微生物学报 *Acta Microbiologica Sinica*
50(9):1185 - 1193; 4 September 2010
ISSN 0001 - 6209; CN 11 - 1995/Q
http://journals.im.ac.cn/actamicrocn

Expression of a modified antimicrobial peptide BhSGAMP-1-S (*Bradysia hygida*) in *Escherichia coli* and characterization of its activity

Jue Wang[2], Qiurong Gao[3], Qiang Liu[2], Linping Xiang[1], Yuqin Wang[2], Dun Wang[1*]

([1] Key Laboratory of Plant Protection Resources and Pest Management, Ministry of Education, [2] College of Forestry, [3] College of Life Science, Northwest A&F University, Yangling 712100, China)

Abstract: [**Objective**] *Bradysia hygida* salivary glands antimicrobial peptide 1 (BhSGAMP-1) is one of the antimicrobial peptides involved in a preventive mechanism of defense of the fly *Bradysia hygida*. To know better about the molecular characterization of this antimicrobial peptide, we expressed and purified the modified BhSGAMP-1-S and investigated its antimicrobial activity. [**Methods**] We synthesized the gene of BhSGAMP-1-S designed with preferred codons of *Escherichia coli* and expressed it as a fusion protein in *E. coli* TB1 by using pMAL-c2X as vector. We purified the fusion protein using amylase resin affinity chromatography. In addition, we cleaved the fusion protein by enterokinase and the released recombinant BhSGAMP-1-S was separated by size exclusion chromatography, then followed by reversed-phase high performance liquid chromatography. We analyzed the antimicrobial activities of the purified recombinant BhSGAMP-1-S by bioassays. [**Results**] The fusion protein was mostly expressed in soluble form under the optimized conditions. The recombinant BhSGAMP-1-S was produced with a pure yield of 0.38 mg/100 mL culture medium. Antimicrobial assays demonstrated that the recombinant BhSGAMP-1-S was active against several Gram-positive and Gram-negative bacteria and fungi. [**Conclusion**] It appears to be the first successful production of the recombinant BhSGAMP-1-S from fly *Bradysia hygida*. Data presented here confirm that the recombinant BhSGAMP-1-S is now ready for further studying and characterizing their antimicrobial properties.

Keywords: antimicrobial assays; antimicrobial peptides; BhSGAMP-1-S; *Bradysia hygida*; purification; soluble expression

CLC number: Q936 **Document code**: A **Article ID**: 0001-6209 (2010) 09-1185-09

Antimicrobial peptides (AMPs) are one of the most important effectors of the innate immune system, which are evolutionarily conserved component of the principal defense system for the majority of living organisms, and are found among all classes of life including insects, amphibians and mammals[1-3]. Insect AMPs are typically cationic and often made of less than 100 amino acid residues. These peptides

Supported by the National Natural Science Foundation of China (30872033) and the Program for New Century Excellent Talents in University (NECT-06-0867)

* Corresponding author. Tel/Fax: +86-29-87091511; E-mail: wanghande@yahoo.com, wanghande@nwsuaf.edu.cn

Received: 24 March 2010/Revised: 20 April 2010

show a broad spectrum of antimicrobial activity which is probably due to the particular capacity of AMPs to disrupt the cell membranes through non-specific peptide - lipid interactions[4]. The most intriguing feature of these molecules is their potential of not to fall victim to the same microbial resistance problems as conventional antibiotics, because their mechanism of action is complicated[5-6]. Furthermore, these peptides suppress bacterial infections but have minimal toxic and allergic effects to the hosts[7]. Therefore, AMPs have received increasing attentions as potential new antimicrobial substances for use in therapeutics[8], animal drugs and food preservatives[9].

Bradysia hygida salivary gland antimicrobial peptide 1 (BhSGAMP-1) is one of the AMPs involved in a preventive mechanism of defense of the fly *Bradysia hygida* which is expressed exclusively in the salivary glands of the larvae to help prevent microbial infection while they are preparing to ecdysis[10]. The gene of BhSGAMP-1 is developmentally regulated by the direct action of 20-OH ecdysone in the salivary gland of *Bradysia hygida* and BhSGAMP-1 expression is in the saliva of these insects, at the end of larval stage, but not in the hemolymph. This preventive defense system probably functions during all larval molts. At least during the third larval molt, BhSGAMP-1 is also active[11]. In this work, the gene of BhSGAMP-1-S was synthesized with preferred codons of *E. coli* to facilitate its expression. Then a recombinant plasmid pMAL-c2X-BhSGAMP-1-S was constructed to express the BhSGAMP-1-S in *E. coli* with maltose-binding protein (MBP) as a fusion partner. The optimal cultivation parameters were determined for expression of the soluble fusion protein. Antimicrobial activity of the purified peptide was confirmed using different assays.

1 MATERIALS AND METHODS

1.1 Materials

Escherichia coli TB1 strain, the expression vector pMAL-c2X and amylose resin were purchased from New England Biolabs. Restriction enzymes, T4 DNA ligase, DL-2000 DNA marker and protein molecular mass standards were obtained from TaKaRa Biotechnology Co. Ltd. (Dalian, China). Protein low molecular mass standards were obtained from SBS Genetech Co. Ltd. (Beijing, China). Enterokinase was purchased from Sinobio Biotech Co. Ltd. (Shanghai, China). Yeast extract, tryptone and beef extract were obtained from Oxoid (England). Anti-Maltose binding and anti-rabbit IgG were purchased from TIANGEN Biotech Co. Ltd. (Beijing, China) and Pioneer Biotechnology Inc. (Xi´an, China), respectively. All other chemicals were of analytical grade from Sangon Biotech Co. Ltd. (Shanghai, China).

1.2 Construction of the recombinant expression vector

The original gene of BhSGAMP-1 (GenBank accession No. DQ180321.1) was modified using codon preference of *E. coli*, resulting a BhSGAMP-1-S gene. An enterokinase (EK) cleavage site was added to the 5'-terminal of the BhSGAMP-1-S so that no vector-derived residue is attached to the protein of interest after tag removal. The new BhSGAMP-1-S gene was synthesized and ligated to the pGE plasmid resulting of a recombinant plasmid, pGE-BhSGAMP-1-S. Gene encoding BhSGAMP-1-S was cleaved from the recombinant pGE-BhSGAMP-1-S with *Bam*HI and *Hin*dIII restriction enzymes and DNA fragment was ligated to pMAL-c2X vector. The recombinant expression vectors were transformed into *E. coli* TB1 cells, resulting of the plasmid TB1/pMAL-c2X-BhSGAMP-1-S. The fusion expression vector was confirmed by restriction endonucleases digestion and sequencing.

1.3 Expression and purification of fusion protein

Overnight cultures of a recombinant clone of BhSGAMP-1-S were inoculated into 100 mL of LB medium with 100 μg/mL ampicillin and cultured at 37℃ with vigorous shaking. When the culture OD_{600} reached about 0.6, the expression of fusion protein MBP-BhSGAMP-1-S was induced by IPTG of a final concentration of 0.4 mmol/L, followed by 8 h shaking at 30℃. The cells were harvested by centrifugation at 4000 × g for 20 min at 4℃. The pellet was resuspended in 4 mL Column Buffer (20 mmol/L Tris-

HCl, 200 mmol/L NaCl, 1 mmol/L EDTA, pH 7.4) and lysed by sonication in short pulses of 10 s for 10 times. Soluble protein fraction was recovered by centrifugation of bacterial extract at 9000 × g for 30 min at 4℃. Dilute the supernatant 1∶5 with the column buffer and applied to a pre-equilibrated amylose resin column at a flow rate of 0.3 mL/min. The fusion protein was eluted with column buffer including 10 mmol/L maltose at a flow rate of 0.15 mL/min. The fractions were collected, monitored by UV absorbance at 280 nm.

1.4 Cleavage and purification of the recombinant BhSGAMP-1-S

After dialyzing overnight at 4℃ with EK reaction buffer [50 mmol/L Tris-HCl, 1 mmol/L $CaCl_2$, 1 % (v/v) Tween-20, pH 8.0], the fusion protein was cleaved by 1.8 U EK at 37℃ for 16 h. The cleavage mixture was desalted by dialysis in 20 mmol/L Tris-HCl (pH 8.0) at 4℃. Released recombinant BhSGAMP-1-S was separated from the mixture by size exclusion chromatography (Superdex™ 75) medium (Amersham Biosciences. Bead size: 11 mm – 15 mm) in a column of 10 mm × 300 mm (column bed volume: 24 mL), which was pre-equilibrated with 20 mmol/L Tris-HCl (pH 8.0). Each sample was loaded with a volume of 0.5 mL and the chromatograms were recorded at 280 nm. Molecular mass was estimated based on their correlation in retention time to standards of various sizes. The elution was performed using 20 mmol/L Tris-HCl (pH 8.0) at a flow rate of 1.5 mL/min. The fractions were collected.

The monomeric BhSGAMP-1-S pooled from the Superdex™ 75 column was subjected to preparative reversed-phase high performance liquid chromatography (RP-HPLC) to further purify the recombinant BhSGAMP-1-S. RP-HPLC was performed on C_{18} column (Symmetry ™ RP -C_{18}, 4.6 nm × 250 mm, 5 μm). The elution was conducted with a linear gradient of 20 % – 90 % (v/v) acetonitrile in 0.1 % (v/v) trifluoroacetic acid (TFA) at the flow rate of 1.5 mL/min for 40 min. The elution was monitored at 225 nm and 280 nm. The eluted fractions were collected corresponding to the absorbance peaks and freeze – dried.

1.5 SDS-PAGE, Tricine – SDS – PAGE and Western blotting

The fractions of affinity chromatography, the cleavage mixture and the fractions of molecular exclusion chromatography were analyzed by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method. After electrophoresis, gels were stained with Coomassie brilliant blue R-250, or peptides were transferred to nitrocellulose membranes. Western blotting was performed as described by Sambrook *et al*.[12], with rabbit anti-Maltose binding protein as the primary antibody and peroxidase-conjugated goat anti-rabbit IgG. The recombinant antimicrobial peptide was analyzed by Tricine – SDS – PAGE according to the method of Schägger and von Jagow[13]. The images of gels and membranes were scanned by GS-800 Calibrated Densitometer (Bio-Rad, USA).

1.6 Antimicrobial activity assay

The antimicrobial activities of purified recombinant BhSGAMP-1-S were analysed against two Gram-negative bacteria (*E. coli*, *Salmonella paratyphi B*), three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus pneumoniae*) and fungi (*Rhizoctonia solani*, *Rhizoctonia cerealis*, *Marssonina zanthoxyli* Chona & Munjal, *Exserohilum turcicurn* (*Pass*) Leonard & Suggs, *Venturia nashicola* Tanaka & Yamamoto, *Puccinia striiformis* f. sp. *tritici*).

The minimal inhibitory concentration (MIC) for liquid growth inhibition assay with bacteria was determined using a 96-well sterile plates as previously described[14]. The sample peptide was dissolved and 2-fold serial dilutions (1 – 10 μmol/L final concentrations) were made in double distilled water, and 10 μL of each dilution were mixed in a 96-well sterile plates with 90 μL of a suspension of mid-log test bacteria culture (7 × 10^5 CFU/mL) in LB medium. The peptides, a non-treated control, and a sterility control were tested in triplicate. The microtiter plates were incubated for 24 h at 37℃. Growth inhibition was determined by optical density measurement at 620 nm and the lowest concentration of peptide at which there was no change in optical density was identified as the

MIC.

For the radial diffusion assay [15], 100 μL of bacterial culture (10^6 CFU /mL) were spread on 90-mm LB plate. A 5-mm diameter gel punch was used to make evenly spaced wells. For the assay, 20 μL of 10 μmol/L recombinant BhSGAMP-1-S was added to each well. For each plate tested, one well was filled with double distilled water as a negative control. The plates were then left undisturbed to allow diffusion of the sample into the agar, and then they were incubated inverted in the dark at the appropriate temperature of each strain for 16 h. The diameter of the clear zone surrounding each well was measured.

The antifungal activity assay was performed on *Puccinia striiformis* f. sp. *tritici* by microscopically examining the growth of spores. 50 μL spore suspension (10^6 spores/mL) was added into 50 μL peptide sample (10 μmol/L) in a 1.5 mL Eppendorf tube. The mixture was spread on 2% agar plates and the plates were incubated at 9℃ for 20 h, and the growth of the spores was monitored microscopically. For the negative control, 50 μL of the double distilled water lacking the peptides was added. The filamentous fungi *Rhizoctonia solani*, *Rhizoctonia cerealis*, *Marssonina zanthoxyli* Chona & Munjal, *Exserohilum turcicurn* (*Pass*) Leonard & Suggs and *Venturia nashicola* Tanaka & Yamamoto were inoculated onto PDA plates containing the peptide (10 μmol/L) for the growth inhibition method. The plates were incubated at 28℃ for 4 – 7 d and the growth of the cells were checked by comparing to that of the negative controls and the inhibitory rate was obtained. For the negative controls, no peptide was added into the solid media.

2 RESULTS

2.1 Construction of expression plasmids

The BhSGAMP-1-S gene designed with preferred codons of *E. coli* was synthesized (GenBank accession no. GQ914992) (Fig. 1). The expression plasmid pMAL-c2X-BhSGAMP-1-S was constructed and it was confirmed by digestion with restriction enzymes (Fig. 2) and sequencing (data not shown).

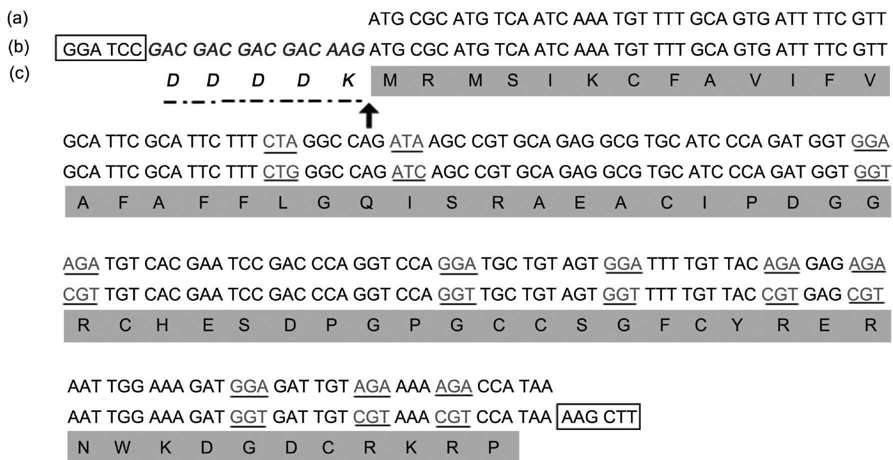

Fig. 1 The cDNA and amino acid sequences of the BhSGAMP-1-S with EK cleavage site. (a) The original DNA sequence of BhSGAMP-1. (b) The BhSGAMP-1-S sequence in which the rare codons for *E. coli* were replaced by preference codons (underlined codons); the *Bam*HI (GGATTC) and *Hin*dIII (AAGCTT) restriction sites are showed in the box; the cDNA sequence of EK recognition sequence is showed in bold italics. (c) The amino acid sequence of modified BhSGAMP-1-S. The EK recognition sequence (DDDDK) is underlined with a dotted line while the EK-cleavage site is indicated by an arrow.

2.2 Expression and purification of the fusion protein

From SDS-PAGE analysis (Fig. 3-A), a dense protein band of fusion protein around 50 kDa was produced in host cells, which was close to the predicted size. Furthermore, induction of the TB1/pMAL-c2X-BhSGAMP-1-S cells under the optimal conditions led to the high level expression of the soluble recombinant protein MBP-BhSGAMP-1-S. Western blot analysis (Fig. 3-B) revealed that the fusion protein MBP-BhSGAMP-1-S was specifically

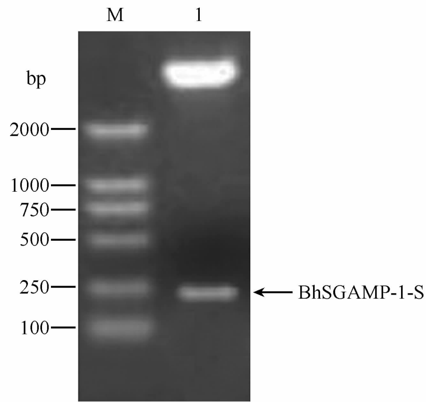

Fig. 2 Electrophoresis analysis of BhSGAMP-1-S gene. Lane M, DL-2000 DNA marker; Lane 1, pMAL-c2X - BhSGAMP-1-S plasmids were digested with *Bam*HI and *Hin*dIII.

recognized by antibody, confirming that the expressed fusion protein was MBP-BhSGAMP-1-S.

2.3 Cleavage and purification of recombinant BhSGAMP-1-S

The SDS-PAGE analysis (Fig. 3-A) shows that the fusion protein produced two new protein bands after EK treatment. The molecular weights of MBP and BhSGAMP-1-S were about 42.5 kDa and 7.2 kDa respectively. The antibodies could immunologically recognize MBP of the cleavage mixture in Western blot (Fig. 3-B). After removing MBP tag by size exclusion chromatography, the recombinant BhSGAMP-1-S was further purifled to homogeneity by RP-HPLC. The peptide was eluted as single symmetrical sharp peak with a retention time of 16.12 min (Fig. 4). From the result of Tricine-SDS-PAGE analysis (Fig. 5), an approximately 7.2 kDa band corresponding to the recombinant monomer BhSGAMP-1-S was observed. The purity and yield of each purification step can be

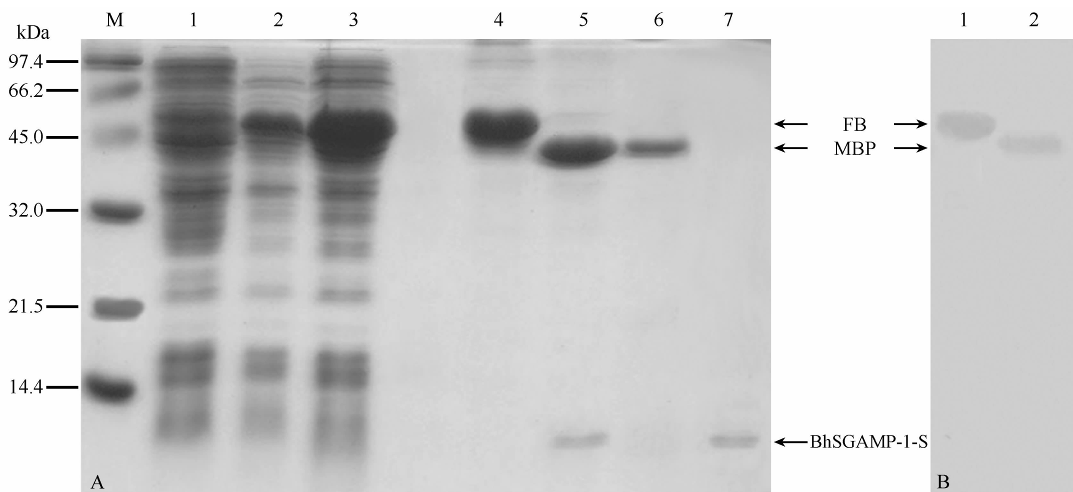

Fig. 3 (A) 12% SDS-PAGE analysis of cleavage and purification of the recombinant BhSGAMP-1-S. Lane M, protein molecular mass standards; Lane1, total proteins prior the IPTG induction; Lane 2, total proteins after induction with 0.4 mmol/L IPTG at 30℃ for 8 h; Lane 3, the soluble protein fractions; Lane 4, MBP-BhSGAMP-1-S fusion protein by affinity chromatography; Lane 5, fusion protein cleaved by EK; Lanes 6 - 7, the first and second fractions separated by size exclusion chromatography. (B) Western blot analysis. Lane 1, the soluble protein fractions; Lane 2, fusion protein cleaved by EK.

seen in Table 1. The highest purity was observed after the step of RP-HPLC with a yield of 0.38 mg of pure recombinant BhSGAMP-1-S from 100 mL of *E. coli* culture.

2.4 Antimicrobial activity assay

Antimicrobial activity of recombinant BhSGAMP-1-S was tested against five bacterial strains using liquid growth inhibition assay and radial diffusion assay. The results of MIC and the diameter of the clear zone were given in Table 2. It was found that the recombinant BhSGAMP-1-S was relatively effective against several Gram-negative and Gram-positive bacteria tested and *E. coli* was most sensitive to the peptide (Fig. 6-A). Fungicidal assays were performed with five filamentous fungi by culturing the strains on solid PDA medium containing 10 μmol/L BhSGAMP-1-S. There were four filamentous fungi sensitive to the peptide and *Rhizoctonia cerealis* was most sensitive to the peptide

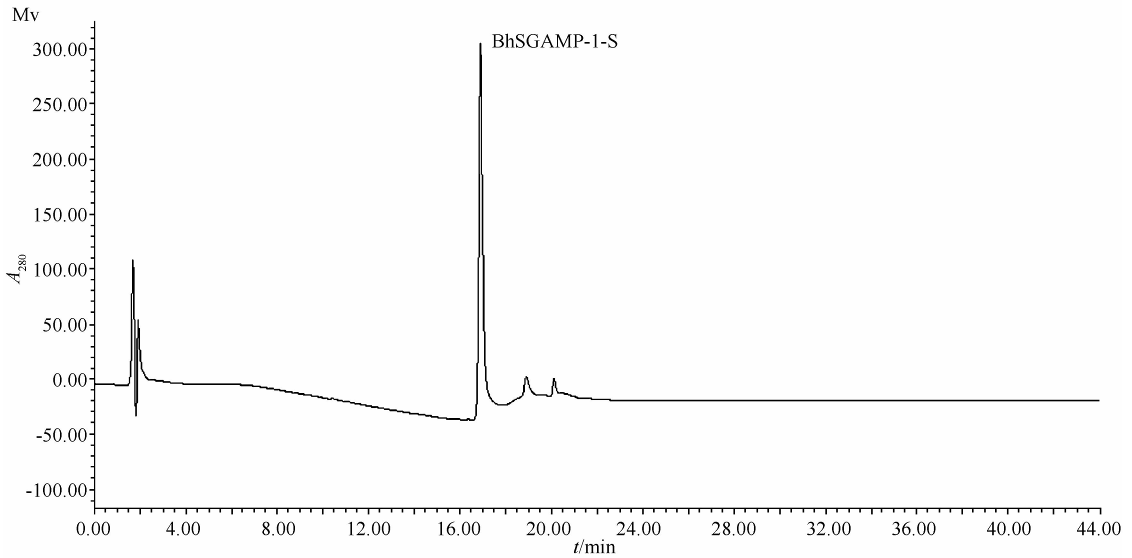

Fig. 4 Purification of BhSGAMP-1-S by RP-HPLC of eluted fractions from size exclusion chromatography.

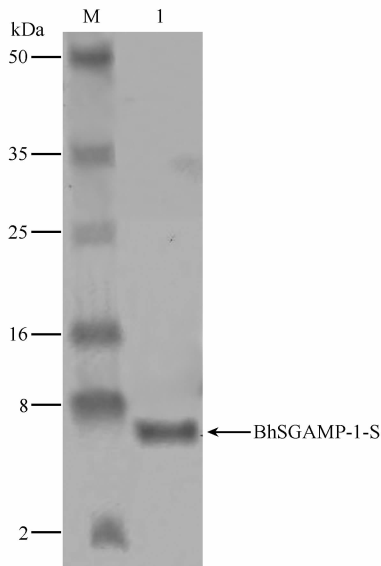

Fig. 5 Tricine-SDS-PAGE analysis of recombinant BhSGAMP-1-S. Lane M, protein low molecular mass standards; Lane 1, the recombinant BhSGAMP-1-S purified by RP-HPLC.

(Fig. 6-B), whereas inhibitation was less pronounced for *Marssonina zanthoxyli* Chona & Munjal (Table 3). By microscopically examining the growth of spores, it was found that the mycelium formation of *Puccinia striiformis* f. sp. *tritici* was strongly inhibited (Fig. 7 and Table 4).

3 DISCUSSION

MBP is a particularly useful fusion parter and affinity tag for the production of recombinant proteins in *E. coli*. Not only it is usually produced in great abundance and affords some degree of protection from intracellular proteolysis in *E. coli*, but also its natural affinity for maltodextrins has been shown to be used for purification. In addition, MBP has a remarkable ability to enhance the solubility and to promote the proper folding of its fusion partners [18], and has been utilized

Table 1 Approximately yield of total and targeted proteins at different steps of purification

Purification step	Total soluble protein/mg[a]	MBP-BhSGAMP-1-S/mg	BhSGAMP-1-S /mg	Purity /%	Recovery /%
Sonicated supernatant	58.10	30.56[b]	4.43[d]	NA[e]	100[d]
Affinity chromatography	27.04	24.01[b]	3.48[d]	88.8[c]	78.6[d]
Size-exclusion chromatography	0.64	NA[e]	0.58[b]	91.0[c]	13.1
RP-HPLC	0.40	NA[e]	0.38[b]	96.9[c]	8.6

Shown in the table are average results from three independent assays. a: The extraction and purification start from 100 mL bacteria cultured. Protein concentration was determined by Bradford protein assay [16] using the bovine serum albumin as standard. b: Percentage of fusion protein (MBP-BhSGAMP-1-S) from total proteins was estimated by SDS-PAGE gel scanning and Bandscan 5.0 [17]. c: Purity of protein or peptide was estimated by SDS-PAGE gel scanning and Bandscan 5.0. d: Theoretical; calculated from the difference in the molecular masses of BhSGAMP-1-S and MBP-BhSGAMP-1-S. e: NA means not applicable.

Table 2　The minimal inhibitory concentration and inhibition zone for the recombinant BhSGAMP-1-S against selected bacteria

	Bacteria	MIC/ (μmol/L)	Inhibition zone/mm
Gram-positive	*Bacillus subtilis*	2.3	10.1 ± 0.6
	Staphyloccocus aureus	4.0	7.3 ± 0.5
	Streptococcus pneumoniae	8.0	5.9 ± 0.6
Gram-negative	*Escherichia coli*	2.2	10.6 ± 0.4
	Salmonella paratyphi B	6.2	8.5 ± 0.5

Shown in the table are average results from three independent assays.

Table 3　The inhibitory rate for recombinant BhSGAMP-1-S against selected fungi

Fungi	Inhibitory rate /%
Rhizoctonia solani	25.56 ± 0.7
Rhizoctonia cerealis	32.76 ± 0.6
Marssonina zanthoxyli Chona & Munjal	7.92 ± 0.8
Exserohilum turcicurn (*Pass*) Leonard & Suggs	11.11 ± 0.5

Shown in the table are average results from three independent assays.

Table 4　Determination of the antimicrobial activity spectrum of bacteria and fungi strains

Gram-positive bacteria		Gram-negative bacteria		Fungi	
Bacillus subtilis	+	*Escherichia coli*	+	*Rhizoctonia solani*	+
Staphyloccocus aureus	+	*Salmonella paratyphi B*	+	*Rhizoctonia cerealis*	+
Streptococcus pneumoniae	−			*Marssonina zanthoxyli* Chona & Munjal	+
				Exserohilum turcicurn (*Pass*) Leonard & Suggs	+
				Venturia nashicola Tanaka & Yamamoto	−
				Puccinia striiformis f. sp. *tritici*	+

Inhibition of the Gram-positive bacteria and Gram-negative bacteria was determined by liquid growth inhibition assay and the radial diffusion assay. Inhibition of the filamentous fungi was detected by microscopic observation of spore growth or by examining the mycelia growth on solid medium. Plus sign indicates a substantial inhibition and minus means no inhibition or slight inhibition.

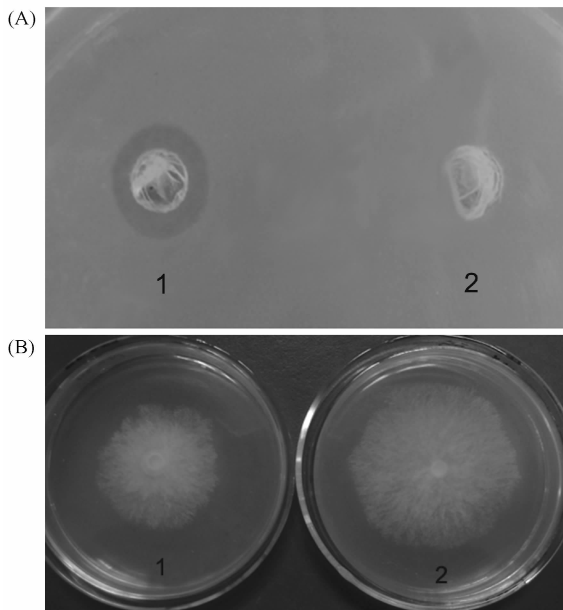

Fig. 6　(A) The antimicrobial activity of the recombinant BhSGAMP-1-S against *Escherichia coli* by the radial diffusion assay. (1) Recombinant BhSGAMP-1-S solution (10 μmol/L). (2) The negative control by sterile water. (B) The antimicrobial activity of the recombinant BhSGAMP-1-S against *Rhizoctonia cerealis by* the growth inhibition method. (1) Recombinant BhSGAMP-1-S solution (10 μmol/L). (2) The negative control by sterile water.

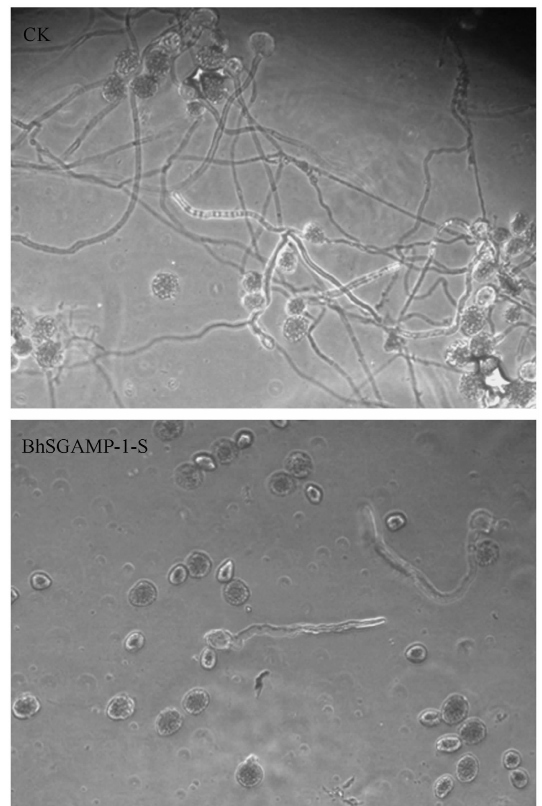

Fig. 7　Determination of the antifungal activity by microscopic detection of spore growth (400 ×). *Puccinia striiformis* f. sp. *tritici* was examed after the incubation of the mixture of their spores with the recombinant BhSGAMP-1-S.

to produce AMPs in previous study [19]. In present study, we cloned the codon optimized gene encoding the BhSGAMP-1-S into pMAL-c2X to produce the expression vector pMAL-c2X-BhSGAMP-1-S, and expressed soluble fusion protein in *E. coli* successfully. However, the fusion protein still

contained some contaminants after affinity chromatography although we add glucose into the media to repress the expression of the amylase. It was similar to those of the previous study[20].

Our results showed that the recombinant BhSGAMP-1-S designed with preferred codons of *E. coli* exhibited high antimicrobial activity against tested bacteria and fungi. The mode of antimicrobial action is not well understood, but this peptide exhibits homology to some AMPs of plant seeds [11]. Comparison with protein sequence data bases showed 57% identity with AMP2 precursor from *Mirabilis jalapa*[21]. These peptides are basic cysteine-rich peptides with typically biological properties that resemble those of defensins, a class of antimicrobial peptides and exhibit a broad spectrum of antibacterial and antifungal activities. The possible mechanism of antimicrobial activities is that BhSGAM-1-S could act on cell membranes by forming voltage-dependent ion-permeable channels[22].

The previous study reported chemically synthesized BhSGAMP-1 was effective in inhibiting the growth of both bacteria and yeast[10]. The recombinant BhSGAMP-1-S was active to fungi while the chemically synthesized was not, though the antibacterial activities against tested bacteria were similar compare to the chemically synthesized BhSGAMP-1. Moreover, the recombinant BhSGAMP-1-S shows higher antimicrobial activity against *E. coli* and *Staphyloccocus aureus* than chemically synthesized. At an economic point, the recombinant BhSGAMP-1-S could be a better approach to produce this protein in large quantity than chemically synthesized peptide that was limited to use for its high cost.

In conclusion, we synthesized the modified gene for BhSGAMP-1-S and expressed fusion proteins in soluble form in *E. coli*. The recombinant BhSGAMP-1-S presents the high antimicrobial activities against several bacteria and fungi. This is the first report of successfully expression of the recombinant BhSGAMP-1-S from the fly *Bradysia hygida*.

REFERENCES

[1] Boman H. Peptide antibiotics and their role in innate immunity. *Annual Review of Immunology*, 1995, 13:61-92.

[2] Hancock RE. Cationic peptides: effectors in innate immunity and novel antimicrobials. *The Lancet Infectious Diseases*, 2001, 1(3):156-164.

[3] Zasloff M. Antimicrobial peptides of multicellular organisms. *Nature*, 2002, 415:389-395.

[4] Epand RM, Vogel HJ. Diversity of antimicrobial peptides and their mechanisms of action. *Biochimica et Biophysica Acta*, 1999, 1462(1-2):11-28.

[5] Kelley KJ. Using host defenses to fight infectious diseases. *Nature Biotechnology*, 1996, 14(5):587-590.

[6] Marshall SH, Arenas G. Antimicrobial peptides: a natural alternative to chemical antibiotics and a potential for applied biotechnology. *Electronic Journal of Biotechnology*, 2003, 6(3):271-284.

[7] Giuliani A, Pirri G, Nicoletto SF. Antimicrobial peptides: an overview of a promising class of therapeutics. *Central European Journal of Biology*, 2007, 2(1):1-33.

[8] Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology*, 2006, 24(12):1551-1557.

[9] Andreu D, Ubach J, Boman A, Wahlin B, Wade D, Merrifield RB, Boman HG. Shortened cecropin A-melittin hybrids significant size reduction retains potent antibiotic activity. *FEBS Letters*, 1992, 296(2):190-194.

[10] Candido-Silva JA, Zanarotti GM, Gallina AP, de Almeida JC. Developmental regulation of BhSGAMP-1, a gene encoding an antimicrobial peptide in the salivary glands of *Bradysia hygida* (Diptera, Sciaridae). *Genesis*, 2007, 45(10):630-638.

[11] Zanarotti GM, Candido-Silva JA, de Almeida JC. BhSGAMP-1, a gene that encodes an antimicrobial peptide, is developmentally regulated by the direct action of 20-OH ecdysone in the salivary gland of *Bradysia hygida* (Diptera, Sciaridae). *Genesis*, 2009, 47(12):847-857.

[12] Sambrook J, Russel DW. *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Lavoratory Press, New York., 1989, pp. 18.1-18.85.

[13] Schägger H, von Jagow G. Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. *Analytical Biochemistry*, 1987, 166(2):368-379.

[14] Shlyapnikov YM, Andreev YA, Kozlov SA, Vassilevski AA, Grishin EV. Bacterial production of latarcin 2a, a potent antimicrobial peptide from spider venom. *Protein Expression and Purification*, 2008, 60(1):89-95.

[15] Xu F, Meng K, Wang YR, Luo HY, Yang PL, Wu NF, Fan YL, Yao B. Eukaryotic expression and antimicrobial spectrum determination of the peptide tachyplesin II. *Protein Expression and Purification*, 2008, 58(2):175-183.

[16] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 1976, 72(2):248-254.

[17] Zhou LF, Zhao ZH, Li BC, Cai YF, Zhang SQ. TrxA mediating fusion expression of antimicrobial peptide CM4 from multiple joined genes in *Escherichia coli*. *Proein Expression and Purification*, 2009, 64(2):225-230.

[18] Routzahn KM, Waugh DS. Differential effects of supplementary affinity tags on the solubility of MBP fusion proteins. *Journal of Structural and Functional Genomics*, 2002, 2(2):83-92.

[19] Pierce JC, Maloy WL, Salvador L, Dungan CF. Recombinant expression of the antimicrobial peptide polyphemusin and its activity against the protozoan oyster pathogen *Perkinsus marinus*. *Molecular Marine Biology and Biotechnology*, 1997, 6(3):248-59.

[20] De Pieri C, Beltramini LM, Selistre-de-Araújo HS, Vettore AL, da Silva FR, Arruda P, Oliva G, de Souza DH. Overexpression, purification, and biochemical characterization of GumC, an enzyme involved in the biosynthesis of exopolysaccharide by *Xylella fastidiosa*. *Protein Expression and Purification*, 2004, 34(2):223-228.

[21] Cammue BP, De Bolle MF, Terras FR, Proost P, Van Damme J, Rees SB, Vanderleyden J, Broekaert WF. Isolation and characterization of a novel class of plant antimicrobial peptides form *Mirabilis jalapa* L. seeds. *The Journal of Biological Chemistry*, 1992, 267(4):2228-2233.

[22] Reddy KVR, Yedery RD, Aranha C. Antimicrobial peptides: premises and promises. *International Journal of Antimicrobial Agents*, 2004, 24(6):536-547.

迟眼蕈蚊抗菌肽 BhSGAMP-1-S 在大肠杆菌中的优化表达及其活性分析

王珏[2],高秋荣[3],刘强[2],项林平[1],王玉芹[2],王敦[1]*

(西北农林科技大学,[1] 植保资源与病虫害治理教育部重点实验室,[2] 林学院,[3]生命科学学院, 杨凌 712100)

摘要:【目的】BhSGAMP-1 是迟眼蕈蚊唾液腺抗菌肽,为了能够更好的了解其分子特性,我们将其表达、纯化并进行了活性测定。【方法】依据大肠杆菌稀有密码子设计并合成了抗菌肽基因 BhSGAMP-1-S,以 pMAL-c2X 作为表达载体在大肠杆菌 TB1 中进行融合表达,融合蛋白通过麦芽糖亲和层析柱进行纯化,获得的融合蛋白经肠激酶切割后,混合物通过分子筛凝胶层析和反相高效液相色谱来获得单体重组抗菌肽 BhSGAMP-1-S,对获得的抗菌肽进行活性测定。【结果】在最优的表达条件下融合蛋白以可溶的形式表达,100 mL 诱导菌液经多步纯化后可得 0.38 mg 的重组抗菌肽 BhSGAMP-1-S,抑菌活性测定表明所获得的抗菌肽对部分测试革兰氏阳性细菌、革兰氏阴性细菌和真菌有较强的抑菌活性。【结论】本研究第一次成功的在大肠杆菌中诱导表达了修饰合成的抗菌肽 BhSGAMP-1-S,纯化后的抗菌肽具有很好的抑菌活性,这为进一步研究和应用奠定了基础。

关键词: 抑菌分析;抗菌肽;BhSGAMP-1-S;迟眼蕈蚊;纯化;可溶性表达

中图分类号: Q936 **文献标识码**:A **文章编号**:0001-6209 (2010) 09-1193-09

(本文责编:王晋芳)

基金项目:国家自然科学基金(30872033);教育部新世纪优秀人才项目(NECT-06-0867)

* 通信作者。Tel/Fax: +86-29-87091511; E-mail: wanghande@yahoo.com, wanghande@nwsuaf.edu.cn

作者简介:王珏(1982 -),女,辽宁普兰店人,硕士,研究方向为森林微生物。E-mail: wangjue1103@hotmail.com

收稿日期:2010-03-24;**修回日期**:2010-04-20