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Heterologous expression and characterization of novel GH12 β -glucanase and AA10 lytic polysaccharide monooxygenase from *Streptomyces megasporus* and their synergistic action in cellulose saccharification

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Abstract

Background The combination of cellulase and lytic polysaccharide monooxygenase (LPMO) is known to boost enzymatic saccharification of cellulose. Although the synergy between cellulases (GH5, 6 or 7) and LPMOs (AA9) has been extensively studied, the interplay between other glycoside hydrolase and LPMO families remains poorly understood.

Results In this study, two cellulolytic enzyme-encoding genes *SmBglu12A* and *SmLpmo10A* from *Streptomyces megasporus* were identified and heterologously expressed in *Escherichia coli*. The recombinant *SmBglu12A* is a non-typical endo- β -1,4-glucanase that preferentially hydrolyzed β -1,3-1,4-glucans and slightly hydrolyzed β -1,4-glucans and belongs to GH12 family. The recombinant *SmLpmo10A* belongs to a C1-oxidizing cellulose-active LPMO that catalyzed the oxidation of phosphoric acid swollen cellulose to produce celloaldonic acids. Moreover, individual *SmBglu12A* and *SmLpmo10A* were both active on barley β -1,3-1,4-glucan, lichenan, sodium carboxymethyl cellulose, phosphoric acid swollen cellulose, as well as Avicel. Furthermore, the combination of *SmBglu12A* and *SmLpmo10A* enhanced enzymatic saccharification of phosphoric acid swollen cellulose by improving the native and oxidized cello-oligosaccharides yields.

Conclusions These results proved for the first time that the AA10 LPMO was able to boost the catalytic efficiency of GH12 glycoside hydrolases on cellulosic substrates, providing another novel combination of glycoside hydrolase and LPMO for cellulose enzymatic saccharification.

Keywords β -Glucanase, Lytic polysaccharide monooxygenase, Synergy, Cellulose saccharification, *Streptomyces megasporus*

Background

Cellulose is the most prevalent renewable carbohydrate in nature, widely found in lignocellulosic biomass, microbes, and animals [1]. It consists of linear chains of several hundred to over ten thousand glucose molecules connected by β -1,4-glycosidic linkages [2]. These long polymer chains are arranged in a highly ordered

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crystalline structure, severely limiting the accessibility of microbial enzymes to the cellulose chains [3]. To efficiently utilize cellulose, cellulolytic microorganisms have evolved multiple strategies to decompose cellulose into sugar monomers, including hydrolysis and oxidation [4]. As for enzymatic hydrolysis of cellulose, three main kinds of glycoside hydrolases such as cellobiohydrolase, endoglucanase, and β -glucosidase, act synergistically to break β -1,4-glycoside linkages. With respect to the oxidation cleavage of cellulose, critical enzymes known as lytic polysaccharide monoxygenases (LPMOs) catalyze the oxidative breakage of the β -1,4-glycosidic linkages in the crystalline regions using O_2/H_2O_2 and electrons [5, 6]. The interplay between glycoside hydrolases and LPMOs in the enzymatic saccharification of cellulose remains to be elucidated [7, 8].

Recently, the synergistic action between glycoside hydrolases and LPMOs on the enzymatic deconstruction of cellulose has attracted more and more attention from researchers [9–13]. Several studies have reported that supplementation of cellulases from glycoside hydrolase families 5, 6 or 7 (GH5, 6 or 7) with LPMOs from the auxiliary activity family 9 (AA9) could significantly enhance enzymatic saccharification of cellulose [14, 15]. Besides, the promoting effect of AA9 LPMOs on cellulase activity is enzyme-dependent [7, 8, 10]. Specifically, C1-oxidizing AA9 LPMOs promote catalytic activity of GH5 endoglucanases and GH6 nonreducing-end cellobiohydrolases, respectively [7, 8]. C1/C4-oxidizing AA9 LPMOs significantly improve hydrolytic efficiency of GH6 nonreducing-end cellobiohydrolases and GH7 reducing-end cellobiohydrolases [10]. Although the synergistic interaction between cellulases (GH5, 6 or 7) and LPMOs (AA9) has been extensively studied, the interplay between other glycoside hydrolase and LPMO families is still poorly understood.

Streptomyces species are a class of efficient cellulose degraders in nature, which produce a variety of extracellular cellulose-degrading hydrolases (GH5, 6 or 12) and oxidative enzymes (AA10) [16–18]. Herein, two adjacent cellulolytic enzyme genes *SmBglu12A* and *SmLpmo10A* were identified from the genome of *Streptomyces megasporus* based on the carbohydrate-active enzymes annotation. In this work, the two genes were successfully cloned and expressed in *Escherichia coli* strains Origami DE3 and Shuffle T7-B, respectively, after screening for multiple molecular chaperones. The purified recombinant *SmBglu12A* and *SmLpmo10A* were biochemically characterized, with particular attention to their performance in degrading PASC (phosphoric acid swollen cellulose). The synergistic action between *SmBglu12A* and *SmLpmo10A* in the enzymatic saccharification of PASC was evaluated. The results demonstrated for the first time

that the combination of *SmBglu12A* and *SmLpmo10A* enhanced enzymatic saccharification of cellulose by improving the native and oxidized cello-oligosaccharides yields.

Results and discussion

Cloning and sequence analysis of *SmBglu12A* and *SmLpmo10A*

Streptomyces species have been reported to efficiently deconstruct natural polymeric macromolecules using extracellular carbohydrate-active enzymes, including cellulose, lignin, and chitin [19, 20]. Herein, two adjacent cellulolytic enzyme genes *SmBglu12A* and *SmLpmo10A* were identified from the genome of *S. megasporus* according to the annotation for carbohydrate-active enzymes against the dbCAN2 database [21]. As shown in Additional file 1, two open reading frames of *SmBglu12A* and *SmLpmo10A* were composed of 1128 bp and 1083 bp nucleotides encoding proteins of 375 and 360 amino acids, respectively. The *SmBglu12A* contained a signal peptide with 37 residues, a catalytic region corresponding to the family 12 of glycoside hydrolases, and a binding domain belonging to the carbohydrate binding module family 2 (Fig. 1A). The BLASTP searches against the UniProt web server showed that *SmBglu12A* exhibited the highest sequence identity to the exoglucanase (51.4%) from *Cellulomonas fimi*. The *SmLpmo10A* harbored a N-terminal signal peptide with 33 residues, a catalytic region corresponding to the auxiliary activity family 10, and a C-terminal binding domain belonging to the carbohydrate binding module family 2 (Fig. 1A). BLAST analysis showed the lytic cellulose monoxygenase from *Streptomyces ambofaciens* as the closest homolog, exhibiting 80.8% amino acid identity. Meanwhile, considering that members of the auxiliary activity family 10 of LPMOs were active on cellulose or/and chitin [6, 22], the evolutionary relationship between *SmLpmo10A* and characterized LPMOs in the AA10 family was further analyzed. The result indicated that *SmLpmo10A* might be a C1 oxidizing LPMO displayed oxidative activity on cellulose (Fig. 1B).

Heterologous expression and purification of *SmBglu12A* and *SmLpmo10A*

SmBglu12A and *SmLpmo10A* were initially expressed as inclusion bodies in the *E. coli* Transetta (DE3), which might be attributed to the reducing environment of bacteria inhibiting the correct formation of disulfide bridges [23]. Based on several strategies that had been developed in recent years to improve the ability of disulfide linkages formation [24, 25], the co-expression of different chaperones plasmids in various host strains of *E. coli* was

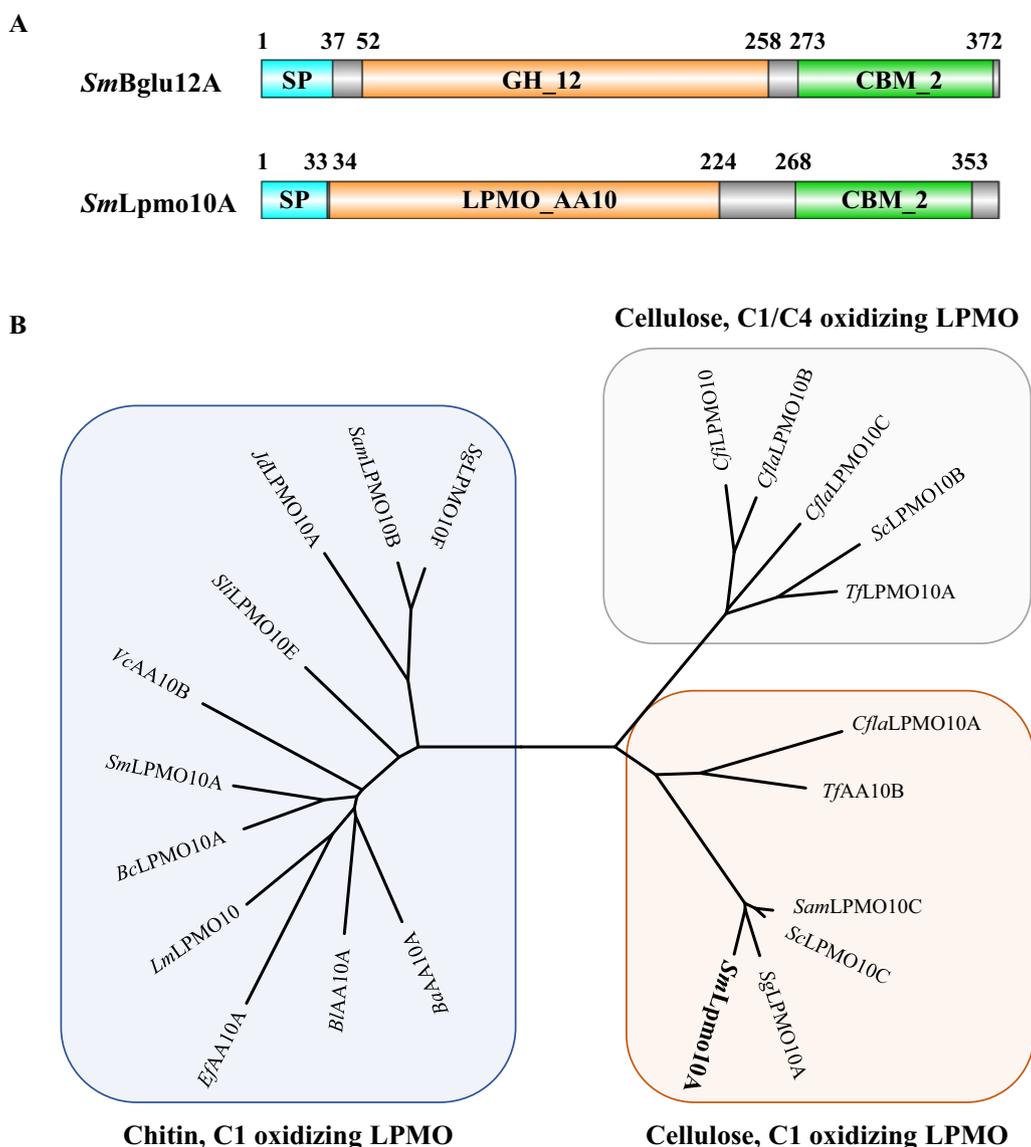


Fig. 1 Schematic illustration of *SmBglu12A* and *SmLpmo10A* domains (A). SP: the signal peptide; GH_12: glycoside hydrolase family 12; CBM_2: carbohydrate binding module family 2; LPMO_AA10: lytic polysaccharide monooxygenase_auxiliary activity family 10. Evolution analysis of *SmLpmo10A* and characterized LPMO_AA10 (B). The maximum likelihood tree was constructed using MEGA7 under the default parameters. *CflaLPMO10A* from *Cellulomonas flavigena*, D5UGB1; *TfAA10B* from *Thermobifida fusca*, QOS58239.1; *SamLPMO10C* from *Streptomyces ambofaciens*, A3KKC4; *ScLPMO10C* from *Streptomyces coelicolor*, Q9RJC1; *SgLPMO10A* from *Streptomyces griseus*, WP_164360609.1; *CflaLPMO10* from *Cellulomonas fimi*, F4H6A3; *CflaLPMO10B* from *Cellulomonas flavigena*, D5UGA8; *CflaLPMO10C* from *Cellulomonas flavigena*, D5UH31; *ScLPMO10B* from *Streptomyces coelicolor*, Q9RJC1; *TfLPMO10A* from *Thermobifida fusca*, Q47QG3; *SgLPMO10F* from *Streptomyces griseus*, B1VN59; *SamLPMO10B* from *Streptomyces ambofaciens*, WP_053126548.1; *JdLPMO10A* from *Jonesia denitrificans*, C7R4I0; *SlLPMO10E* from *Streptomyces lividans*, WP_003975967.1; *VcAA10B* from *Vibrio cholerae*, Q9KLD5; *SmLPMO10A* from *Serratia marcescens*, WP_060560026.1; *BcLPMO10A* from *Bacillus cereus*, Q81CE4; *LmLPMO10* from *Listeria monocytogenes*, Q8Y4H4; *EfAA10A* from *Enterococcus faecalis*, Q838S1; *BIAA10A* from *Bacillus licheniformis*, Q62YN7; *BaAA10A* from *Bacillus amyloliquefaciens*, WP_065521218.1

attempted to achieve soluble expression of *SmBglu12A* and *SmLpmo10A*.

After the induction and lysis using sonication, SDS-PAGE analysis of these cell lysate supernatants showed that *SmBglu12A* and *SmLpmo10A* were successfully

expressed as the soluble form in two different *E. coli* strains Origami DE3 and Shuffle T7-B with plasmids pTf16 and pG-KJE8 co-expression, respectively. The cell lysates of recombinant *SmBglu12A* and *SmLpmo10A* were applied on nickel column affinity chromatography

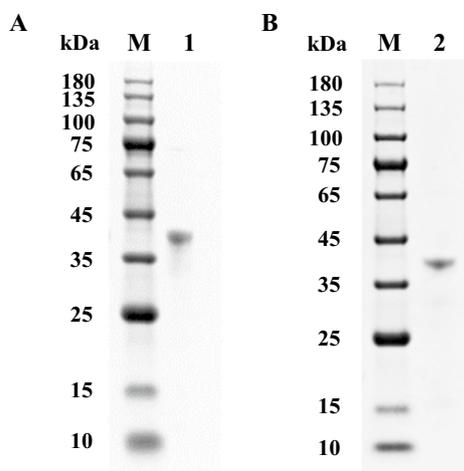


Fig. 2 SDS-PAGE analysis of the purified *SmBglu12A* (A) and *SmLpmo10A* (B). Lanes M, 1, and 2 indicated protein marker, *SmBglu12A*, and *SmLpmo10A*, respectively

and anion-exchange chromatography. SDS-PAGE analysis of purified recombinant *SmBglu12A* and *SmLpmo10A* showed single bands at 35–45 kDa (Fig. 2), which were similar to their calculated molecular weight of 38.7 kDa and 38.0 kDa, respectively.

Biochemical characterization of purified *SmBglu12A*

The purified *SmBglu12A* exhibited optimal pH at 6.0, with relative activities of more than 70% between pH values of 5.0 and 9.0 (Fig. 3A). In contrast, when pH was below 5.0, the enzyme activity of *SmBglu12A* showed a remarkable drop, exhibiting only 25% (pH 4.0) and 13% (pH 3.0) of its maximum activity. *SmBglu12A* was stable throughout a wide pH range of 3.0–9.0, maintaining over 80% of its initial activity after 1 h of treatment (Fig. 3C). The optimal temperature of *SmBglu12A* was 50 °C, and more than 95% of the maximum activity was retained at 60 °C (Fig. 3B). *SmBglu12A* retained 84% and 66% of its original activity when incubated at 40 °C and 50 °C for 1 h, respectively. When the temperature was raised above

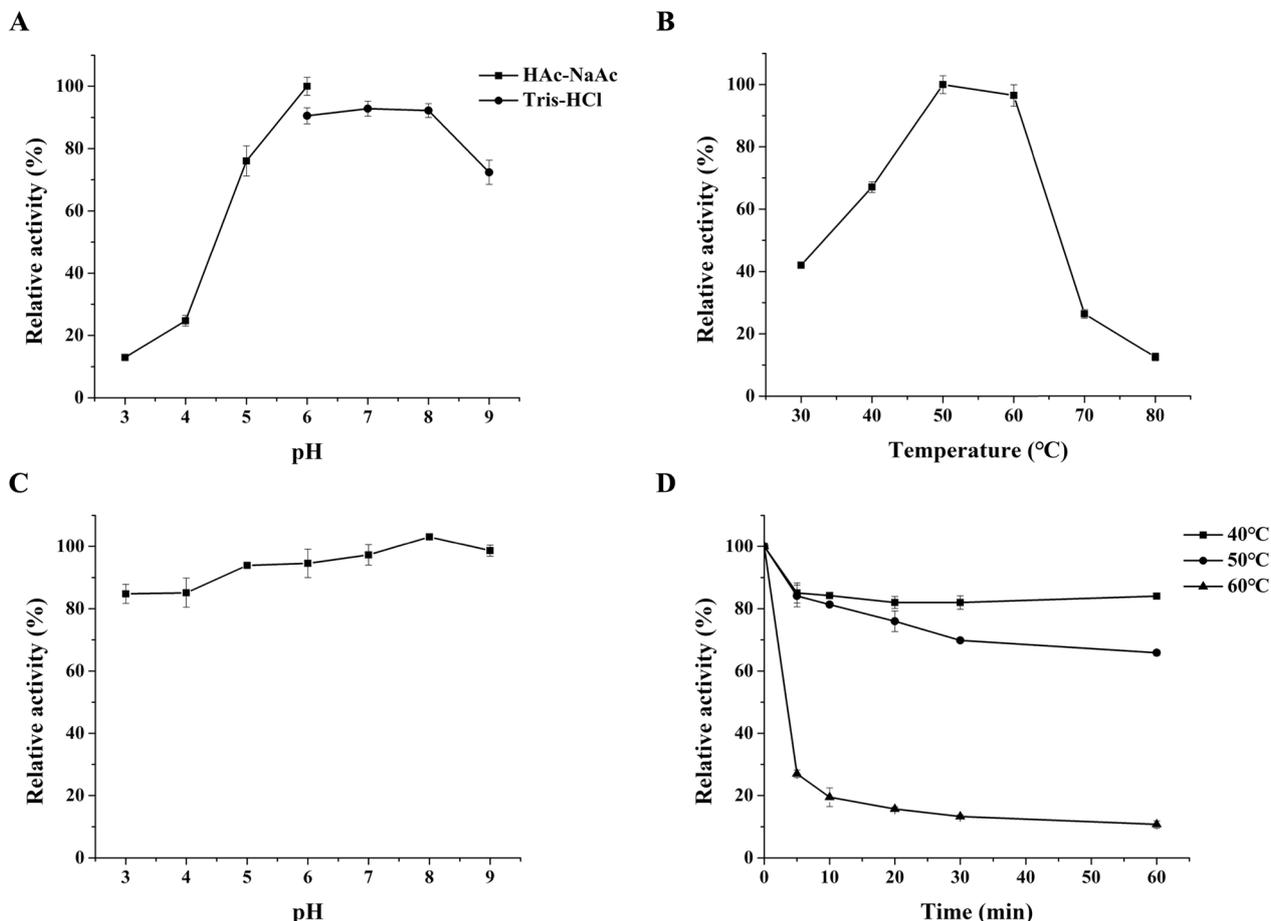


Fig. 3 Effects of pH and temperature on the activity and stability of the purified recombinant *SmBglu12A* using the substrate β -1,3-1,4-glucan. **A** Optimum pH; **B** Optimum temperature; **C** pH stability. The initial activity before treatment was defined as 100%; **D** thermostability. The initial activity before treatment was defined as 100%

60 °C, the enzyme activity of *SmBglu12A* decreased significantly (Fig. 3D).

The purified recombinant *SmBglu12A* could catalyze the hydrolysis of various substrates containing β -1,4-glycosidic bonds, including β -1,3-1,4-glucan, lichenan, carboxymethylcellulose sodium salt (CMCNa), PASC, as well as Avicel. The specific activity of *SmBglu12A* at pH 6.0 and 50 °C for β -1,3-1,4-glucan, lichenan, CMCNa, PASC, as well as Avicel was 239.9 ± 12.5 , 189.7 ± 9.3 , 49.5 ± 0.7 , 43.6 ± 1.4 , and 18.5 ± 4.2 U/mg (Table 1), suggesting that *SmBglu12A* preferentially hydrolyzed β -1,3-1,4-glucans but slightly hydrolyzed β -1,4-glucans. This feature was different from typical glucanases in GH5 or 7 families that preferentially break down β -1,4-glucans such as CMCNa [26]. Moreover, *SmBglu12A* was not active on laminarin (β -1,3-glucan), suggesting that it strictly hydrolyzed the β -1,4-glycosidic bonds not β -1,3-glycosidic bonds in polysaccharides. Meanwhile, the values for K_m , V_{max} , and k_{cat} of *SmBglu12A* at pH 6.0 and 50 °C were 0.3 mg/mL, 331.4 μ mol/mg/min, and 201.3 s⁻¹, respectively.

Table 1 Substrate specificities of the purified recombinant *SmBglu12A* for the hydrolysis of various polysaccharide substrates in the pH 6.0 Tris–HCl buffer at 50 °C

Substrate	Specific activity (U/mg)
β -1,3-1,4-Glucan	239.9 ± 12.5
Lichenan	189.7 ± 9.3
CMCNa	49.5 ± 0.7
PASC	43.6 ± 1.4
Avicel	18.5 ± 4.2

To further elucidate the β -glucans degradation pattern of *SmBglu12A*, hydrolytic products of β -1,3-1,4-glucan and PASC were analyzed during different time periods. As shown in Fig. 4, cellobiose, cellotriose and cellotetraose were the major degradation products β -1,3-1,4-glucan and PASC at the initial stages of β -1,3-1,4-glucan and PASC hydrolysis. With the increase of reaction time, the concentration of cellobiose, cellotriose and cellotetraose increased. These results revealed that *SmBglu12A* was an endo-type glucanase.

Biochemical characterization of purified *SmLpmo10A*

The purified recombinant *SmLpmo10A* was incubated with PASC to assess LPMO activity and regioselectivity by analyzing the oxidized cello-oligosaccharides. As shown in Additional file 2, *SmLpmo10A* exhibited optimal pH at 6.0, with relative activities of more than 40% between pH values of 3.0 and 9.0. The optimal temperature of *SmLpmo10A* was 30 °C, and more than 60% of the maximum activity was retained at 50 °C. Besides, the purified *SmLpmo10A* catalyzed the oxidation of PASC to yield new chromatographic peaks (Fig. 5A), corresponding to C1 oxidized cello-oligosaccharides produced by recombinant cellobiose dehydrogenase from *Irpex lacteus* acting on cello-oligosaccharides [27]. These results indicated that the recombinant *SmLpmo10A* belonged to a C1-oxidizing cellulose-active LPMO.

In addition to PASC, *SmLpmo10A* was also found to act on various cellulosic substrates, including Avicel, CMCNa, lichenan, and β -1,3-1,4-glucan (Fig. 5). Specifically, the action of *SmLpmo10A* towards Avicel and PASC produced natural and oxidized cello-oligosaccharides, exhibiting a relative low specific activity on Avicel. In contrast, *SmLpmo10A* exhibited weak endo-glucanase-like activity towards CMCNa, lichenan,

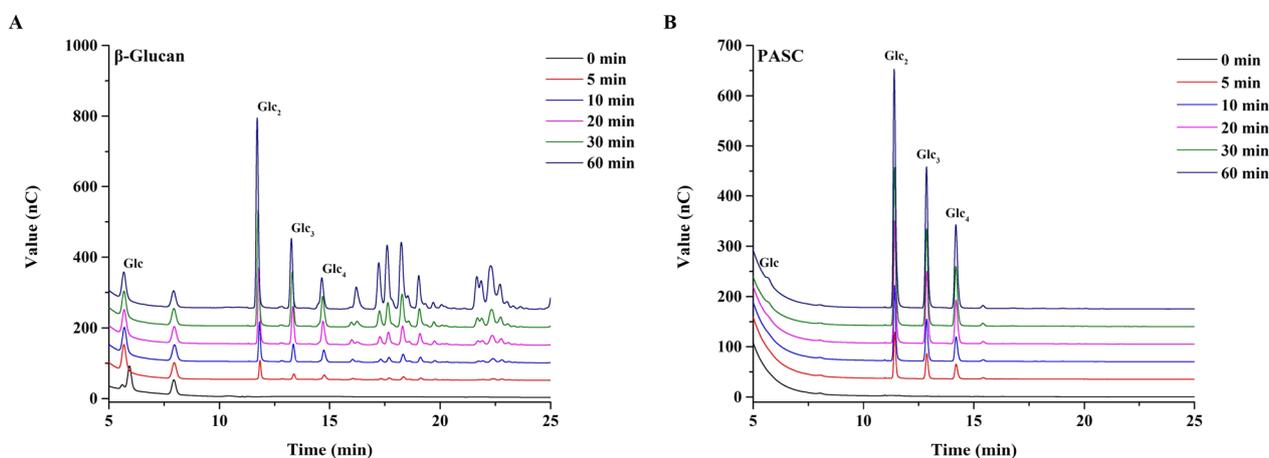


Fig. 4 HPAEC analysis of hydrolytic products of β -1,3-1,4-glucan (A) and PASC (B) by *SmBglu12A* in the pH 6.0 Tris–HCl buffer at 50 °C. Glc, Glc₂, Glc₃, and Glc₄ indicated glucose, cellobiose, cellotriose, and cellotetraose, respectively

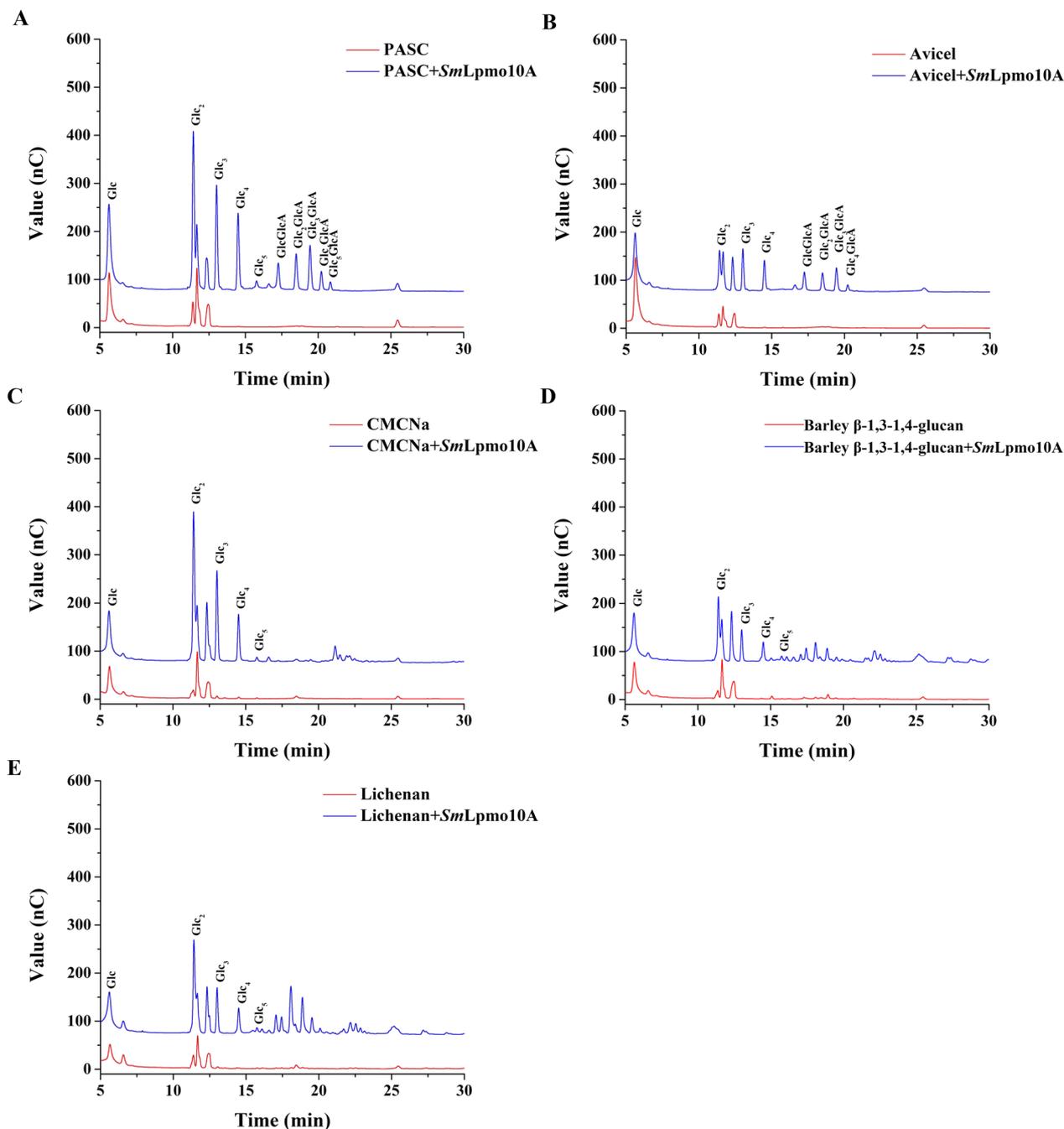


Fig. 5 HPAEC analysis of degradation products of PASC (A), Avicel (B), CMCNa (C), β -1,3-1,4-glucan (D), and Lichenan (E) by *SmLpmo10A* in the pH 6.0 acetate buffer at 50 °C for 24 h. Glc: glucose; Glc_2 : cellobiose; Glc_3 : cellotriose; Glc_4 : cellotetraose; Glc_5 : cellopentaose; $GlcGlcA$: cellobionic acid; Glc_2GlcA : cellotronic acid; Glc_3GlcA : cellotetraonic acid; Glc_4GlcA : cellopentaonic acid; Glc_5GlcA : cellohexaonic acid

and β -1,3-1,4-glucan, which was similar to the typical characteristics of AA9 LPMOs [6, 28].

Synergistic effect between *SmBglu12A* and *SmLpmo10A* on the cellulose saccharification

To evaluate whether AA10 LPMO could boost enzymatic saccharification of cellulosic substrates by GH12

β -glucanase, the synergistic effect of purified *SmBglu12A* and *SmLpmo10A* on PASC was investigated. As shown in Fig. 6A, it was observed that the production of natural cello-oligosaccharides in different groups gradually increased as time progressed. The production of natural cello-oligosaccharides obtained with the enzymes *SmBglu12A* and *SmLpmo10A* significantly

exceeded the total amount produced with the sole enzyme *SmBglu12A* and *SmLpmo10A* in the whole process of saccharification, which might be attributed to more cellulose chain ends generation and crystalline cellulose surface amorphization with the action of *SmLpmo10A* [14]. This indicates that the presence of *SmLpmo10A* boosts the turnover of *SmBglu12A* on cellulosic substrates. After 12 h of incubation at pH 6.0 and 50 °C, the production of natural cello-oligosaccharides obtained with mixture enzymes increased by approximately 8.8% compared to the sum of natural cello-oligosaccharides produced with individual *SmBglu12A* and *SmLpmo10A*. Apart from that, there was also a significant improvement in the production of oxidized cello-oligosaccharides at the early-stage degradation (Fig. 6B). This result showed that the turnover of *SmLpmo10A* was also enhanced by the presence of *SmBglu12A*, which was similar to the phenomenon between endoglucanase (GH5 or 7) and LPMO (AA9) [7, 29]. Generally speaking, the combination of *SmBglu12A* and *SmLpmo10A* resulted in a synergistic effect on the degradation of PASC by improving the natural and oxidized cello-oligosaccharides yields.

Conclusions

In this work, two novel cellulolytic enzyme genes *SmBglu12A* and *SmLpmo10A* from *S. megasporae* were discovered, cloned, and successfully expressed in *E. coli* strains Origami DE3 and Shuffle T7-B, respectively, after screening for multiple molecular chaperones. The recombinant *SmBglu12A* and *SmLpmo10A* are a non-typical GH12 endo- β -1,4-glucanase and a C1-oxidizing cellulose-active

AA10 LPMO, respectively. Individual *SmBglu12A* and *SmLpmo10A* were both active on various cellulosic substrates, including barley β -1,3-1,4-glucan, lichenan, CMCNa, PASC, and Avicel. Most importantly, *SmBglu12A* and *SmLpmo10A* exhibited a significant synergistic activity on PASC degradation by improving the native and oxidized cello-oligosaccharides yields. This study proved for the first time that AA10 LPMO could boost the activity of GH12 glycoside hydrolases on cellulose, providing another efficient combination of glycoside hydrolase and LPMO for cellulose enzymatic saccharification.

Methods

Strains and plasmids

S. megasporae ACCC 41475 was obtained from the Agricultural Cultural Collection of China (ACCC, Beijing, China). Different *E. coli* strains containing Origami DE3 and Shuffle T7-B were purchased from ZOMANBIO Co., Ltd (Beijing, China). The expression vector pET22b and chaperone plasmids pTf16 and pG-KJE8 were purchased from Novagen Co., Ltd (Madison, USA) and Takara Bio Inc. (Beijing, China), respectively.

Substrates and chemicals

Substrates Icelandic moss lichenan, barley β -1,3-1,4-glucan, and cello-oligosaccharides were acquired from Megazyme Ltd (Dublin, Ireland). Avicel PH-101, CMCNa, glucose, cellobiose, and beechwood xylan were obtained from Sigma–Aldrich Co., Ltd (Saint Louis, USA). Avicel PH-101 was used to prepare PASC based on the previous method reported by Zhou et al [30]. All

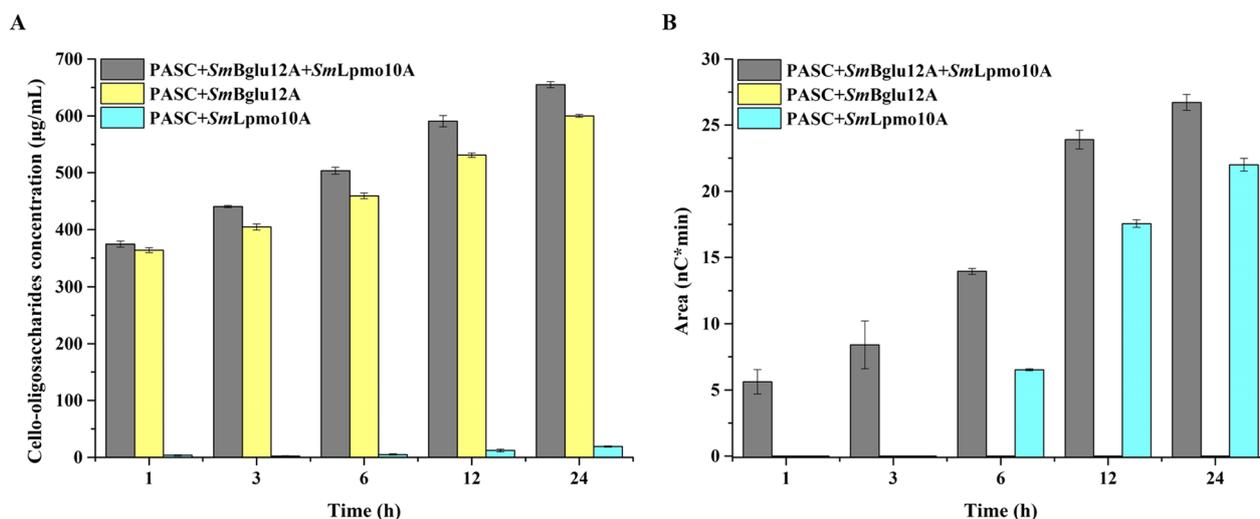


Fig. 6 The production of native (A) and oxidized (B) cello-oligosaccharides released from PASC by *SmBglu12A* and *SmLpmo10A* in the pH 6.0 acetate buffer at 50 °C for different time periods

other analytical research grade reagents and chemicals were purchased from biotech companies in China.

Cloning, expression, and purification of *SmBglu12A* and *SmLpmo10A*

SmBglu12A and *SmLpmo10A*-encoding genes in the absence of signal peptide sequence were amplified from the *S. megasporae* genome with gene-specific primers (Additional file 3). Then PCR amplification products were assembled into pET22b vector digested with *Nco* I and *Not* I using the DNA assembly and cloning kit from TransGen biotech Co., Ltd (Beijing, China). After sequencing verification, the recombinant plasmids and different chaperones plasmids, including pTf16, pG-KJE8, pKJE7, pGro7, as well as pG-Tf2, were separately co-transformed into various expression hosts containing *E. coli* Origami DE3 and Shuffle T7-B for achieving soluble expression of *SmBglu12A* and *SmLpmo10A*. And the optimal combination of recombinant plasmids, chaperones plasmids, and expression hosts was selected according to the recombinant protein production through SDS-PAGE analysis.

The positive transformants of recombinant *SmBglu12A* and *SmLpmo10A* were pre-cultured in Luria broth medium at 37 °C for 12 h and used as the inoculum of 300 mL Luria broth medium supplemented with corresponding molecular chaperone inducers. *SmBglu12A* transformants used 0.5 mg/mL L-arabinose for inducing the expression of the molecular chaperone tig. *SmLpmo10A* transformants utilized 5 ng/mL tetracycline and 0.5 mg/mL L-arabinose to induce expression of multiple molecular chaperones [31]. When the cells reached exponential growth phase at OD₆₀₀ of 0.8–1.0, a final IPTG concentration of 0.5 mM was applied.

Cells were collected after 12 h of IPTG induction at 16 °C, and sonicated at 130 W for 0.5 h. Then sonicated cell lysates were purified using nickel column affinity chromatography with 200 mM imidazole and 500 mM NaCl dissolved in the 20 mM phosphate buffer (pH 7.4). As for *SmLpmo10A*, the crude enzyme was further purified via HiTrap Q HP column anion exchange chromatography with a linear gradient of 0–1 M NaCl dissolved in the 20 mM phosphate buffer (pH 7.4) to remove nonspecific binding molecular chaperones. Finally, the purity of recombinant *SmBglu12A* and *SmLpmo10A* was verified by 12% SDS-PAGE. In addition, purified *SmLpmo10A* was saturated with CuSO₄ for 0.5 h under room temperature using a 1:3 molar ratio, followed by removing excess copper using a PD MidiTrap desalting column with Sephadex G-25 resin from Cytiva according to the gravity protocol using the 20 mM Tris–HCl buffer (pH 7.0) [32].

Measurement of β -glucanase and LPMO activity

β -glucanase activity was quantified by 3,5-DNS method using the substrate β -1,3-1,4-glucan [33]. The assay mixture contained 0.5% β -1,3-1,4-glucan and the diluted enzyme solution in the pH 6.0 acetate buffer. LPMO activity was evaluated through monitoring the oxidized and non-oxidized oligosaccharides released from PASC [34]. The reaction was performed at pH 6.0 acetate buffer containing 0.5% PASC and 1 mM ascorbic acid for 24 h incubation at 50 °C.

Characterization of *SmBglu12A* and *SmLpmo10A*

The impact of pH on purified *SmBglu12A* was measured in the acetate buffer between 3.0 and 6.0 and Tris–HCl buffer between 6.0 and 9.0 at 50 °C. The impact of temperature on purified *SmBglu12A* was measured in the pH 6.0 Tris–HCl buffer ranging from 30 to 80 °C. To measure the pH stability, purified *SmBglu12A* was pre-incubated within the pH range from 3.0 to 9.0 at 4 °C for 1 h, and then residual activity was measured at 50 °C in the pH 6.0 Tris–HCl buffer. To determine the thermostability, purified *SmBglu12A* was pre-incubated at 40, 50, and 60 °C for different time periods, and then residual activity was measured at 50 °C in the pH 6.0 Tris–HCl buffer. The initial activity before treatment was defined as 100%.

The substrate specificity of *SmBglu12A* was studied for the hydrolysis of five different substrates in the pH 6.0 Tris–HCl buffer at 50 °C for 1 h, including β -1,3-1,4-glucan, lichenan, CMCNa, PASC, as well as Avicel. All kinetic parameters of *SmBglu12A* were measured using different concentrations of β -1,3-1,4-glucan varying between 1.0 and 10.0 mg/mL in the pH 6.0 Tris–HCl buffer at 50 °C for 1 h. Besides, degradation patterns of *SmBglu12A* on β -1,3-1,4-glucan and PASC were carried out in the pH 6.0 Tris–HCl buffer at 50 °C. High-performance anion-exchange chromatography (HPAEC) was used to monitor their corresponding degradation products.

The substrate specificity of *SmLpmo10A* was determined through the degradation of various cellulosic substrates, including β -1,3-1,4-glucan, lichenan, CMCNa, PASC, as well as Avicel, in the pH 6.0 acetate buffer containing 1 mM ascorbic acid at 50 °C for 24 h. The generated natural and oxidized cello-oligosaccharides were determined using HPAEC using a CarboPac PA1 analytical column (2×250 mm) connected to a CarboPac PA1 guard column (2×50 mm) at 30 °C based on the previous method reported by Westereng et al. [35]. The elution program was as follows: 0–10% B (1 M NaOAc in 0.1 M NaOH), 10 min; 10–30% B, 15 min; 30–100% B, 5 min; 100–0% B, 5 min; 100% A (0.1 M NaOH) 5 min at a flow rate of 0.25 mL/min.

Synergistic degradation of PASC by *SmBglu12A* and *SmLpmo10A*

The synergistic action between *SmBglu12A* and *SmLpmo10A* on the enzymatic deconstruction of cellulose was evaluated in the pH 6.0 acetate buffer containing 1 mM ascorbic acid using 0.5% PASC as the substrate by addition of 0.5 μ M *SmBglu12A* and 0.5 μ M *SmLpmo10A*. Reactions were carried out at 50 °C for five different incubation periods (1, 3, 6, 12, and 24 h) in triplicate. The control group was set up without enzymes. The production of natural and oxidized cello-oligosaccharides from PASC was determined and quantified by HPAEC using standard mixtures of cello-oligosaccharides, including glucose, cellobiose, cellotriose, cellotetraose, cellopentaose, and cellohexose. The statistical analysis was performed using the SPSS software.

Abbreviations

LPMOs	Lytic polysaccharide monooxygenases
AA9	Auxiliary activity family 9
PASC	Phosphoric acid swollen cellulose
CMCNa	Carboxymethylcellulose sodium salt
HPAEC	High-performance anion-exchange chromatography

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13068-023-02332-0>.

Additional file 1. Nucleotide sequence analysis of two adjacent cellulolytic enzyme genes *SmBglu12A* and *SmLpmo10A* in the genome of *S. megasporae*. Genomic DNA sequences encoding *SmBglu12A* and *SmLpmo10A* were highlighted in blue and green, respectively. **Additional file 2.** Effects of pH and temperature on the activity of the purified recombinant *SmLpmo10A* using the substrate PASC. **A:** Optimum pH; **B:** Optimum temperature. **Additional file 3.** Primers used in this study.

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Author contributions

XQ: Funding acquisition, Data Curation, Visualization, Investigation, Conceptualization, Writing—original draft, Methodology. KY: Investigation. JZ: Investigation. XW: Methodology. TT: Resources. YW: Visualization. XS: Curation. BY: Funding acquisition, Project administration. HH: Funding acquisition. HL: Writing—review and editing, Project administration, Conceptualization. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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