneous hydrolyses of cellulose and xylan, providing an applicable strategy for high biomass enzymatic saccharification and bioethanol conversion in bioenergy crops.

1. Introduction

Lignocellulose represents the most abundant biomass resource that can be transformed for biofuels and biochemicals [1]. As lignocellulose is mainly composed of cellulose, hemicellulose and lignin [2], it is increasingly implemented for syntheses of diverse biochemicals and bioproducts such as ethanol, 3-hydroxypropionic, propionic and succinic acids, isoprene, and nanomaterials [3–5]. In particular, cellulosic ethanol has been evaluated as a perfect additive in petrol fuels to reduce net carbon liberation [6]. The bioconversion of cellulosic ethanol involves three essential stages: biomass pretreatment for wall polymer destruction, sequential enzymatic hydrolysis for fermentable sugars, and yeast fermentation for ethanol production [7]. However, lignocellulose recalcitrance limits enzymatic hydrolysis and chemical conversion in a

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cost-effective and environmentally-friendly manner. Although physical and chemical pretreatments have been employed to enhance enzymatic saccharification of biomass, lignocellulose-degradation enzymes with higher activities must be explored [8,9].

Lignocellulolytic enzymes include cellulases (endoglucanases, cellobiohydrolases, β-glucosidase), hemicellulases (xylanases, β-1,4-xylosidases, arabinofuranosidases), carbohydrate esterases (feruloyl esterase, acetyl xylan esterases), and auxiliary activity enzymes [10,11]. The major cellulases secreted by fungi are derived from family 7 glycoside hydrolases (GH7) (EC3.2.1.176). Owing to their effectiveness in cellulose degradation, GH7 enzymes have been used in the enzymatic saccharification of biomass [12]. As a typical *Agaricomycetes* class in the *Basidiomycota phylum*, *Lentinula edodes* belongs to the glycoside hydrolase families GH5, GH7, and GH10, and they can secrete many lignocellulolytic enzymes that enable efficient lignin and cellulose hydrolysis [13]. However, much remains unknown about its activity in hemicellulose and other wall polymer degradations.

Trichoderma reesei (Syn. *Hypocrea jecorina*) is a major industrial fungus used for the production of biomass-degrading enzymes [14–16]. *T. reesei* strains mainly secrete two cellobiohydrolases (CBHI and CBHII), two endoglucanases (EGI and EGII), β-glucosidase I (BGLI), xylanases, and auxiliary proteins [17]. Over the past few years, *T. reesei* strains have been engineered to improve cellulases yield and activity. For example, *PaCel3A* from *Polyporus arcularius* was introduced into *T. reesei* to increase filter paper activity, carboxymethyl cellulase activity, and biomass saccharification [18], whereas the bacterial *BsEXLE1* gene was overexpressed in *T. reesei* (Rut-C30) to synergistically enhance enzymatic saccharification in corn and *Miscanthus* straw [19].

In the most cellulolytic fungi, the GH7s consist of diverse CBHs and EGs, which forms the powerhouse of cellulose degradation in nature [20]. Particularly, the xylanase-like activity is a common feature shared by the EGs of GH7 family, and they are also of dual functional xylanase-cellobiohydrolase activities [21]. In this study, *LeGH7_3643* was initially cloned from *Lentinula edodes* W1 and overexpressed in wild-type *T. reesei* (Rut-C30). Two engineered *T. reesei* strains were then selected as GH7–5 and GH7–6, due to their secreted enzymes with much raised activities including FPase, pNPCase, CMCase, β-glucosidase, and xylanase. Unexpectedly, we found that *Le*GH7 purified from the engineered strain exhibited enzymatic activity for both cellulose and xylan hydrolysis. Using the total crude enzymes secreted from the two engineered strains, this study further examined the remarkably enhanced biomass enzymatic saccharification under physical and chemical pretreatments in five major bioenergy crops, providing a novel biotechnology applicable to lignocellulose-degradation enzyme productivity and biomass saccharification.

2. Materials and methods

2.1. Biomass samples and enzymes

Straws of rice (NPB), corn (B73), *Miscanthus* (*M. lutarioriparius*/Mlu04), cotton (C201), and rapeseed (ZH11) were harvested from an experimental field at Huazhong Agricultural University. The stem samples were dried and ground into powders, stored in a container until use. Avicel was purchased from China National Pharmaceutical Group Co., Ltd. β-xylanase, endoglucanase, and exoglucanase were bought from Megazyme Company. The *T. reesei* Rut-C30 strain was selected for this study, which was obtained from the ARS (NRRL) Culture Collection.

2.2. Bioinformatics analysis

The sequence of *Lentinula edodes* W1 was obtained from the Joint Genome Institute (GJI) Genome Portal (http://genome.jgi.doe.gov/portal/). A homology model of *Le*GH7 three-dimensional structures was created using SWISS-MODEL (http://swissmodel.expasy.org/) with SMTL ID 4v20.1.

2.3. Vector construction for LeGH7_3643 expression

The sequence of *Lentinula edodes* W1 was synthesized *in vitro* according to the codon preference of *T. reesei* [13]. This vector was used for *LeGH7_3643* expression in *T. reesei* (Rut-C30) (Figs. S1 and S2), which contained the Ppdc promoter, hygromycin B resistance gene, CBH I signal peptide, and His-tag-encoding sequence. The amplified pdc1 promoter, *LeGH7_3643*, and pdc1 terminator were fused to pCZF according to the manufacturer's instructions (Sangon Biotech, Shanghai, China), and the pCZF-GH7_3643 vector was generated and verified by sequencing.

2.4. Transformation and heterologous expression in T. Reesei

The pCZF-GH7_3643 vector was transformed into *T. reesei* (Rut-C30) *via A. tumefaciens*-mediated transformation, as previously described [22]. Eight transformants were verified using PCR and Western blot analyses (Fig. S3). The *T. reesei* transformants were grown on potato dextrose agar (PDA) at 30 °C for 7 days, and the flask cultivation conidia were harvested with ddH_2O and counted on a hemocytometer. The spores were collected and adjusted to a density of 10^7 spores/mL in a liquid cellulase-inducing medium containing 4 % (*w/v*) Avicel as the carbon source. The culture filtrate was harvested as a crude cellulase complex for enzyme activity assays and recombinant protein purification.

2.5. LeGH7 protein characterization

The crude cellulase complex of the engineered *T. reesei* strain was collected after a 7-d induction period with Avicel. After centrifugation (12,000 ×*g* for 10 min at 4 °C), the supernatant solution was purified using Ni-NTA Beads 6FF column at 25 °C for 30 min [18]. Bound proteins were eluted using imidazole and concentrated using Amicon Ultra centrifugal filters (cutoff, 30 kDa; Millipore). Protein samples were analyzed using SDS-PAGE and visualized using colloidal Coomassie blue staining. Individual enzyme assays were performed as previously described [23].

2.6. Filter paper activity and protein assay

The engineered strains were incubated with Avicel as a carbon source to secrete crude enzymes, as previously described [19]. The crude enzyme solution was centrifuged at 3000 ×*g* for 5 min to collect the supernatant for analysis of filter paper activity (FPA), protein level, and pH. The FPA assay was performed as previously described [24]. The reaction mixture with Whatman No.1 filter paper (50 mg) was conducted at 50 °C for 60 min, 2 mL DNS was added and boiled for 10 min. Protein concentration was determined using the Coomassie Brilliant Blue G250 dye assay, as previously described [25,26]. The absorbance of the protein-dye complex was measured at 595 nm using a UV–vis spectrometer (V—1100D, Shanghai MAPADA Instruments Co., Ltd. Shanghai).

2.7. Determination of cellulolytic activity

Exoglucanase (CBH), endoglucanase (EG), β-glucosidase (BG), and xylanase (XYN) activities *in vitro* were detected using p-nitrophenol-D-cellobioside (pNPC), carboxymethylcellulose (CMC—Na), D(−)-salicin, and beechwood xylan (Sigma-Aldrich Co. LLC., China National Pharmaceutical Group Co., Ltd., Shanghai Yuanye Bio-Technology Co., Ltd., China), as previously described [19]. The amount of reducing sugars released was determined using the dinitrosalicylic acid method (DNS).

2.8. Saccharification of pretreated biomass

For the saccharification assay, biomass samples were treated by 0.5

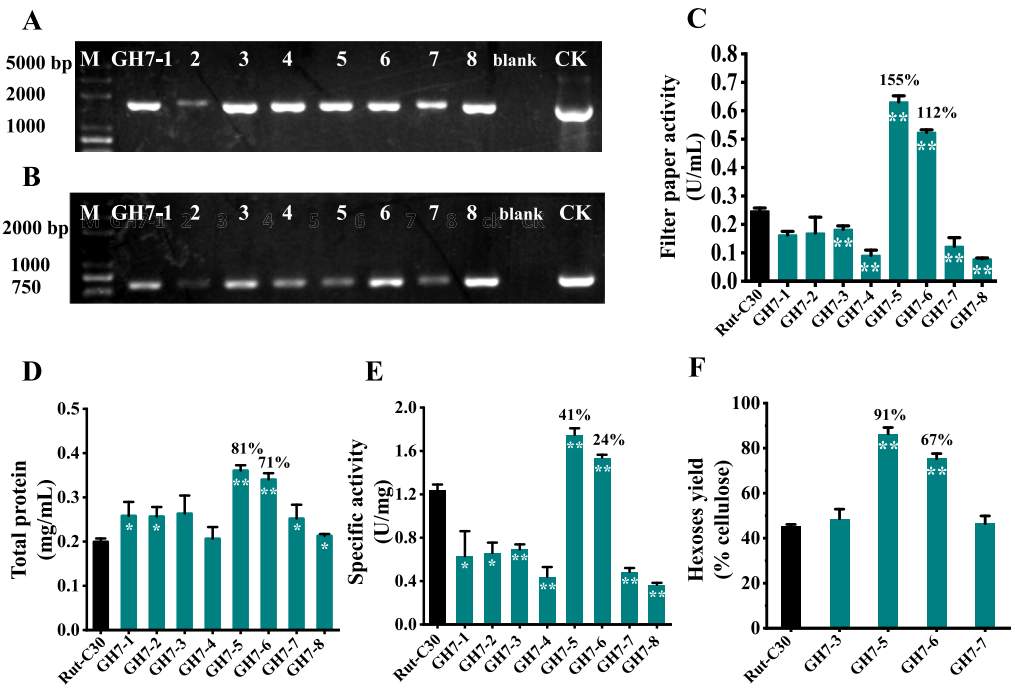

Fig. 1. Selection of desirable engineered *T. reesei* strains. (A, B) PCR detection of genomic DNA for mushroom *LeGH7_3643* gene identification in eight engineered strains by using two primers; (C-E) FPA, total protein content and specific activity of crude enzymes secreted by engineered strains and wild type (Rut-C30) using Avicel as carbon source; (F) Hexoses yield (% cellulose) released from enzymatic hydrolyses of steam-exploded lignocellulose of corn straw by using crude enzymes secreted from engineered strains; Data as mean ± SD; * and ** as *t*-test at $p < 0.05$ and $p < 0.01$ levels ($n = 3$).

%, 1.0 %, and 2.0 % NaOH (*w/v*), washed to reach neutral pH as previously described [27]. Steam explosion (SE) was performed using corn straw [28]. After biomass pretreatment, lignocellulose residues were collected by centrifugation (3000 ×*g* for 5 min) and incubated with total crude enzymes secreted from engineered *T. reesei* strains, as previously described [19]. After hydrolysis for 48 h, the supernatants were collected to determine their hexoses and pentoses according to the anthrone/H_2SO_4 and orcinol/HCl methods [29].

2.9. Yeast fermentation and bioethanol assay

The supernatants of enzymatic hydrolyses as described above was sterilized at 120 °C for 20 min, then incubated by *Saccharomyces cerevisiae* strain (Angel yeast Co., Ltd., Yichang, China) at 37 °C for 48 h. The fermentation liquid was distilled at 100 °C to collect ethanol liquor. The ethanol yield was determined using the $K_2Cr_2O_7$ method as previously described [28,30].

2.10. SDS-PAGE and Western blot analysis

Crude enzymes and eluted proteins were analyzed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using stain-free precast gels (Beijing Zoman Biotechnology Co., Ltd.). Western blotting was performed using a polyvinylidene difluoride (PVDF) membrane for separated proteins transformation. The membrane blocking was done by incubating it with 5 % nonfat dry milk for 1.5 h, rinsed with TTBS buffer (0.05 % Tween-20 in TBS). The blot was then incubated with HRP Rabbit Anti-His Tag diluted 1:1000 for 1 h at room temperature. After TTBS wash for three times, the blot was treated for 1 h with goat anti-rabbit secondary antibody IgG-HRP (Beijing Zoman Biotechnology Co., Ltd.) which had been diluted (1:5000) and prepared in the blocking solution. The color development was detected using ECL Plus Western Blotting Detection and scanned with GeneGnome XRQ (Syngene Inc., Maryland, US).

2.11. Statistical analysis

All experimental assays were performed in independent triplicate unless indicated, and Student's *t*-test was performed using SPSS 23 software (Inc., Chicago, IL).

3. Results and discussion

3.1. Desirable engineered T. Reesei strains selected for secreting high-activity enzymes

In this study, *LeGH7_3643* gene was initially isolated from *Lentinula edodes* W1, a typical mushroom organism, and the cloned *LeGH7* was effectively introduced into wild-type *T. reesei* (Rut-C30) under the control of the Ppdc promoter (Fig. S1). Using our previously established approach [19], eight transgenic *T. reesei* lines were screened for GH7–1 to GH7–8 based on genomic and western blot analyses (Fig. 1A-B; Figs. S2 and S3), and the secreted crude enzymes were collected for filter paper activity (FPA), total protein and specific activity assays *in vitro* (Fig. 1C-E). As a result, largely varied FPA, protein levels and specific activity were determined among the eight engineered lines, but only two GH7–5 and GH7–6 lines showed significantly higher FPA, protein content and specific activity than Rut-C30 did at $p < 0.01$ levels ($n = 3$). Furthermore, the crude enzymes of four representative engineered lines were incubated with steam-exploded residues of corn straw, and only the GH7–5 and GH7–6 lines had significantly higher hexose yields (% cellulose) than those of Rut-C30 from enzymatic hydrolysis (Fig. 1F), suggesting that the GH7–5 and GH7–6 lines are desirable engineered strains for the secretion of biomass-degrading enzymes with high activities. Although the other five transgenic lines also produced *Le*GH7 proteins, as detected by Western blot analysis, they had relatively low enzymatic activities, probably because of their spatial structure alteration from protein assembly and secretion. In addition, the large variations in transcript levels that occurred in the other five engineered *T. reesei* strains need to be explored by genomic analysis in the future.

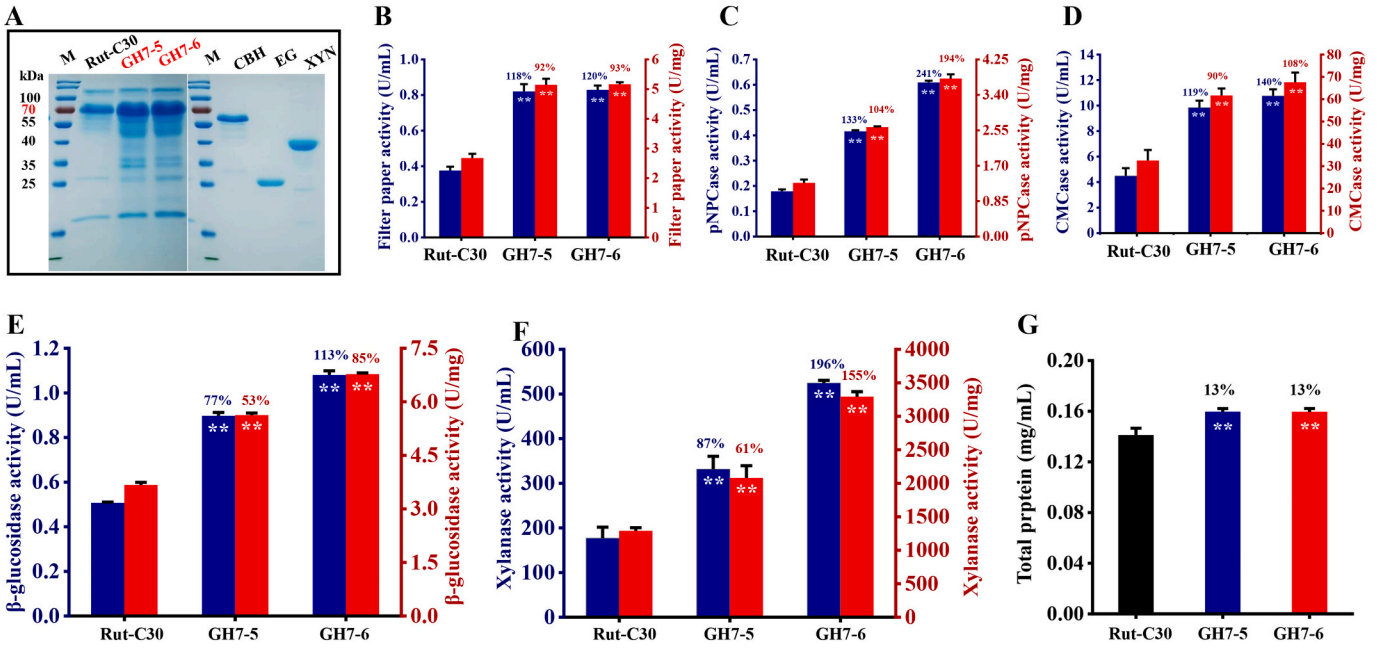

Fig. 2. Characterization of two desirable engineered GH7–5 and GH7–6 strains incubated with Avicel as carbon source for secreting high activity enzymes. (A) SDS-PAGE separation of engineered strains secreted enzymes and commercial enzymes (CBH, EG, XYN); (B—F) Individual enzyme activity assay *in vitro* for FPA, pNPCase, CMCase, β-glucosidase and xylanase activity; (G) Total protein content of crude solution secreted; Data as mean ± SD; * and ** as *t*-test at $p < 0.05$ and $p < 0.01$ levels ($n = 3$).

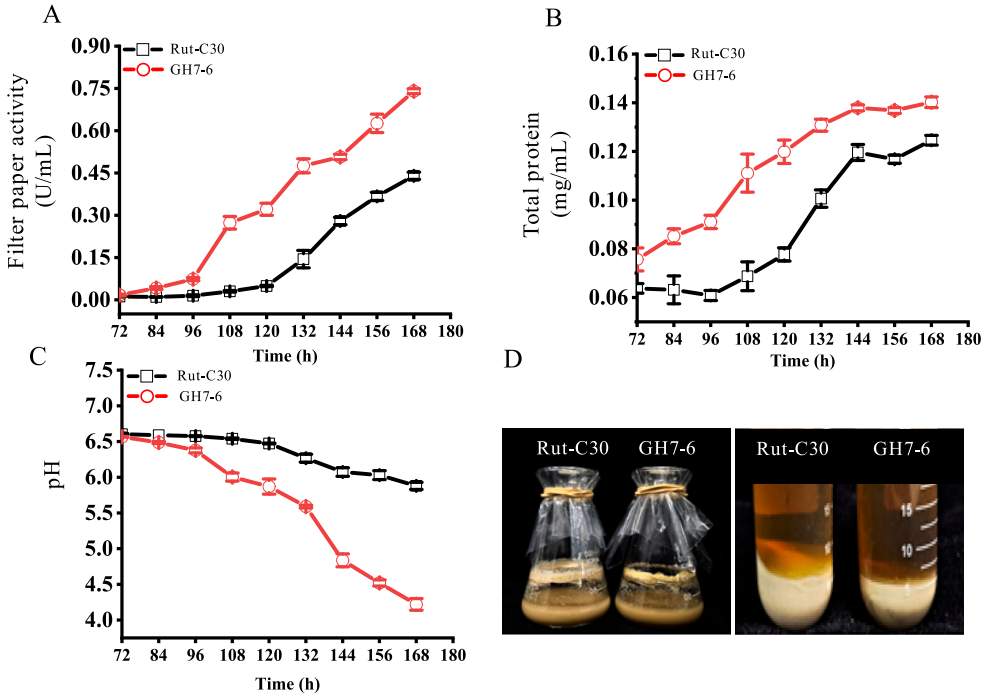

Fig. 3. Time-course observation of engineered GH7–6 strain for enzymes secretion from engineered GH7–6 strain and Rut-C30 by incubating with Avicel as carbon source. (A) FPA assay *in vitro*; (B, C) Total protein levels and pH values measurements; (D) Images of biomass residues after 168 h incubation. Data as mean ± SD ($n = 3$).

3.2. Significantly raised multiple enzyme activities in two desirable engineered strains

To test the desirable GH7–5 and GH7–6 strains, we performed SDS-gel protein separation of the crude enzymes secreted by the engineered GH7–5 and GH7–6 strains, and both strains displayed much stronger bands than Rut-C30 (Fig. 2A). Using our previously established enzyme assays *in vitro* [23,31], we determined significantly higher activities of five enzymes in two engineered strains at $p < 0.01$ levels ($n = 3$), compared to Rut-C30 (Fig. 2B-F). In particular, the GH7–6 strain was of the pNPCase, xylanase, CMCase, and β-glucosidase activities raised by 241 %, 196 %, 140 %, and 113 %, respectively. However, while the enzyme activity was measured as U/mg protein, the pNPCase, xylanase, CMCase, FPase and β-glucosidase activities were respectively raised by

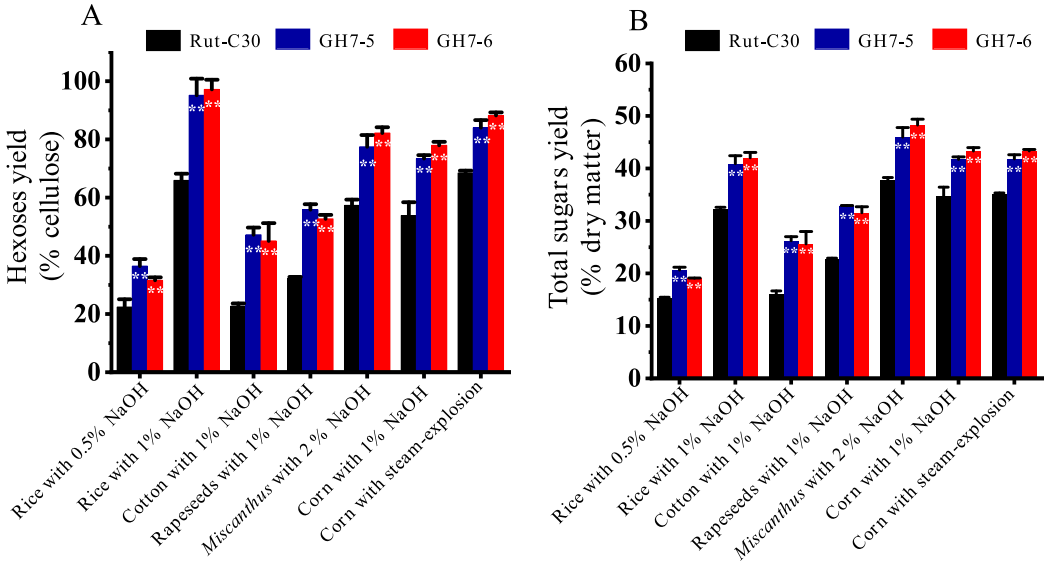

Fig. 4. Biomass saccharification of alkali-pretreated and steam-exploded lignocelluloses of five bioenergy crops by using crude enzymes secreted by engineered strains (GH7–5, GH7–6) and wild type (Rut-C30). (A) Hexoses yields (% cellulose); (B) Total sugars yields (hexoses and pentoses); Data as mean ± SD; * and ** as *t*-test at $p < 0.05$ and $p < 0.01$ levels ($n = 3$).

194 %, 155 %, 108 %, 93 % and 85 % (Fig. 2B-F). The two engineered strains secreted significantly more protein than Rut-C30, with an increased rate of 13 % (Fig. 2G). The results thus indicated that the two desirable engineered strains could secrete *Le*GH7 with high cellulases and xylanase activities, which was similar to the previous findings on *T. reesei* (Rut-C30) incubation with either the desirable de-lignin substrate of *Miscanthus* or the distinct cellulose nanofibril substrate of a natural rice mutant [23,31]. The two independent GH7–5 and GH7–6 strains showed similar enzyme activities and protein yields, suggesting that *Le*GH7 is consistently functional in the engineered *T. reesei* strains. Nevertheless, it would be interesting to test whether *Le*GH7 activity could be further improved using optimally inducing substrates such as cellulose nanofibers and xylan as carbon sources.

3.3. Stable secretion of high-activity enzymes by engineered GH7–6 strain

To understand the desirable GH7–6 strain secreting enzymes with higher activities, we conducted a time-course assay for enzyme activities from GH7–6 and Rut-C30 incubated with Avicel as the carbon source (Fig. 3). After 72 h of incubation, GH7–6 began to secrete enzymes with higher FPAs than Rut-C30, and the largest difference in FPAs was observed between GH7–6 and Rut-C30 after 108 h of incubation (Fig. 3A). Accordingly, the GH7–6 strain consistently secreted higher protein levels than Rut-C30 from 72 to 180 h of incubation, and the greatest differences in protein levels were observed at 108 h (Fig. 3B). Furthermore, this study consistently measured the reduced pH values of the GH7–6 secreted solution compared to that of Rut-C30 (Fig. 3C). In particular, the lowest pH value of the GH7–6 secreted solution was close to 4.22, whereas the pH of Rut-C30 remained at 5.88 after 108 h of incubation, which should account for the distinct FPAs and protein levels examined in the GH7–6 and Rut-C30 strains. As several fungal GH7 families have been characterized with cellulase or xylanase activities at an optimal pH of 3.0–6.0 [32–34], this may explain why the engineered GH7–6 strain secreted the enzyme solution under acidic conditions, which should be one of the major causes for the relatively higher cellulases and xylanase activities examined. Finally, significantly fewer biomass residues were observed in GH7–6 than in Rut-C30 after 186 h of incubation (Fig. 3D), indicating that much more of the Avicel substrate was consumed by the GH7–6 strain because of its higher FPA and protein levels. Hence, the time-course observation indicated the stable secretion of higher-activity enzymes in the engineered GH7–6 strain.

3.4. Consistently enhanced biomass enzymatic saccharification in five bioenergy crops

As the GH7–5 and GH7–6 strains could secrete multiple cellulases and xylanase with high activities, this study supplied their crude enzymes into the enzymatic hydrolyses of mature straws/stalks in five major bioenergy crops, including rice, corn, rapeseeds, cotton, and *Miscanthus* (Fig. 4), which have been characterized with different lignocellulose compositions and features [35–43]. After steam explosion and mild alkali (NaOH) pretreatments, seven pretreated lignocellulose substrates were incubated with the enzymes secreted by engineered GH7–5 and GH7–6 strains, resulting in significantly higher hexoses yields (% cellulose) than the enzymes secreted by Rut-C30 (Fig. 4A). Notably, after 1 % NaOH pretreatment, the rice straw sample showed near-complete cellulose hydrolysis with hexoses yield of 97 % (% cellulose) by GH7–6 secreted enzymes, whereas only 66 % hexoses yield was obtained by Rut-C30 secreted enzymes. Furthermore, lignocellulose hydrolysis by GH7–5 and GH7–6 secreted enzymes also led to much higher total sugar (hexoses and pentoses) yields (% dry matter) than those of Rut-C30 ($p < 0.01$ levels ($n = 3$)) in all bioenergy crops examined (Fig. 4B). Because pentoses are released by xylanase hydrolysis [34], these results support the significantly increased activities of both cellulases and xylanase secreted by the GH7–5 and GH7–6 strains, as described above. Taken together, even though those five bioenergy crops represent distinct lignocellulose substrates after alkali and steam-explosion pretreatments are commonly effective for partial lignin and hemicellulose extractions [7,23,29,36,38,39,44], the enzymes secreted by the GH7–5 and GH7–6 strains should have consistent and stable catalytic activities for biomass saccharification.

3.5. Distinct yeast fermentation and bioethanol conversion

Using all hexoses released from enzymatic hydrolysis of representative rice and *Miscanthus* straw after NaOH pretreatment, yeast fermentation was conducted based on our previously established method [45,46]. The GH7–5 and GH7–6 samples achieved significantly higher bioethanol yields (% dry matter) than the Rut-C30 sample at $p <$ 0.01 levels (Fig. 5A), which is consistent with the increased hexoses yields examined above. In particular, the bioethanol yield achieved from

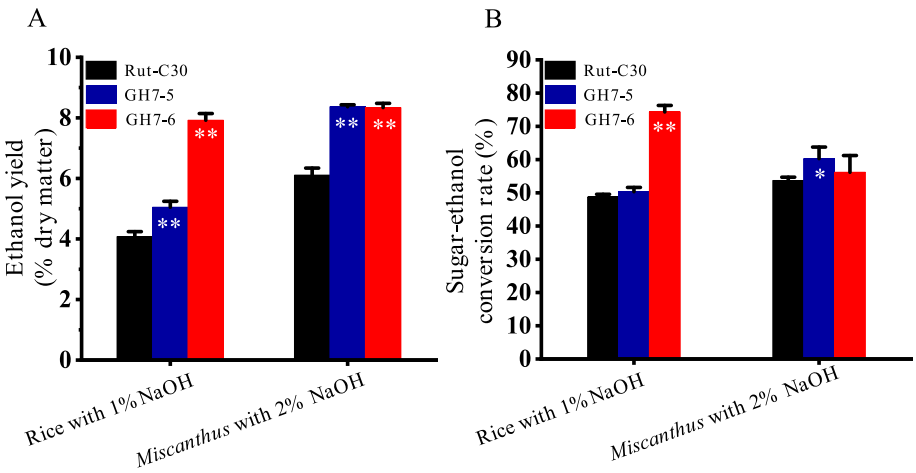

Fig. 5. Bioethanol production by yeast fermentation with hexoses released as shown in Fig. 4. (A) Bioethanol yield (% dry matter); (B) Sugar-ethanol conversion rate; Data as mean ± SD; * and ** as *t*-test at $p < 0.05$ and $p < 0.01$ levels ($n = 3$).

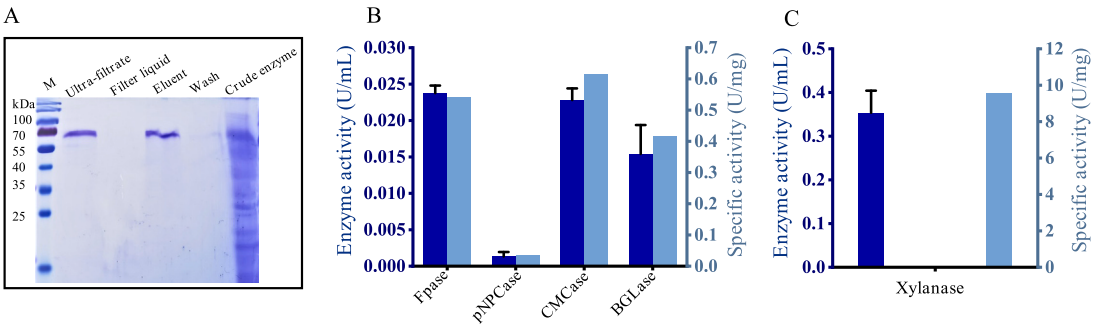

Fig. 6. Characterization of individual *Le*GH7 enzyme purified from crude solution secreted by engineered (GH7–6) strain. (A) SDS-gel profiling of *Le*GH7 protein purified by His-tag; (B, C) Individual enzyme assay *in vitro* using purified *Le*GH7 enzyme; Data as mean ± SD ($n = 3$).

yeast fermentation with rice straw increased by 1.9 fold in the GH7–6 sample compared to that in the Rut-C30 sample. Regarding the calculation of hexose-ethanol conversion, the GH7–6 showed a significantly higher conversion rate than Rut-C30 ($p < 0.01$) from NaOH-pretreated rice straw, whereas GH7–5 had a slightly higher conversion rate ($p < 0.05$) from pretreated *Miscanthus* straw (Fig. 5B). Since the sugar-ethanol conversion rate is responsible for the toxic compounds that inhibit yeast fermentation [47], the results suggest that biomass saccharification catalyzed by GH7–5 and GH7–6 secreted enzymes could produce relatively less toxic compounds than Rut-C30, which may be due to the engineered GH7 strains secreting proteins that are active for the adsorption of toxic compounds. In addition, based on xylose-ethanol conversion rate in theory, this study estimated that total bioethanol yield could be increased to 12.4 % (% dry matter) (Table S1) [48,49].

3.6. Dual enzymatic activities of mushroom LeGH7 for cellulose and xylan hydrolyses

To determine the mechanism by which the engineered GH7 strain secretes higher-activity enzymes to consistently enhance biomass saccharification in diverse bioenergy crops, we purified *Le*GH7 from the crude proteins secreted by the engineered strain (Fig. 6A). By performing individual enzyme assays *in vitro*, the purified *Le*GH7 enzyme was found to have high activity for three major cellulases, FPase, CMCase, and BGLase (Fig. 6B), providing direct evidence of the effectiveness of cellulose digestion. However, the purified *Le*GH7 enzyme retained a relatively low activity for pNPCase/CBH, which differs from previous reports on other microorganisms [50,51]. Unexpectedly, the *Le*GH7 enzyme showed the highest xylanase activity (Fig. 6C), which should be accountable for the efficient lignocellulose hydrolyses examined in bioenergy crops with varied hemicellulose levels [33,34]. As purified *Le*GH7 could act as multiple cellulases and an active xylanase, it should play a synergistic enhancement role in the biomass enzymatic saccharification examined in the five bioenergy crops. However, it remains interesting to determine how *Le*GH7 has relatively low CBH and high xylanase activity by exploring its catalytic and binding domains.

4. Conclusions

By screening desirable GH7–5 and GH7–6 strains that overproduce mushroom *Le*GH7 in *T. reesei* (Rut-C30), we examined the significantly elevated enzyme activities for efficient lignocellulose digestion. By supplying crude enzymes secreted by the engineered strains, this study determined consistently enhanced biomass saccharification and bioethanol production of distinct lignocellulose substrates after steam explosion and mild alkali pretreatments of five bioenergy crops. Notably, individual *Le*GH7 enzymes were purified with relatively higher cellulases and xylanase activities, which should be responsible for the synergistic enhancement of biomass saccharification. Therefore, the findings of this study provide an applicable biotechnology for high-activity enzyme production and biomass saccharification of bioenergy crops.

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CRediT authorship contribution statement

Peng Liu: Methodology, Investigation, Formal analysis, Software, Writing – original draft. **Yihong Wang:** Investigation, Methodology, Formal analysis. **Heng Kang:** Validation, Data curation. **Yanting Wang:** Validation, Project administration. **Hua Yu:** Investigation. **Hao Peng:** Formal analysis, Methodology. **Boyang He:** Methodology. **Chengbao Xu:** Formal analysis, Methodology. **Kai-Zhi Jia:** Formal analysis, Methodology. **Shilin Liu:** Writing – review & editing, Formal analysis. **Tao Xia:** Methodology, Formal analysis, Investigation, Writing – review & editing. **Liangcai Peng:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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