Supporting materials

Destruxin A induces and binds HSPs in *Bombyx mori* Bm12 cells

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Prokaryotic expression of BmHSPs

For cloning of BmHSP genes, the total RNA was isolated by Total RNA Kit II (OMEGA, USA) from the Bm12 cells in stable phase according to the manufacturer's protocol. The purity and concentration of total RNA were determined by SimpliNano micro spectrophotometer (GE Heathercare Life Science, Beijing, China). The cDNA was synthesized using the Reverse Transcriptase (Takara, Japan) following the manufacturer's protocol. The PCR specific primers were designed based on the *Bombyx mori* HSPs gene sequences (from NCBI data base) with restriction enzyme sites (Table S1). The PCR reaction system of 25μL contained LA PremixTaq (12.5μL), forward primer (1μL), reverse primer (1μL), template cDNA (1μL), and distilled water (9.5 μL). The amplification conditions consisted of a pre-denaturation at 94°C for 4 min, followed by 30 cycles of 94°C for 30 s, 56~58°C for 30 s and 72°C for 2.5 min, with a final extension at 72°C for 10 min. The PCR products were examined with 1% (w/v) gel electrophoresis and analyzed by sequencing. Additionally, the PCR products of BmHSP genes were gel purified with Universal DNA Purification Kit (Tiangen, Bejing, China) and used for structuring prokaryotic expression system.

Table S1. Primers of the silkworm HSP genes for PCR amplification

Gene	Gene Accession	Protein Accession	Primer (5′−3′)
BmHSP70-3 (heat shock protein 70-3)	JF836796.1	Bm_nscaf 2888_391	F: TAGGTACCATGGCAGCGAAGGAAAAGGCA R: CCGCTCGAGTGGTGGTGGTGTGGTGTAATTC
BmHSP75 (heat shock protein 75)	NM_001279432	Bm_nscaf 1898_379	F: TAGGTACCATGTTTGCAGTCCGTCCCAG R: CCGCTCGAGGTGTTTTTCTAAAACCTTGACTAGA
BmHSP83 (heat shock protein 83)	NM_001043411.1	Bm_nscaf 2801_21	F: CGATATCATGCCGGAAGAAATGGAG R: CCTCGAGATCAACTTCCTCCATGCG
BmHSCP (heat shock cognate protein 70)	NM_001043427.1	Bm_nscaf 2511_180	F: GTCTAGAATGGCTGCTATGTCCGTCAT R: CGAATTCAATTCAACATCCATTTGCTCTTGAG

To construct the prokaryotic expression system, the Cloning Vector or PET-30a plasmids (BioVector NTCC, Beijing, China) and *Escherichia coli* BL21 strain were used. Firstly, target gene fragments were cloned into the linear purpose vehicle PET-30a plasmids by T4 DNA ligase (TaKaRa, Takara Bio Inc., Shiga 525-0058, Japan) linking for static overnight at 16°C. Subsequently, the recombinant plasmids were transformed into competent host *Escherichia coli* BL21 cells, then, the positive clones were picked up and validated by sequencing.

The results indicated that the cells of *E. coli* BL21 were transformed successfully by recombinant plasmids. The electrophoretic profiles of PCR products showed the positive clones which contain the target BmHSPs genes (Figure S1), meanwhile the genes were confirmed by sequencing. Evidently, prokaryotic

expression system of BmHSPs were successfully constructed.

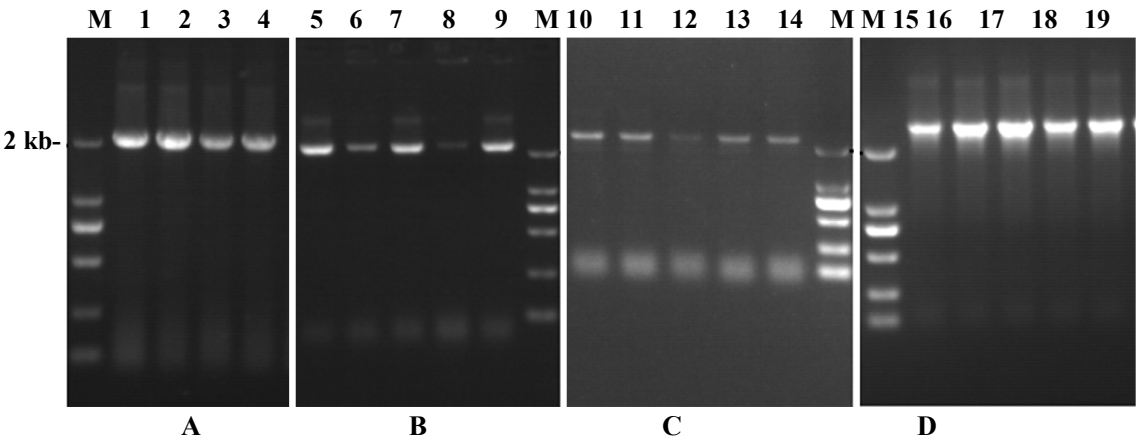

Figure S1 Electrophoresis profiles of recombinant clone by PCR identification. **A,** BmHSP70-3; **B,** BmHSP75; **C,** BmHSP83; **D,** BmHSCP; **M,** Marker; **1-19,** positive clones. The fragment sizes of BmHSP70-3, BmHSP75, BmHSP83 and BmHSCP were respectively about 1.95kb, 2.08kb, 2.15kb and 2.5kb.

Preparation of BmHSPs

Firstly, the recombinant *E.coli* strains were cultured and induced with IPTG in LB liquid medium (kanamycin resistance) at 37°C and 200 rpm. Then, the broths were centrifuged at 4100×g and 4°C for 10 min to respectively collect pellets and supernatants. The following steps were extracted total proteins from the pellets through ultrasonication on ice with cold phosphate buffered saline (PBS) and from the supernatants by centrifugation at 16500×g and 4°C. The total protein samples were dialyzed in the phosphate buffered saline at 4°C and passed through a 0.22 μm micro-pore membrane filter for removing impurities. Then, the samples were used to purify the target BmHSPs by the Nickel affinity chromatography employing BioLogic Duo-Flow system (Bio-Rad, Hercules, CA, USA). The target HSPs were validated by stability test (freeze thawing test), quantification (Bradford) and western blotting [1]. The test results showed the four HSPs were all eligible (Figure S2).

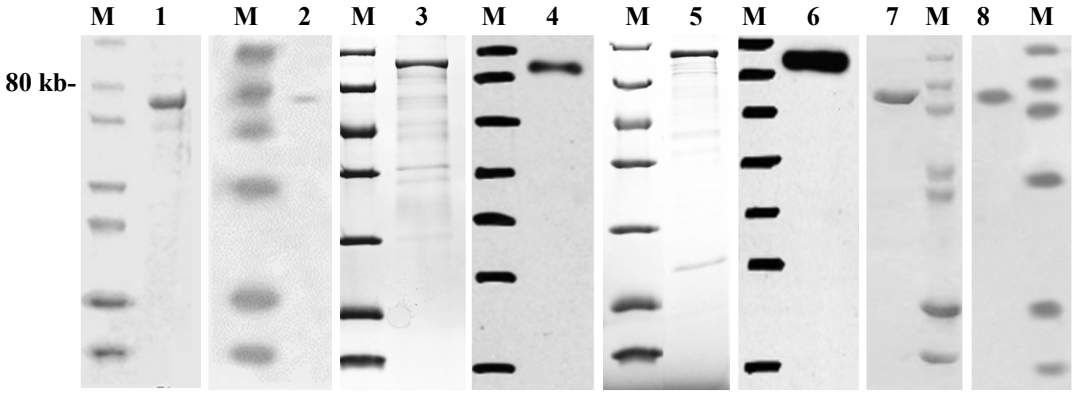

Figure S2. Profiles of gel electrophoresis and western blotting (WB) for target proteins. **1/2**, Gel / WB of BmHSP70-3; **3/4,** Gel / WB of BmHSP75; **5/6,** Gel / WB of BmHSP90; **7/8,** Gel / WB of BmHSCP; **M,** protein marker

Referrences

1. Guo, Y., Protein electrophoresis experiment technology - Second Edition. China Science Publishing and Media Ltd., Beijing, **2005**.