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Identification and expression profiling of five chemosensory protein genes in the whitefly MED, *Bemisia tabaci*

Ran Wang [a,1], Xiao-man Zhang [a,1], Hong-liang Li [b], Xiao-jun Guo [a], Chen Luo [a,*]

[a] Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, People's Republic of China
[b] College of Life Sciences, China Jiliang University, Hangzhou, 310018, People's Republic of China

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ABSTRACT

Insect chemosensory proteins (CSPs) are small, water-soluble proteins involved in detection and recognition of chemical cues and semiochemicals. A better understanding of CSPs and their function could aid in the development of pest control strategies that are based on chemosensory regulation. We screened our unpublished transcriptome data of *Bemisia tabaci* and identified five CSP genes (*BtabCSPs*) including two novel *BtabCSPs* viz. *BtabCSP4* and *BtabCSP5*. Phylogenetic analysis of amino acid sequences of the proteins encoded by these genes revealed that *BtabCSPs* are distinct from CSPs of other hemipterans. Quantitative PCR analysis showed differential expression of these genes in different stages. In the adult stage, *BtabCSP1*, *2* and *4* had significantly higher expression than *BtabCSP3* and *5* respectively. On the other hand, *BtabCSP2* and *5* were relatively highly expressed in stage of pseudopupae compared to *BtabCSP1*, *3* and *4*. In the third instar *BtabCSP3*, *4* and *5* exhibited higher expression compared to other CSP genes. Expression of all CSP genes was relatively low in the first and second instars compared to later developmental stages. The identification of novel *BtabCSPs* and the expression profiling of CSP genes in general may help facilitate development of pest management strategies based on disruption of CSP-driven behaviors.

Introduction

The tobacco whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most devastating agricultural pests of tropical and subtropical areas and is considered to be a highly cryptic species complex with more than 36 described biotypes (Dinsdale et al., 2010; De Barro et al., 2011). Within this complex, the Mediterranean biotype (MED, formerly known as biotype 'Q') is of particular concern and it is often found to be resistant to IPM-compatible insecticides including neonicotinoids and the juvenile hormone analog, pyriproxyfen (Horowitz et al., 2005; Luo et al., 2010). Control of *B. tabaci* relies heavily on use of pesticides resulting in rapid development of resistance in certain populations (Luo et al., 2010; Roditakis et al., 2005; Houndété et al., 2010; Vassiliou et al., 2011). The continued use of pesticides can cause significant harm to public health and the environment. Thus, it is of paramount importance to develop bio-rational and eco-friendly alternatives for controlling this pest rather than using toxic chemical insecticides (Luo and Liu, 2011). Since odors and tastes profoundly influence insect behavior, one ideal strategy would be to target the molecules and processes involved in the chemosensory pathways for the development of novel, more effective insect control products. In order to do this, it is important to first identify and characterize such proteins or molecules.

Chemosensory proteins (CSPs) are expressed over the entire insect body suggesting that CSPs not only exert chemosensory functions, but may also be involved in other roles such as in limb regeneration and immune responses (Kitabayashi et al., 1998; Oduol et al., 2000; Gonzalez et al., 2009;). An intensive investigation of CSPs in *B. tabaci* would help reveal the relationship between these pests and exotic chemicals and also provide insight into the molecular mechanisms by which insects in general can capture and transport ambient chemical signals. These data will also help elucidate the mechanisms involved in behavioral reactivity in pests and increase the efficiency of pest management. Compared with odorant binding proteins (OBPs), CSPs are more conserved resulting from a specific motif of four cysteines that form two disulfide bonds (Sánchez-Gracia et al., 2009). Similar to some well-study OBPs, the hydrophobic pocket in CSPs can recognize and transport ambient chemical signals to receptors (Sun et al., 2014). The structure of the *Mamestra brassicae* antennal protein, CSPMbraA6, showed that it has a globose shape and there is one hydrophobic channel inside which could bind with 12-bromo-dodecanol, 15-bromo-pentadecanoic acid as well as 9-bromo-stearic acid (Lartigue et al., 2002). On the other hand, the locus *Schistocerca gregaria* CSP4 has high affinity to 2-amylcinnamadehyde (Ban et al., 2002).

* Corresponding author.
E-mail address: luochen1010@126.com (C. Luo).
[1] These authors contributed equally to this work.

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Table 1
Real-time quantitative PCR primers.

Primer name	Sequence (5′-3′)	
BtabCSP1	Forward	TTGTCTTGGTCGGCTGTG
	Reverse	GGTCGTATTTCTGCTGGAGT
BtabCSP2	Forward	ATCCGCCTAATCCATTCC
	Reverse	CAGATTGCTCAGAGAATGAC
BtabCSP3	Forward	TTTCGGTTTCTCATCTGC
	Reverse	GTGCCCTTTGGGTCATAT
BtabCSP4	Forward	AGCAGAGCGACCTGGAGA
	Reverse	CCTTGAACTTGGCCGAAC
BtabCSP5	Forward	AAACGCCTCATCCGCAACTA
	Reverse	TCCTTCCATTCCTCCGGGTA
actin	Forward	TCAACCAGCCATCCTTCTTG
	Reverse	CGGTGATTTCCTTCTGCATT

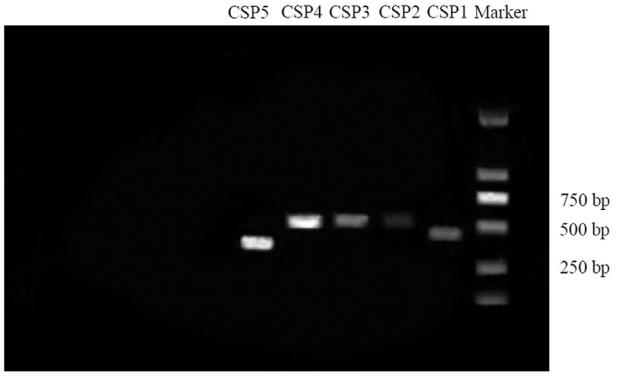

Fig. 1. Diagnostic PCR products for identify the five CSPs genes of *B. tabaci*.

The molecular functions of CSPs remain poorly understood. They have been reported to participate in olfactory recognition indicating the principal basis of interactions between insects and their environment. They may also be involved in physiological processes other than chemosensory functions (Dani et al., 2011; Gu et al., 2012). The increasing number of studies on CSPs from a variety of insect species has revealed additional chemoreceptive functions (Yi et al., 2014). Till date, most studies on *B. tabaci*, chemosensory protein genes have revealed only CSP1, even when based on de novo characterization of a whitefly transcriptome (Wang et al., 2010a, 2010b; Bai et al., 2012; Wu et al., 2015). In this investigation, we report the identification of five *B. tabaci* MED chemosensory protein genes and provide an analysis of their relative transcription levels during different development stages.

Materials and methods

Insects

B. tabaci MED were obtained from the Institute of Vegetables and Flowers in the Chinese Academy of Agricultural Sciences, and established in the laboratory at the Institute of Plant and Environment Protection, Beijing Academy of Agriculture and Forestry Sciences, China. All colonies were maintained on cotton plants (*Gossypium hirsutum* L. var. 'Shiyuan 321') under a 16 h: 8 h, light: dark photoperiod at 25–28 °C and 60–80% humidity. Adult whiteflies used for RNA extraction were less than 7 days old. Approximately 500 adults for each sample was frozen in liquid nitrogen and kept at −80 °C until RNA isolation.

Identification, diagnostic PCR and analysis of CSP genes

One *B. tabaci* transcriptome data set was constructed in the authors' laboratory (Based on Illumina paired-end sequencing technology, unpublished), using a mixture of RNA derived from heads of *B. tabaci* MED. Five CSP genes were identified by searching with keywords (chemosensory protein and CSP), and by using the basic local alignment search tool (BLAST) (Altschul et al., 1997). The ORFs of five *B. tabaci* CSP genes were predicted by utilizing the tool of ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). To validate the reliability of the sequences, five pairs of primers (Table S1) were designed to amplify complete open reading frames (ORFs) of the five CSP genes (Table S2). PCR amplification were conducted under the following conditions:1.0 μL template of cDNA, 12.5 μL of Premix Taq (TaKaRa Taq™ Version 2.0 plus dye, Takara, Japan), 1.0 μL of forward and reverse primers (10 pmol/mL), and 4.5 μL of sterile water, for a total reaction volume of 20 mL PCR. PCR amplification was carried out in a Bio-Rad thermocycler (Bio-Rad DNA Engine Peltier Thermal Cycler, Bio-Rad, USA) with the following thermal profiles:94 °C for 3 min; 35 cycles of 94 °C for 30 s, 52 °C for 30 s, and 72 °C for 1 min, followed by incubation at 72 °C for 10 min. The agarose gel electrophoresis was conducted with 1% agarose gels and DL2000 DNA Marker (Takara, Japan). We separated PCR products and purified the products of expected size with the Wizard DNA purification system (Promega, WI, USA). The DNA fragments from PCR amplification were cloned into a pMD18-T (Takara, Japan) and sequenced by Sunbiotech (Beijing, China). The cDNA sequence assembling and multiple sequence alignment were performed with DNAMAN (DNAMAN 5.2.2, Lynnon BioSoft). ExPASy Proteomics Server (http://cn.expasy.org/tools/pi_tool.html) was used to compute isoelectric point and molecular weight of deduced protein sequences. The matured CSP protein sequences from *B. tabaci*, and other hemipteran pest species were aligned by using ClustalX 1.83 and a phylogenetic tree was constructed in MEGA5.05 using the neighbor-joining method with 1000-fold bootstrap resampling. Location of signal peptide cleavage sites was predicted using the SignalP 4.1 server (Thompson et al., 1997; Tamura et al., 2011).

Real-time quantitative PCR

The relative transcription levels of five CSPs in different development stages were examined using real-time quantitative PCR. Total RNA collected from 500 adults for each sample was extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. After RNase free DNaseI (1000u, Fermentas) treatment, the single stranded cDNA templates, which were used as the templates for qPCR, were synthesized using primer script™ 1st strand cDNA Synthesis System (Takara, Japan) according to kit instructions. RNA quantity was determined on a Nanodrop ND-2000 spectrophotometer (NanoDrop products, Wilmington, DE, USA) and the integrity was verified by gel electrophoresis. Actin gene was used as control to check the quantity of cDNA templates. Real-time quantitative PCR was performed using gene-specific primers and SYBR Premix EX TaqTM (Takara, Japan) in three biological replicates with different

Table 2
In silico characterization of CSP genes identified in *B. tabaci*.

Gene name	Nucleotide length (bp)	ORF length (bp)	Complete protein (aa)	Signal peptide (aa)	Molecular weight (KDa)	Isoelectric point	Accession numbers
BtabCSP1	438	378	126	19	14.17	8.22	KT694344
BtabCSP2	521	393	131	18	14.74	7.52	KT694345
BtabCSP3	508	414	138	20	15.61	9.03	KT694346
BtabCSP4	496	384	128	19	14.26	5.42	KT694347
BtabCSP5	390	372	124	21	13.99	6.31	KT694348