

## Short Communication

# Overexpression of *amyA* and *glaA* substantially increases glucoamylase activity in *Aspergillus niger*

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## Abstract

The purpose of this study was to obtain an engineered *Aspergillus niger* strain with high glucoamylase activity by overexpressing the glucoamylase gene *glaA* and  $\alpha$ -amylase gene *amyA* in *A. niger* CICC2462. Three recombinant strains containing a single copy of *amyA* (1A), containing two copies of *amyA* (2A), and coexpressing *amyA* and *glaA* (AG), respectively, were constructed. The transcript levels of *amyA* in 1A and 2A were increased by 2.95 folds and 3.09 folds, respectively. The levels of *amyA* and *glaA* in AG were increased by 1.21 folds and 2.86 folds, but the maximum extracellular glucoamylase activities did not differ significantly. In addition, after 1% casein phosphopeptides (CPPs) was added to the fermentation medium, the maximum extracellular glucoamylase activities for strains 1A, 2A, and AG were 35,200, 37,300, and 40,710 U/ml, respectively, which were significantly higher than that of the parental strain CICC2462 (28,250 U/ml), while CPPs alone had no effect on the parental strain CICC2462. We demonstrate that overexpression of *amyA* and *glaA* substantially increases the expression and secretion of glucoamylase in *A. niger*, and CPPs effectively improves the yield of glucoamylase in recombinant *A. niger* strains overexpressing *amyA* and *glaA*. The newly developed strains and culture methods may have extensive industrial applications.

**Key words:** glucoamylase,  $\alpha$ -amylase, casein phosphopeptides, *Aspergillus niger*, secretory expression

## Introduction

*Aspergillus niger*, a common filamentous fungus, has been identified as Generally Recognized As Safe (GRAS) by the US Food and Drug Administration and is characterized by luxuriant growth, short fermentation cycle, and lack of toxins [1]. It is commonly used as a fermentative strain in food industry. The *A. niger* expression systems exhibit more efficient protein expression and secretion and improved modification ability when compared with the *Escherichia coli* and *Pichia pastoris* expression systems, and the recombinants have a high genetic stability [2].

Glucoamylase ( $\alpha$ -1,4-glucan glucohydrolase, EC 3.2.1.3) is the main enzyme in the hydrolysis of starch [3]. In general,  $\alpha$ -amylase and glucoamylase act together to convert starch into monosaccharides

in industrial glucoamylase production. Thus,  $\alpha$ -amylase and glucoamylase exert a synergistic effect on the hydrolysis of starch. This enzymatic reaction involves a two-step process to release glucose. The first reaction liquefies starch via amylases to produce soluble oligosaccharides, and the resulting products act as a substrate for the glucosylation of glucose by glucoamylase [4]. Increasing the concentration of  $\alpha$ -amylase provides more non-reducing ends for glucoamylase [5], thereby increasing its catalytic efficiency. However, this two-step enzymatic hydrolysis also presents some challenges, requiring an additional step of pH and temperature adjustment, which involves considerable time and costs. Preparation of the  $\alpha$ -amylase and glucoamylase from *A. niger* with compatible pH and temperature in proportion facilitates the one-step saccharification

of starch [6]. As an enzyme preparation product with large output and high export in China, the market price of glucoamylase is almost equal to its level of production. Therefore, cultivation of a strain with a high glucoamylase yield is of great significance.

Many methods have been adopted to improve glucoamylase production. It is particularly common to increase the transcription of target genes for enhanced protein secretion. For example, Liu *et al.* [7] used this approach to optimize *Rhizopus oryzae* glucoamylase secretion in *Pichia pastoris* (yield, 12.619 IU/ml). Parashar *et al.* [6] used chimeric acid-stable  $\alpha$ -amylase-glucoamylase in *E. coli* for one-step starch saccharification. The resulting strain more efficiently glycosylates wheat and corn starch while retaining the parent enzyme activity.

Casein phosphopeptides (CPPs) are derived from bovine casein, which are biologically active polypeptides that contain clusters of phosphoserine residues. High expression of exogenous or endogenous proteins results in unfolded protein response (UPR) caused by abnormal folding or secretion disorders due to the excessive accumulation of nascent polypeptides in the endoplasmic reticulum cavity [8]. Adding CPPs can alter the organellar calcium concentration. In particular, altered vesicle-mediated secretory pathway of filamentous fungi increases the secretion of homologous and heterologous proteins [9].

In this study, we constructed recombinant strains carrying one or two copies of *amyA* or coexpressing *amyA* and *glaA* in the same vector to obtain a strain with efficient glucoamylase secretion and expression for one-step saccharification. In addition, the use of CPPs further improved the secretion of glucoamylase and  $\alpha$ -amylase; this was the first attempt to study the effects of CPPs on

the overexpression-mediated secretion of endogenous proteins in *A. niger*, with clear practical applications.

## Materials and Methods

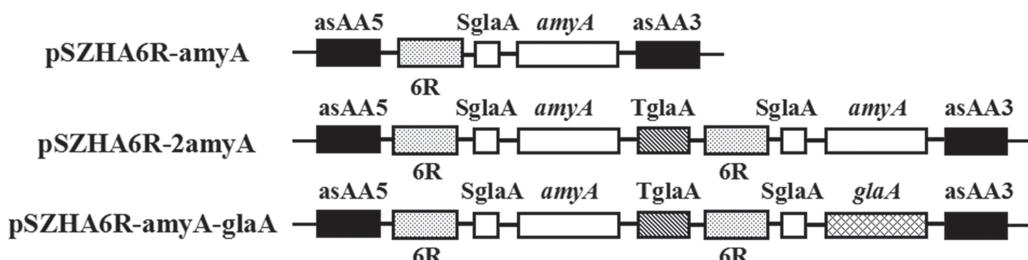
### Fungi, plasmids, and cultivation

*Aspergillus niger* strain CICC2462 was obtained from Zhaodong Richeng Enzyme Preparation Co., Ltd (Zhaodong, China). *Escherichia coli* strain DH5 $\alpha$  and *Agrobacterium tumefaciens* strain AGLI were used for DNA manipulation. The vectors pSZHA and pT-6R were constructed and stored in our laboratory. pT-6R contains the *glaA* promoter, a modified *glaA* promoter, and the highly efficient signal peptide of glucoamylase. Luria-Bertani (LB) medium containing 5 g/l yeast extract, 10 g/l peptone, and 10 g/l NaCl was used to culture the plasmid-carrying *E. coli*. *Agrobacterium tumefaciens* was cultivated using Yeast Extract Beef (YEB) medium (pH 6.5) composed of 1 g/l yeast extract, 5 g/l peptone, 0.493 g/l MgSO<sub>4</sub>·7H<sub>2</sub>O, 5 g/l beef extract paste, and 5 g/l sucrose. Potato Dextrose Agar (PDA) medium (20 g/l glucose, 3 g/l KH<sub>2</sub>PO<sub>4</sub>, 1.5 g/l MgSO<sub>4</sub>·7H<sub>2</sub>O, and 200 g/l potato chips) was used for *A. niger* co-culture. Czapek-Dox (CD) medium (pH 5.5) containing 20 g/l glucose, 3 g/l NaNO<sub>3</sub>, 2 g/l KCl, 0.5 g/l MgSO<sub>4</sub>·7H<sub>2</sub>O, 1 g/l K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, and 0.01 g/l FeSO<sub>4</sub>·7H<sub>2</sub>O was used to screen for *A. niger* homozygous transformants. The fermentation medium consisted of 100 g/l glucose, 20 ml/l corn syrup, and 20 g/l soy flour (pH 5.5–6.0). Primer STAR DNA polymerase, restriction endonuclease and pMD-19T were from Dalian Bao Biotech Co. (Dalian, China). Plasmid extraction kit, gel recovery kit, genome extraction kit, and RNA extraction kit were purchased from Kangwei

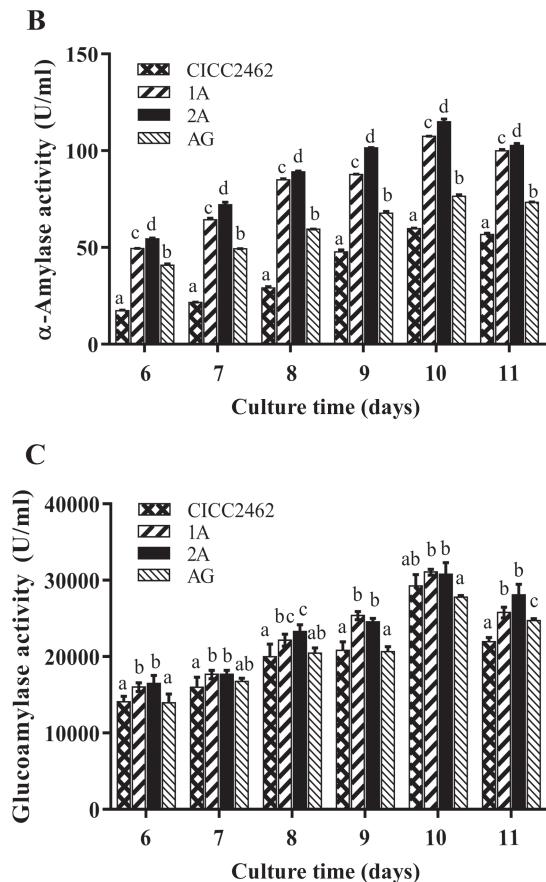
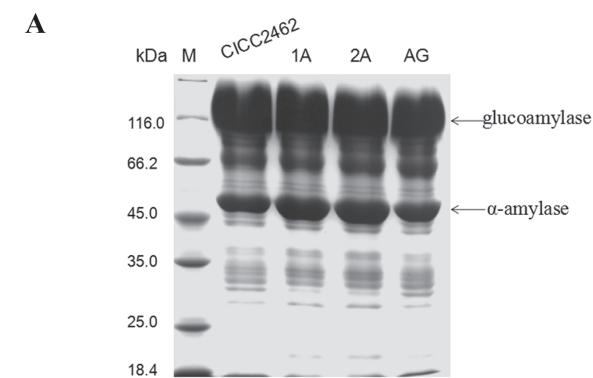
**Table 1. Primer name and sequence used in this study**

Primers	Sequence (5'→3')	Restriction enzymes
P1:amyA-sence	<u>TCTAGAGCAACGCCTGCGGACTG</u>	XbaI
P2:amyA-antisense	<u>AAGCTTGGATCCACCGAGCTACTACAGATCTTG</u>	HindIII; BamHI
P3:glaA-sence	<u>TCTAGACCATGTCGTTCCGATCTCTA</u>	XbaI
P4:glaA-antisense	<u>AAGCTTACCGCCAGGTGTCAGTCAC</u>	HindIII
P5:asAA-sence	TCGCCTATCTACTCCTCATCC	
P6:asAA-antisense	CTTCAACCCCTGCTGCTAAATC	
glaA-RT-sense	GACAATGGCTACACCAGCAC	
glaA-RT-antisense	AATCACACCAGGAGCAGGAC	
amyA-RT-sense	GCCCATCTACAAAGACGACA	
amyA-RT-antisense	ACATTTCCATCCGAACCAAAC	
actA-RT-sense	CCACGAGACCACCTCAACTCCA	
actA-RT-antisense	CCACCGATCCAGACGGAGTACTTGC	

Restriction sites are underlined.



**Figure 1. Expression cassette of pSZHA6R-amyA, pSZHA6R-2amyA, and pSZHA6R-amyA-glaA** asAA5 and asAA3 are homologous arms for homologous recombination. asAA3 contains a terminator.

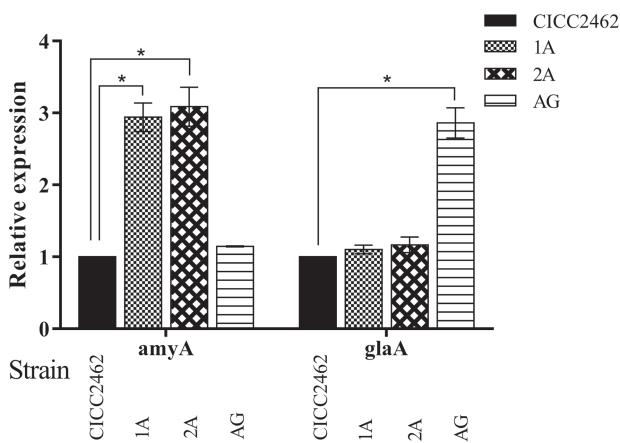


**Figure 2.** Secretory protein and enzyme activity analysis of recombinant strains in the fermentation medium (A) The proteins of the strains, *A. niger* CICC2462, 1A, 2A, and AG, were identified by SDS-PAGE. (B)  $\alpha$ -Amylase activity in CICC2462 and the recombinant strains 1A, 2A, and AG in the fermentation medium for 6–11 days. (C) Glucoamylase activity in CICC2462 and the recombinant strains. Data are presented as the mean  $\pm$  SD of three independent replicates. Different letters indicate significant differences among strains at  $P < 0.05$ .

Century Biotechnology Co. (Beijing, China). Ampicillin, Kanamycin, and Rifampicin were from Sangon Biotech (Shanghai, China). 5-Fluoroorotic acid (5-FOA) and uridine were purchased from Sigma (St Louis, USA). Other chemical reagents were of analytical grade.

#### Gene cloning and expression vector construction

Primers were designed based on the *amyA* and *glaA* gene sequences in the *A. niger* genome using Primer 5.0. Primer sequences used for

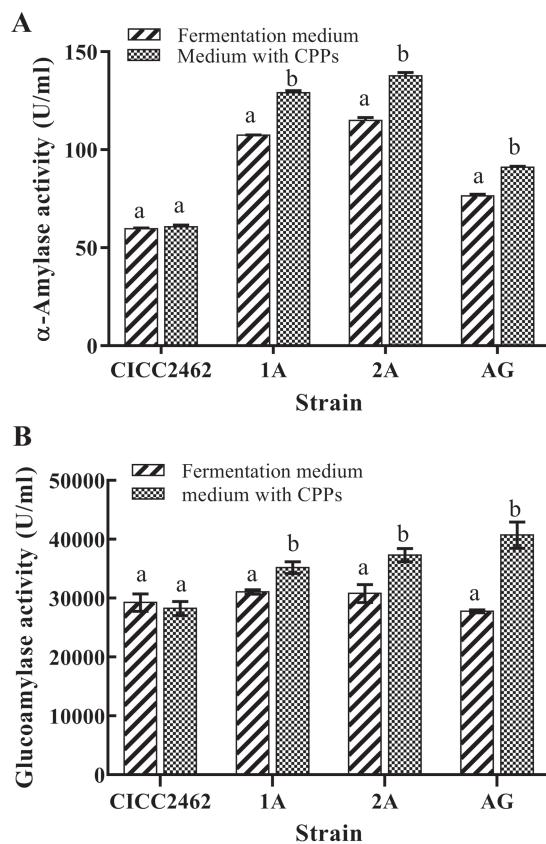


**Figure 3.** Real-time quantitative PCR analysis of the glucoamylase gene (*glaA*) and amylase gene (*amyA*) of the parental strain, 1A, 2A, and AG. Data are shown as the mean  $\pm$  SD of three replicates. The statistical significance was determined by equal variance *t*-test and indicated with an asterisk if  $P < 0.05$ .

polymerase chain reaction (PCR) are shown in Table 1. With the *A. niger* genome as a template, the target *amyA* and *glaA* fragments were amplified using primers P1/P2 and P3/P4, respectively, cloned into pMD-19T, and verified by sequencing. The gene fragments digested with *Xba*I and *Hind*III were recovered and ligated with plasmid pT-6R digested with *Nhe*I and *Hind*III to obtain the plasmids pT-6R-*amyA* and pT-6R-*glaA*. The *amyA* gene fragment digested with *Xba*I and *Bam*HI, the *TglA*A terminator fragment digested with *Bam*HI and *Hind*III, and the pT-6R vector fragment digested with *Nhe*I and *Hind*III were ligated to obtain the plasmid pT-6R-*amyA-TglA*A. The plasmids pT-6R-*amyA* and pT-6R-*glaA* were digested with *Xba*I and *Hind*III. The fragments containing promoter and gene were ligated with pT-6R-*amyA-TglA*A digested with *Nhe*I and *Hind*III to obtain plasmids pT-6R-*amyA-TglA*A-6R-*amyA* and pT-6R-*amyA-TglA*A-6R-*glaA*. Finally, the *amyA* single-copy expression cassette 6R-*amyA* from pT-6R-*amyA*, the *amyA* two-copy expression cassette 6R-*amyA-TglA*A-6R-*amyA* from pT-6R-*amyA-TglA*A-6R-*amyA*, and the *amyA* and *glaA* co-expression cassettes 6R-*amyA-TglA*A-6R-*glaA* from pT-6R-*amyA-TglA*A-6R-*glaA* were ligated into the expression vector pSZHA digested with *Xba*I/*Hind*III, thus pSZHA6R-*amyA*, pSZHA6R-2*amyA*, and pSZHA6R-*amyA-glaA* were obtained, respectively (Fig. 1).

#### Acquisition and identification of *A. niger* transformants

*Aspergillus niger* transformants were obtained by the homologous gene replacement technique using *Agrobacterium*-mediated transformation [10]. The entire process is as follows: the recombinant plasmid was transferred into AGLI by freeze-thaw method, and the obtained *Agrobacterium* recombinant transformant was inoculated in YEB liquid medium, and it was rotated and shaken at 200 rpm for 24 h at 28°C. The primary activated *Agrobacterium* transformant was inoculated in fresh YEB liquid (20 ml) medium in a 1:10 ratio for secondary activation, when the OD600 reached 0.4–0.6, 100 µl of *Agrobacterium* was centrifuged, and the pellet was mixed with 50 µl of *A. niger*-inoculated hyphae and coated on PDA-coated glassine paper (200 µM acetosyringone) at 28°C for incubation for 2 days. Mycelium-containing cellophane was transferred onto new CD plates (200 mM cephalosporin, 20 g/l maltose, 1 M uridine, and

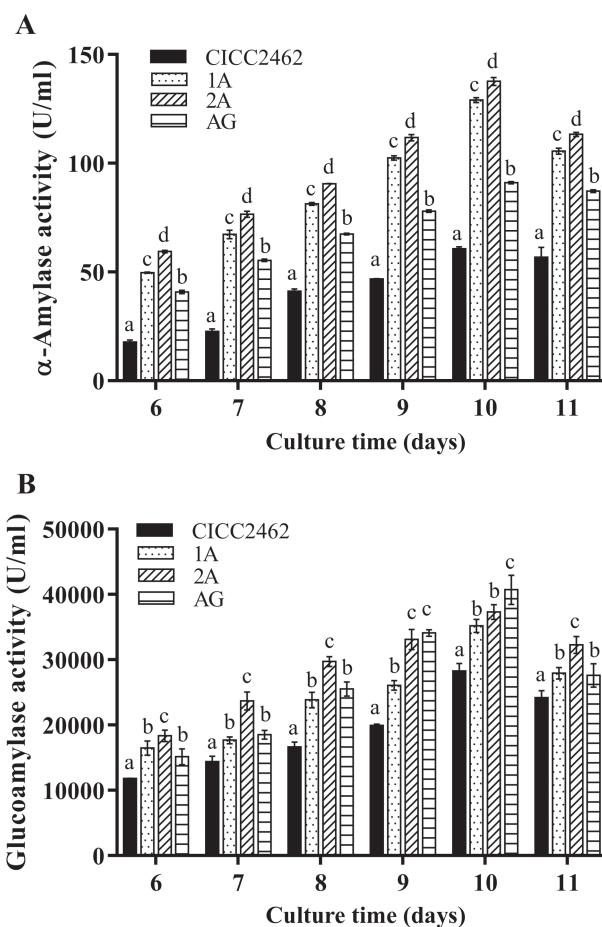


**Figure 4.** Enzyme activity of recombinant strains in the fermentation medium with and without CPPs. (A)  $\alpha$ -Amylase enzyme activity of strains 1A, 2A, and AG. (B) Glucoamylase enzyme activity of strains 1A, 2A, and AG. The sample was cultured for 10 days at 250 rpm and 30°C. Data are shown as the mean  $\pm$  SD of three replicates. Different letters indicate significant differences among strains at  $P < 0.05$ .

2 M 5-FOA). After 1 day of culture, the cellophane was removed and further cultured at 30°C for 5–7 days to form colonies. A single colony obtained on the plate was inoculated in CD liquid medium (200 mM cephalosporin, 20 g/l maltose, 1 M uridine, and 1 M 5-FOA) and cultured for 6–8 days. Genomic DNA was extracted from the *A. niger* transformants, and a homozygous transformant was obtained and verified by PCR using the primers P5 and P6.

#### Protein and enzyme assays

Three homozygous transformant strains 1A, 2A, and AG were used for fermentation. Recombinant strains cultures (10 ml) were inoculated into the industrial fermentation medium (100 ml) and shaken at 30°C and 250 rpm in flasks. The supernatant was obtained for enzyme activity analyses and protein detection. The samples were subjected to SDS-PAGE using a 4% stack gel and a 12% separation gel in a micro vertical electrophoresis system (Bio-Rad Laboratories, Hercules, USA). The loading volume is 10  $\mu$ l, the gel was stained with CBB-R250 for 12 h and decolorized for another 4–6 h.  $\alpha$ -Amylase was determined using an iodine starch colorimetric method [11]. One enzyme unit is defined as the amount of enzyme that liquefies 1 g of soluble starch at 60°C and pH 6.0 in 1 h and is expressed as 'U/ml'. The 3,5-dinitrosalicylic acid (DNS) method was used to determine the level of glucoamylase activity [12]. One enzyme unit is defined as the amount of enzyme



**Figure 5.** Enzyme activity assay of recombinant strains in the fermentation medium with CPPs on different days. (A)  $\alpha$ -Amylase activity in CICC2462 and the recombinant strains 1A, 2A, and AG after 6–11 days. (B) Glucoamylase activity in CICC2462 and the recombinant strains. Data are presented as the mean  $\pm$  SD of three independent replicates. Different letters indicate significant differences among strains at  $P < 0.05$ .

that hydrolyzes starch per hour at 40°C and pH 4.6 and releases 1 mg of glucose and is expressed as 'U/ml'.

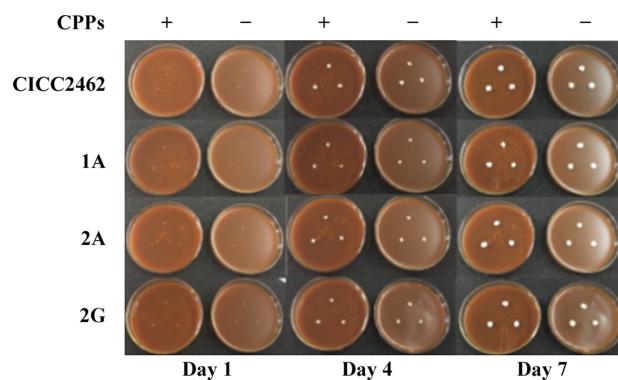
#### RNA isolation, cDNA synthesis, and quantitative RT-PCR

RNA was extracted from the homozygous transformant strain of *A. niger* on the fifth day of fermentation using a CWBIO's RNA kit (Kangwei Century Biotechnology Co.) according to the manufacturer's protocol; cDNA was synthesized using the Prime-Script RT Kit containing gDNA Eraser, and quantitative real-time PCR was performed using a Stratagene Mx3000P instrument (San Diego, USA) using SYBR Premix Ex Taq. The oligonucleotide sequences of the primers used for this analysis are listed in Table 1. The internal quantification standard was the transcript abundance of the *actin* gene from the same sample.

## Results and Discussion

#### Construction of recombinant expression vectors

Expression vectors were obtained by restriction enzyme ligation for overexpression of *amyA* and *glaA*. The *amyA* and *glaA* gene



**Figure 6.** Growth of the parental strain CICC2462 and recombinant strains 1A, 2A, and AG after different days of fermentation on medium plates with or without 1% CPPs

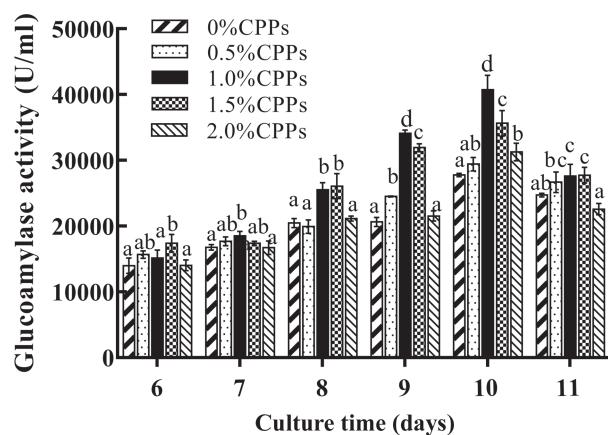
fragments of the amplification products were 1996 and 2254 bp, respectively (Supplementary Fig. S1). The recombinant expression vectors pSZHA6R-*amyA*, pSZHA6R-2*amyA*, and pSZHA6R-*amyA-glaA* were identified by double digestion with *Xba*I and *Hind*III.

#### Successful screening of the homozygous transformants of *A. niger*

The development of beneficial filamentous fungi has been a major focus of recombinant protein research. Increasing the production of recombinant proteins in the filamentous fungus *Aspergillus* has gained enormous commercial interest [13,14]. In this experiment, the recombinant strains were constructed by using the industrially produced glucoamylase strain *A. niger* CICC2462 as a recipient strain [15]. According to the principle of homologous recombination, bands for the homozygous transformants of *A. niger* were identical to those of the positive control, and there was no band identical to that of the parental strain. The results indicated that CICC2462 (pSZHA6R-*amyA*), CICC2462 (pSZHA6R-2*amyA*), and CICC2462 (pSZHA6R-*amyA-glaA*) were homozygous transformants of *A. niger* (Supplementary Fig. S2). The recombinant strain containing a single copy of the *amyA* expression cassette was renamed 1A ( $\Delta$ asAA::*amyA* in *A. niger* CICC2462), the recombinant strain containing two copies of the *amyA* expression cassette was renamed 2A ( $\Delta$ asAA::2*amyA* in *A. niger* CICC2462), and the recombinant strain containing one copy of the *amyA* expression cassette and one copy of the *glaA* expression cassette was renamed AG ( $\Delta$ asAA::*amyA-glaA* in *A. niger* CICC2462).

#### Secretion of $\alpha$ -amylase and glucoamylase protein in recombinant strains

To investigate the expression of  $\alpha$ -amylase and glucoamylase, the secretory proteins of recombinant strains 1A, 2A, and AG were analyzed by SDS-PAGE. The glucoamylase protein band was detected above 66 kDa, and the  $\alpha$ -amylase band was detected at  $\sim$ 48 kDa (Fig. 2A). The  $\alpha$ -amylase bands of 1A and 2A became more prominent, while that of AG showed no significant change; no significant changes were observed in the glucoamylase bands in any of the strains when compared with those of CICC2462. The result indicated that the secretion of  $\alpha$ -amylase and glucoamylase in AG may be inhibited.



**Figure 7.** Effects of different concentrations of CPPs on glucoamylase activity of strain AG. Fermentation was continued for 6–10 days. Data are presented as the mean  $\pm$  SD of three independent experiments. Different letters indicate significant differences among strains at  $P < 0.05$ .

In the fermentation medium,  $\alpha$ -amylase and glucoamylase activities were further analyzed. The  $\alpha$ -amylase activities of strains 1A, 2A, and AG were increased by 79.8%, 92.5%, and 27.9%, respectively, but the increased levels did not correspond to the copy number (Fig. 2B). The highest glucoamylase activities of the recombinant strains 1A, 2A, and AG were observed on the 10th day, with enzyme activity levels of 31,080, 30,800, and 27,780 U/ml, respectively (Fig. 2C). After the analysis of the differences in statistical significance, compared with the enzyme activity of the CICC2462 (29,250 U/ml), there was no significant increase in the enzyme activity after increasing the gene copy number. This result can presumably be explained by the excessive gene dose, resulting in misfolding of the endoplasmic reticulum cavity, unfolded protein aggregation, secretion stress, and UPR upregulation [16]. As a result, the protein cannot be secreted or expressed normally and protein expression tends to decrease, remain steady, or increase slowly. These data indicated that the  $\alpha$ -amylase activity of the recombinant strain is increased after increasing the gene copy number, but the glucoamylase activity is not significantly increased, and the strain may cause folding or secretion pressure.

#### Improved transcription levels of *amyA* and *glaA* in recombinant strains

To analyze the rate-limiting steps for the overexpression of *amyA* and *glaA*, we analyzed the levels of *amyA* and *glaA* transcription. The quantitative real-time PCR results for the recombinant strains are summarized in Fig. 3. The transcript levels of *amyA* in strains 1A, 2A, and AG were 2.95 folds, 3.09 folds, and 1.21 folds higher than those in the parental strain, respectively. The transcript levels of *glaA* in 1A and 2A were almost the same as those in the parental strain. These results are consistent with the results obtained for enzyme activity and protein levels. However, the level of *glaA* in AG was 2.86 folds higher than that in the parental strain, indicating significant post-transcriptional regulation of *glaA* expression in AG.

#### CPPs increase the overexpression of $\alpha$ -amylase and glucoamylase

Research has shown that CPPs increase glyceraldehyde-3-phosphate dehydrogenase protein with microtubule binding and vesicle fusion

activity, thereby increasing the secretion of secretory vesicles [17]. At the same time, CPPs have the ability to carry  $\text{Ca}^{2+}$  and play an important role in the  $\text{Ca}^{2+}$  homeostasis of cells, effectively regulating the transport pathway of proteins, alleviating the secretion pressure, thereby enhancing the enzyme levels of the recombinant strain. To improve the folding and secretion of  $\alpha$ -amylase and glucoamylase in each strain, 1% CPPs was included in the fermentation medium.  $\alpha$ -Amylase activities of strains 1A, 2A, and AG were increased by 20.2%, 19.8%, and 19.3%, respectively; the glucoamylase activities of 1A and 2A strains were increased by 13.2% and 21.1%, respectively, and glucoamylase activity in the AG strain was increased by 46.5% (Fig. 4). CPPs have no effect on parental strains (Fig. 4), probably because the transcription, translation, and transport of  $\alpha$ -amylase and glucoamylase in the parental strain are in a coordinated state, and no additional  $\alpha$ -amylase or glucoamylase is detained in hypha. These data indicated that both glucoamylase activity and  $\alpha$ -amylase activity were significantly improved by CPPs in strains 1A, 2A, and AG.

The  $\alpha$ -amylase activity levels of 1A, 2A, and AG were increased significantly after addition of CPPs, but the  $\alpha$ -amylase activity of strain AG was lower than that of 1A (Fig. 5A). The glucoamylase activities of recombinant strains 1A, 2A, and AG peaked at 35,200, 37,300, and 40,710 U/ml, respectively, and the glucoamylase activity was significantly higher than that of the parental strain (Fig. 5B). In particular, activity in the strain AG was increased by 44.1%. Accordingly, the increase in glucoamylase activity in the recombinant strain AG was mainly attributed to the overexpression of glaA. In addition, the increase in glucoamylase activities of strains 1A and 2A further demonstrates that increasing the  $\alpha$ -amylase concentration provides more non-reducing ends to the glucoamylase [6,18], which contributes to the improvement of glucoamylase activity. These results demonstrate that CPPs have a positive effect on the overexpression of  $\alpha$ -amylase and glucoamylase.

Theoretically, after relieving secretion pressure, protein and enzyme activity levels of the recombinant strain should be proportionated. However, the enzyme activity results were inconsistent with this hypothesis. It is possible that the integration sites of genes, the quantity of transcriptional regulatory proteins, and other factors can also limit secretion. Further exploration is needed to evaluate these factors.

### Overexpression of amyA and glaA and CPPs have no effect on mycelium growth

To observe the growth of the parental strain and the recombinant strain, we cultured *A. niger* CICC2462 and strains 1A, 2A, and AG on fermentation medium plates without CPPs or containing 1% CPPs. As shown in Fig. 6, there was no significant difference in the growth of the parent strain and the recombinant strain, indicating that overexpression of amyA and glaA did not inhibit mycelium growth. Furthermore, the three recombinant strains did not differ in the size and morphology after addition of CPPs. These data indicated that CPPs had no effect on the growth rate of the mycelium, which further proved that CPPs play a major role in regulating the secretory pathway.

### CPPs have the optimal effect in strain AG

The AG strain was used as a reference to explore whether different concentrations of CPPs have different effects. Homozygous AG strains were inoculated into a fermentation medium containing 0%–2% CPPs (per 0.5% increment). After 6–11 days, fermentation

broth was collected for the enzyme activity assay. Results showed that the enzyme activity levels on Day 10 in the medium containing 1%–1.5% CPPs were similar and the highest among all concentrations (Fig. 7). The enzyme activity was decreased when the concentration of CPPs reached 2%, and there was no significant difference compared with the activity at 0%, suggesting titration effects. These results showed that the optimal effect of CPPs on the expression of the *A. niger* glucoamylase gene occurred at concentrations of 1%–1.5% in strain AG.

## Conclusion

In summary, the results clearly show that the modification of amyA copy number and co-expression of amyA and glaA in *A. niger* CICC2462 result in increased transcription in the recombinant strain. Furthermore, the addition of CPPs to the fermentation medium significantly increased the levels of  $\alpha$ -amylase and glucoamylase secretion. Therefore, this work highlights the effectiveness of overexpressing amyA and glaA to increase glucoamylase production in *A. niger*. This study provides an effective approach for the industrial production of glucoamylase in *A. niger*.

## Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

## Funding

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