Subtilisin-like serine protease gene \textit{TghSS42} from \textit{Trichoderma ghanense} ACCC 30153 was successfully expressed in \textit{Escherichia coli} and recombinant protease r\textit{TghSS42} exhibited antifungal ability to five phytopathogens

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The subtilisin-like serine protease gene \textit{TghSS42} was cloned from biocontrol agent \textit{Trichoderma ghanense} ACCC 30153. Its coding region is 1302 bp in length, encoding 433 aa with a predicted protein molecular weight of 42.5 kDa and \( pI \) of 5.53. The accession number of cDNA sequence of \textit{TghSS42} gene is KJ740359. Furthermore, the transcription of the \textit{TghSS42} gene was all up-regulated under nine different treatments by RT-qPCR analysis, and the highest transcription level of \textit{TghSS42} approached 177.29-fold at 4 h under induction with 1\% (w/v) \textit{Alternaria alternata} cell walls, indicating that \textit{TghSS42} could be induced by the plant or phytopathogen. Furthermore, \textit{Escherichia coli} recombinant strain BL21-\textit{TghSS42} was constructed. The recombinant protease r\textit{TghSS42}, with an expected molecular weight of approximately 68.5 kDa (containing 26.0 kDa GST tag), has been successfully expressed and purified from BL21-\textit{TghSS42}. The purified protease r\textit{TghSS42} activity reached a peak of 18.7 U/mL at 4 h following 1.0 mM IPTG induction. The optimal enzyme reaction temperature was 40°C and the optimal \( pH \) was 7.0. The recombinant protease r\textit{TghSS42} exerted broad-spectrum antifungal ability against \textit{Rhizoctonia solani}, \textit{Fusarium oxysporum}, \textit{A. alternata}, \textit{Sclerotinia sclerotiorum} and \textit{Cytospora chrysosperma}. The inhibition rate of mycelial growth varied between 21.2\% and 50.0\%.

Key words: \textit{Trichoderma ghanense} / Subtilisin-like serine protease / Prokaryotic expression / Enzymatic property.

\section*{INTRODUCTION}

\textit{Trichoderma} are effective biocontrol agents against many soilborne plant pathogenic fungi through antibiosis, nutrient competition and secreting cell wall-degrading enzymes (Viterbo et al., 2004; Shoresh et al., 2005). Biocontrol fungal proteases take part in the fungal phytopathogen cell wall breakdown process or act as proteolytic inactivators of pathogen enzymes. Some biocontrol proteases have been recognized, including aspartic protease (Viterbo et al., 2004), serine protease, and trypsin-like protease, etc. The biocontrol functions of \textit{Trichoderma} proteases have also been researched. The aspartic protease gene \textit{SA76} from \textit{T. harzianum} T88 was expressed in \textit{Saccharomyces cerevisiae}, and the culture supernatant of recombinant yeast was able to inhibit the growth of five phytopathogenic fungi (Liu and Yang, 2007). The aspartic protease gene \textit{TaAsp} from \textit{T. asperellum} T4 was expressed in \textit{Pichia pastoris},
and the recombinant protease TAASP can inhibit mycelial growth of the pathogenic fungi (Yang et al., 2013). Aspartic protease gene Asp55 from T. asperellum ACCC 30536 was all up-regulated in media containing cell wall fragments or fermentation supernatant of Alternaria alternata (Dou et al., 2014).

Furthermore, overexpression of serine protease gene tvsp1 in T. virens strains significantly increased the survival rate of cotton seedlings against Rhizoctonia solani (Pozo et al., 2004). Purified serine protease SprT from the crude extract of biocontrol T. pseudokoningii SMF2 is the first well-characterized subtilase with nematicidal activity from Trichoderma (Chen et al., 2009). The expression level of the subtilisin-like serine protease gene SS10 from T. harzianum T88 was all up-regulated in the presence of five pathogenic fungi cell walls (Liu and Yang, 2009). The subtilisin serine protease gene Spm 1 from T. asperellum T4 was all up-regulated in response to different fungal phytopathogen induction (Liu et al., 2010). The transcription of serine protease gene in T. harzianum was up-regulated under Fusarium solani cell walls treatment (Vieira et al., 2013). Northern blot analysis indicated that the serine protease gene SL41 from T. harzianum T88 could be induced by phytopathogenic fungicide cell walls and the protease SL41 from T. harzianum T88 exerted broad-spectrum antifungal activity against phytopathogenic fungi (Liu and Yang, 2013). The transcription of the serine protease gene ThSS45 from T. harzianum ACCC 30371 was slightly up-regulated in the presence of cell walls or fermentation supernatant from A. alternata and the purified serine protease ThSS45 from recombinant E. coli BL21-ThSS45 obviously inhibited the growth of A. alternata mycelia (Fan et al., 2014). However, the biocontrol function of serine protease of T. ghanense has not been reported.

In the study, the subtilisin-like serine protease gene TghSS42 was cloned from T. ghanense ACCC 30153 and its sequence was analyzed. The transcription of TghSS42 was also studied by RT-qPCR after T. ghanense ACCC 30153 induced by nine different culture conditions. The recombinant vector pGEX-TghSS42 and strain BL21-TghSS42 were constructed. Recombinant protease rTghSS42 and its purified product were analyzed by SDS-PAGE. The temperature stability and pH dependence of rTghSS42 were also evaluated. The antifungal properties of the recombinant protease rTghSS42 to five different phytopathogens were also researched. The results may provide a theoretical support and a practical reference for the development of biological protease pesticides from T. ghanense ACCC 30153.

### MATERIALS AND METHODS

#### Strains, plasmids and plant materials

T. ghanense ACCC 30153 was from the Agricultural Culture Collection of China. E. coli strain TOP10 and vector pMD18-T (TaKaRa Biotechnology Co., Ltd., Dalian, China) were used for the genetic manipulation. E. coli BL21 and vector pGEX-4T-2 (Novagen, Madison, USA) were employed for the prokaryotic expression experiment. Fungal phytopathogens A. alternata (poplar leaf witter) and Cytospora chrysosperma (poplar bark rot) were used to prepare carbon source in inducing media for T. ghanense ACCC 30153. The aseptic Populus davidiana × P. alba var. pyramidalis (Pdpap poplar) seedlings were cultured in liquid woody plant medium (WPM) (Faisal et al., 2012).

Five species of the fungal plant pathogens, R. solani (rice sheath blight), Fusarium oxysporum (soybean root rot), A. alternata, Sclerotinia sclerotiorum (sclerotium disease on soybean) and C. chrysosperma, were used in antifungal assays. These strains were stored at School of Forestry, Northeast Forestry University (Harbin, China).

#### Cloning of the TghSS42 gene

Primers for TghSS42 cloning were SS42-1 (5'-ATGGGCACCCCTCAACACCAT-3') and SS42-2 (5'-TTAAAGTTCGGCTTGTT-3'). Genomic DNA was extracted from mycelia of T. ghanense ACCC 30153 according to the procedures of Huang et al. (2015). The PCR conditions were 94°C for 5 min; 35 cycles of 94°C for 30 s, 58°C for 40 s, 72°C for 80 s; 72°C for 7 min. The amplified fragment was purified and ligated into the pMD18-T vector according to the manufacturer’s instructions and then sequenced (Shanghai Sangon Co., China).

#### Differential expression analysis of TghSS42 in T. ghanense

Spores of T. ghanense ACCC 30153 were inoculated into Potato Dextrose (PD) medium and cultured at 28°C and 200 rpm for 36 h. Mycelia were filtered, washed, and grown in mineral medium (MM) containing 15.0 g/L NaH_{2}PO_{4}, 5.0 g/L (NH_{4})_{2}SO_{4}, 600.0 mg/L CaCl\textsubscript{2}·2H_{2}O, 600.0 mg/L MgSO\textsubscript{4}·7H_{2}O, 5.0 mg/L FeSO\textsubscript{4}, 2.0 mg/L CoCl\textsubscript{2}, 1.6 mg/L MnSO\textsubscript{4}, 1.4 mg/L ZnSO\textsubscript{4}, and 0.5% (w/v) glucose for 2 h. And then the mycelia were treated with nine different culture conditions: MM, MM without the glucose carbon source (MM-C), MM without nitrogen ammonium sulfate (MM-N), MM containing 1% (w/v) Pdpap poplar stem powder (MM-stem), MM containing 1% (w/v) Pdpap poplar leaf powder (MM-leaf), MM containing 1% (w/v) cell walls of A. alternata (MM-CW-Aa), MM containing 1% (w/v) cell...
walls of *C. chrysosperma* (MM-CW-Cc), MM containing 5% (v/v) fermentation supernatant of *A. alternata* (MM-ferm-Aa) and MM containing 5% (v/v) fermentation supernatant of *C. chrysosperma* (MM-ferm-Cc). The mycelia were harvested after inducing for 0, 0.5, 1, 2, 4, 8, 12, and 24 h for RNA extraction. The mycelia cultured for 0 h were used as the control.

Total RNA was extracted from the mycelia using Trizol reagent (Invitrogen, USA) and digested with DNase I (Promega, USA). Total RNA (0.5 µg) from each pooled sample was reverse transcribed into cDNA in the presence of oligo (dT) in a volume of 10 µL. The synthesized cDNA was diluted with 90 µL of water and used as a template for real-time reverse transcription RT-qPCR. The α-tubulin, β-tubulin and actin genes were used as internal references to normalize the amount of total RNA present in each reaction. PCR primers for amplifying TghSS42 cDNA fragment are listed in Table 1. The reactions were performed on an OpticonTM2 PCR Detection System (BioRad, Hercules, CA, USA), and each reaction was conducted, at a minimum, in triplicate. PCR was performed using a total volume of 20 µL, containing 10 µL of SYBR Premix Ex Taq (TaKaRa Biotechnology), 0.5 µmol L⁻¹ of each forward and reverse primers and 2 µL of cDNA template (equivalent to 0.1 µg of total RNA). The amplification was conducted with the following cycling parameters: 95°C for 3 s; 45 cycles of 95°C for 5 s, 59°C for 15 s, 72°C for 10 s; 82°C for 1 s. A melting curve was generated for each sample at the end of each run to assess the purity of the amplified products. The expression level of the TghSS42 was calculated from the threshold cycle according to $2^{-ΔΔCT}$ (Livak and Schmittgen, 2001).

### Table 1. Primers for RT-qPCR

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primers name</th>
<th>Sequence (5'→3')</th>
<th>Tm (°C)</th>
<th>Size of product (bp)</th>
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<td>TGCTCTGCTGGTTGATGGTGTCTG</td>
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<tr>
<td>β-tublin</td>
<td>βt-L</td>
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<td>232</td>
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<tr>
<td></td>
<td>βt-R</td>
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<td>59.4</td>
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<tr>
<td>actin</td>
<td>Act-L</td>
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<td>59.0</td>
<td>247</td>
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<tr>
<td></td>
<td>Act-R</td>
<td>AGTTGAGGCGCAGCGGATA</td>
<td>58.7</td>
<td></td>
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</tbody>
</table>

Expression and purification of recombinant protease rTghSS42

Recombinant transformant BL21-TghSS42 and control BL21-pGEX (E. coli BL21 transformed with empty plasmid pGEX-4T-2), were induced following the procedures as described in the Glutathione S-transferase (GST) Gene Fusion System Handbook (GE Healthcare UK Ltd, Buckinghamshire HP7 9NA, England). The isopropyl-β-D-thiogalactopyranoside (IPTG) was added into the LB medium at a final concentration of 1.0 mM. The E. coli cells were harvested after inducing for 2 and 4 h at 37°C, respectively. The induced cells were processed using the *E. coli* Protein Extraction Solution (HalGene, Harbin, China) following the manufacturer’s instructions. After the addition of 1 × loading buffer, the supernatants and cell pellets were boiled for 5 min, centrifuged for 10 min at 8,000 rpm and loaded into a 12% (v/v) polyacrylamide slab gel. The recombinant protein rTghSS42 was purified using the method of Sun et al. (2012).

The detection of enzymatic activity of rTghSS42

The transformant BL21-TghSS42 was induced with 1.0 mM IPTG at 40°C. The enzyme activity was measured at different inducing times ranging from 1 to 9 h at 1-h intervals; The activity of recombinant protease rTghSS42 induced at 4 h was measured at different temperature ranging from 30 to 60°C. The enzyme activity was measured at different pH values ranging from 4.0 to 8.5
The DNA sequence of serine protease gene TghSS42 is 1516 bp in length, containing four exons and three introns. The exons are 633, 303, 196 and 170 bp and the introns are 86, 56 and 72 bp in length. The splice sites between exon and intron follow the GT-AG splicing rule. The cDNA sequence of the protease gene TghSS42 is 1302 bp in length, encoding 433 aa with a calculated molecular weight of 42.5 kDa and pI of 5.53. The BlastP search indicates that the TghSS42 amino acid sequence shares 98% identity with a known protease (XP-006966748) from T. reesei. The cDNA sequence of the TghSS42 gene was deposited into the GenBank database with the accession number of KJ740359.

RESULTS

Cloning and sequence analysis of serine protease gene TghSS42

Antifungal property of purified rTghSS42

A 5 mm diameter disk of fungal phytopathogen mycelia was placed at the center of PDA plate that contained the 10% (v/v) purified rTghSS42. As a control, the rTghSS42 was heat-denatured and then added to Potato Dextrose Agar (PDA) medium at a concentration of 10% (v/v). After cultivating for 5 d at 28°C, the antagonistic activity of rTghSS42 was evaluated as described by Liu and Yang (2007). Six replicates were performed.

Transcription of TghSS42 in response to nine different treatments

The RT-qPCR analysis indicated that the TghSS42 gene was differentially regulated by different treatments at 0.5-unit intervals. All the experiments were performed three times. The activity of rTghSS42 was detected as described by Fan et al. (2014) and Lowry et al. (1951).

FIG. 1. Expression of TghSS42 in T. ghanense in response to nine different treatments. X-axis: time points, Y-axis: expression level=log2 (fold change in expression), namely gene expression in T. ghanense under different treatments. (a) cultured in MM; (b) cultured in MM-C (MM without the glucose carbon source); (c) cultured in MM-N (MM without nitrogen ammonium sulfate); (d-e) MM-stem and MM-leaf (adding 1% (w/v) stem and leaf powder of Pdpap poplar), respectively; (f-g) induced by MM-CW-Cc and MM-ferm-Cc (adding 1% (w/v) C. chrysosperma cell walls and 5% (v/v) C. chrysosperma fermentation supernatant), respectively; (h-i) induced by MM-CW-Aa and MM-ferm-Aa (adding 1% (w/v) A. alternata cell walls and 5% (v/v) A. alternata fermentation supernatant), respectively.
in *T. ghanense* ACCC 30153. The transcription of *TghSS42* gene was up-regulated under MM, MM-C and MM-N treatments and the peak transcription levels were 28.25 (2.33-fold), 10.19 (2.33-fold) and 3.59 (2.33-fold) at 8, 4, and 4 h, respectively (Figs.1a, 1b, 1c). Further, the transcription of *TghSS42* gene was also up-regulated by 1% (w/v) stem and leaf powder of *Pdpap* poplar treatments, and finally the peak transcription levels were 22.16 (4.43-fold) and 17.51 (4.13-fold) at 2 and 8 h, respectively (Figs.1d, 1e). Moreover, the transcription of *TghSS42* was also up-regulated under 1% (v/v) *C. chrysosperma*, *A. alternata* cell walls or 5% (v/v) *C. chrysosperma*, *A. alternata* fermentation supernatant treatment. The transcription maximum was 19.97 (2.32-fold), 177.29 (2.47-fold), 3.81 (2.03-fold) and 16.68 (2.06-fold) respectively (Figs.1f, 1h, 1g, 1i). In brief, the transcription of *TghSS42* gene was affected by nine different treatments. Both plant and plant pathogenic fungus treatments could trigger *TghSS42* transcription.

**Expression and purification of rTghSS42**

SDS-PAGE analysis was conducted to determine whether the recombinant transformant BL21-*TghSS42* could synthesize recombinant protease rTghSS42. Compared with the control transformant BL21-*pGEX*, the transformant BL21-*TghSS42* showed a clear protein band with a molecular weight of approximately 68.5 kDa (containing 26.0 kDa GST tag, Fig.2). The result indicated that protease TghSS42 had been successfully synthesized in *E. coli* BL21.

**The property of the purified recombinant protease rTghSS42**

The activity of purified recombinant protease rTghSS42 from the transgenic *E. coli* strain BL21-*TghSS42* reached a peak of 18.7 U/mL at 4 h following 1.0 mM IPTG induction (Fig.3a). The activity of purified rTghSS42 reached a peak of 20.1 U/mL at 40°C and was dramatically reduced at temperature more than 40°C. Therefore, the optimal reaction temperature for rTghSS42 was 40°C (Fig.3b). At 40°C, the activity of recombinant protease rTghSS42 was stable in the pH range of 4.0 to 8.5, with a peak of 20.1 U/mL at pH 7.0 (Fig.3c).

**Antifungal property of purified rTghSS42**

The purified rTghSS42 showed different affection to five phytopathogens growth and spores production (Fig.4). The rTghSS42 exerted broad-spectrum anti-
species could be regulated by plant. The aspartic proteases PapA and PapB of *T. asperellum* were induced in response to plant root attachments (Viterbo et al., 2004). The expression of aspartic protease gene *Asp55* from *T. asperellum* was down-regulated under 1% (w/v) root and leaf powder of *Pdpap* poplar treatments (Dou et al., 2014). The transcription of the subtilisin-like serine protease gene *ThSS45* from *T. harzianum* was up-regulated under 1% (w/v) root, stem, and leaf powder of *Pdpap* poplar treatments, with peak transcription level of 3.47-, 2.28-, and 2.65-fold at 6, 12, and 6 h, respectively (Fan et al., 2014). Compared with the gene *ThSS45* (Fan et al., 2014), the gene *TghSS42* was obviously up-regulated under 1% (w/v) stem powder (Fig. 1a) and 1% (w/v) leaf (Fig. 1b) powder of *Pdpap* poplar treatments, reaching to 22.16 (2^4.28)- and 17.51 (2^4.13)-fold at 2 and 8 h, respectively. This suggests that the gene *TghSS42* can respond to the poplar induction more strongly than *ThSS45*. In a word, the plant can induce the biocontrol activity of *Trichoderma* species.

The transcription level of serine protease in *Trichoderma* species was also regulated by phytopathogenic fungi. The serine protease gene *tvsp1* from *T. virens* was strongly up-regulated under the *R. solani* cell walls, with the maximum level of expression observed at 24 h (Pozo et al., 2004). The subtilisin-like protease gene *SS10* from *T. harzianum* reached the highest levels at 4 h in the presence of fungal cell walls (*C. chrysosperma, R. solani* or *F. oxysporum*) (Liu and Yang, 2009). The serine protease gene *ThSS45* from *T. harzianum* was slightly up-regulated under 1% (w/v) *A. alternata* cell walls at 6 h with peak transcription level of 1.4 times of pretreatment (Fan et al., 2014). Similar to *prb1, tsp1* and *SS10* (Cortés et al., 1998; Pozo et al., 2004; Liu and Yang, 2009), the gene *TghSS42* of *T. ghanense* was strongly induced by the fungal cell walls (*A. alternata* and *C. chrysosperma*) at 4 h, suggesting that *TghSS42* could participate in early stages of the mycoparasitic process. Moreover, the transcription of the serine protease gene *ThSS45* was slightly up-regulated under 1% (w/v) fermentation supernatant of *A. alternata* (Fan et al., 2014). In our study, the subtilisin-like serine protease gene *TghSS42* from *T. ghanense* was also up-regulated under 5% (v/v) fermentation supernatant of *A. alternata* and *C. chrysosperma* at 4 h and 4 h, respectively. In conclusion, the subtilisin-like serine protease *TghSS42* has biocontrol functions in the interactions of *Trichoderma* species with pathogen or plant.

The proteases play roles in the interactions of *Trichoderma* species with plant or pathogen in the environment (De Marco and Felix, 2002; Viterbo et al., 2004; Benítez et al., 2004). The protease functions of the biocontrol *T. asperellum* and *T. harzianum* have been studied, but there is no report of the biocontrol function of serine protease of *T. ghanense*.

**TABLE 2**. Inhibition rate of five phytopathogens growth under rTghSS42 stress

<table>
<thead>
<tr>
<th>Plant phytopathogen</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. solani</em></td>
<td>67.0</td>
<td>85.0</td>
<td>21.2</td>
</tr>
<tr>
<td><em>F. oxysporum</em></td>
<td>32.0</td>
<td>46.0</td>
<td>30.4</td>
</tr>
<tr>
<td><em>A. alternata</em></td>
<td>48.0</td>
<td>61.0</td>
<td>21.3</td>
</tr>
<tr>
<td><em>S. sclerotiorum</em></td>
<td>6.0</td>
<td>12.0</td>
<td>50.0</td>
</tr>
<tr>
<td><em>C. chrysosperma</em></td>
<td>65.0</td>
<td>88.0</td>
<td>26.1</td>
</tr>
</tbody>
</table>

A. The diameter of mycelia under treated with rTghSS42 (mm)
B. The diameter of mycelia under treated with denatured rTghSS42 (mm)
C. Inhibition rate of mycelia growth (%)

**DISCUSSION**

The proteases are important secreted proteins of *Trichoderma* species. The proteases play roles in the interactions of *Trichoderma* species with plant or pathogen in the environment (De Marco and Felix, 2002; Viterbo et al., 2004; Benítez et al., 2004). The protease functions of the biocontrol *T. asperellum* and *T. harzianum* have been studied, but there is no report of the biocontrol function of serine protease of *T. ghanense*. The transcription of protease genes from *Trichoderma* species could be regulated by plant. The aspartic proteases PapA and PapB of *T. asperellum* were induced in response to plant root attachments (Viterbo et al., 2004). The expression of aspartic protease gene *Asp55* from *T. asperellum* was down-regulated under 1% (w/v) root and leaf powder of *Pdpap* poplar treatments (Dou et al., 2014). The transcription of the subtilisin-like serine protease gene *ThSS45* from *T. harzianum* was up-regulated under 1% (w/v) root, stem, and leaf powder of *Pdpap* poplar treatments, with peak transcription level of 3.47-, 2.28-, and 2.65-fold at 6, 12, and 6 h, respectively (Fan et al., 2014). Compared with the gene *ThSS45* (Fan et al., 2014), the gene *TghSS42* was obviously up-regulated under 1% (w/v) stem powder (Fig. 1a) and 1% (w/v) leaf (Fig. 1b) powder of *Pdpap* poplar treatments, reaching to 22.16 (2^4.28)- and 17.51 (2^4.13)-fold at 2 and 8 h, respectively. This suggests that the gene *TghSS42* can respond to the poplar induction more strongly than *ThSS45*. In a word, the plant can induce the biocontrol activity of *Trichoderma* species.

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The serine proteases of biocontrol *Trichoderma* could inhibit phytopathogen growth. The subtilisin-like protease gene *SS10* from *T. harzianum* T88 was expressed in *S. cerevisiae* and recombinant protease rSS10 has broad-spectrum antifungal activity against *F. oxysporum, S.*
sclerotiorum, R. solani, C. chrysosperma and A. alternata with at 50- and 100-fold concentrated culture (Liu and Yang, 2009). The serine protease gene SL41 from T. harzianum T88 was expressed in S. cerevisiae and the recombinant protease RSL41 can inhibit the mycelial growth of phytopathogenic fungi (Liu and Yang, 2013). In our study, the recombinant protease rTghSS42 was added to PDA medium to evaluate its antagonistic activity. The recombinant protease rTghSS42 exerted broad-spectrum antifungal activity against R. solani, F. oxysporum, A. alternata, S. sclerotiorum and C. chrysosperma with the inhibition rate of 21.2%, 30.4%, 21.3%, 50.0% and 26.1%, respectively. The inhibition property of rTghSS42 may be related with cell wall components of the pathogenetic fungi. The major components of cell walls of phytopathogenic fungi are chitin, glucan, and protein (Jones, 1970). Protease can digest the fungal cell wall proteins to inhibit the growth of phytopathogens (De Marco and Felix, 2002; Liu and Yang, 2007; Liu and Yang, 2009). The recombinant protease rTghSS42 could also digest fungal cell walls. Especially, the rTghSS42 showed the highest inhibition rate against S. sclerotiorum and the reason might be that there are more proteins in the cell wall of S. sclerotiorum or the cell wall proteins of S. sclerotiorum is more easily degraded. The S. sclerotiorum can infect at least 400 plant species and cause serious yield losses on many important crops throughout the world (Atallah et al., 2004; Hegedus and Rimmer, 2005). The S. sclerotiorum can produce sclerotia under adverse environmental conditions and its scleroria can survive for up to 8 years in soil (Willetts, 2005; Bae and Knudsen, 2007), so it is difficult to control the disease caused by S. sclerotiorum. The strain of S. sclerotiorum used in the study can cause sclerotiorum disease. So the research on the protease TghSS42 is significant for future control of soybean sclerotiorum disease. Furthermore, from the transcription level of TghSS42, the cell wall (Figs.1f, 1h) and fermentation supernatant inductions (Figs.1g, 1i) of A. alternata was higher than those of C. chrysosperma. The inhibition rate of the rTghSS42 to C. chrysosperma was higher than that of A. alternata (Table 1). So the inhibition rate did not agree with the transcription of TghSS42 induced by A. alternata and C. chrysosperma. It could be that there are many genes participating in the Trichoderma mycoparasitic process. In total, the subtilisin-like protease gene TghSS42 from T. ghanense has biocontrol function and the recombinant rTghSS42 could antagonize phytopathogens by inhibiting mycelial growth.

In the study, the subtilisin-like serine protease gene TghSS42 was cloned from T. ghanense and expressed in E. coli. The antifungal ability of the rTghSS42 was assayed. The expression of TghSS42 from T. ghanense provides a theoretical support for further studies of protease involving biocontrol and practical reference for applications of the genus Trichoderma.

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