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Functional characterizations of one odorant binding protein and three chemosensory proteins from *Apolygus lucorum* (Meyer-Dur) (Hemiptera: Miridae) legs

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ABSTRACT

Chemoreception plays an important role in insects for sensing information when searching for host and oviposition sites. An understanding of the chemosensory mechanism could aid in the development of new methods to effectively prevent damage from insects in agriculture. We have constructed a legs cDNA library for *Apolygus lucorum* and sequenced 1584 ESTs, from which we identified 669 unigenes. From this collection we identified one putative odorant binding protein (AlucOBP5) and three chemosensory proteins (AlucCSP2, AlucCSP3, AlucCSP4) genes. Using real-time PCR method, we assessed the expression of these genes in the head, thorax, abdomen, wing, antenna and mouthparts. Results indicate that the expression of these genes had tissue- and gender-specificity. AlucCSP2 and AlucCSP3 were specifically expressed in female wings. AlucCSP4 was expressed relatively highly in female wings but also expressed in other tissues. AlucOBP5 was expressed in female abdomen and male legs with high levels in the latter. Expression vectors for these proteins were constructed and expressed in BL21(DE3). The purified proteins were then tested for binding properties using bis-ANS as the fluorescent ligand. AlucOBP5 could bind strongly with phenyl acetaldehyde, 1-hexanol, 3-hexenal and β-ionone. AlucCSP2 and AlucCSP3 had low affinity with all general odorants. AlucCSP4 did not bind with any of the standards. All four proteins could bind with gossypol, meletin with high affinity and could also bind with rutin hydrate, although AlucCSP4 had weak binding capacity. AlucCSP3 and AlucCSP4 could bind weakly with catechin, while AlucCSP2 and AlucOBP5 could not.

1. Introduction

Apolygus lucorum (Meyer-Dür) (Hemiptera: Miridae) is currently one of the major pierce-sucking insects in China. Along with the commercial planting of BT cotton in China and the decreasing application of pesticide, the occurrence of *A. lucorum* has dramatically increased in the cotton fields of in the Yellow River and Yangtze River regions causing increased economic losses. *A. lucorum* has become the major pest in cotton fields (Wu et al., 2002). Thus, it is valuable to study the molecular mechanism of its chemosensory system in order to develop effective prevention and control strategies. The chemosensory systems of insect perceive information from the environment for searching mating partners, food and oviposition sites. Both odorant binding proteins (OBPs) and chemosensory proteins (CSPs) are small water soluble extracellular proteins containing a hydrophobic pocket (Kim et al., 1998; Krieger and Breer, 1999; Li et al., 2008; Pelosi, 1998; Pelosi et al., 2006; Spinelli et al., 1998; Vogt et al., 1999). OBPs are essential for the transduction of information. The selective binding of odorant and nonvolatile agents to these proteins is the first step in the reaction of the chemosensory system. Several numbers of the CSPs are highly expressed in the lymph of chemosensilla and exhibit binding activity to odorants and pheromones, while those expressed in pheromone glands might be involved in storing and releasing pheromone molecules (Pelosi et al., 2006).

Chemosensory receptors include olfactory and gustatory receptors. Insects have independent receptors in the antennae, mouthparts, legs, wings and ovipositor (van Naters and Carlson, 2006). Olfactory and gustatory receptor cells are found in chemosensory sensilla. Olfactory sensors mainly exist in antennae, and gustatory sensors are found in antennae, mouthparts and legs (Abdel-Latief, 2007; Wanner and Robertson, 2008).Flies sense tastants and nonvolatile pheromones through gustatory sensilla and pegs

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Table 1
Oligonucleotide primers used for gene expression analysis of the chemosensory protein and odorant binding proteins from *Apolygus lucorum*.

Primer name	Sequence (5′–3′)	Position (bp)
CSP2-Forward	GCCGAGCTGAAAAAGAATATTCC	179–200
CSP2-Reverse	CTTCTGCTTTTCGCTGCACTT	226–246
CSP2-Probe	FMA-AAGCCCTGCAAACCGATTGCGC-Eclipse	203–224
CSP3-Forward	CGACCAAGTACGACAACATCGA	65–86
CSP3-Reverse	GGCGAGGCAGTCGTAGTACTTC	123–139
CSP3-Probe	FAM-TCCTGTCGAACCAGCGGCTGTACA-Eclipse	98–121
CSP4-Forward	GGGCGTCGAGAAAGTTCTGA	249–268
CSP4-Reverse	TTCGACCTTCTTCTTGAGGTGTT	353–375
CSP4-Probe	FAM-CCTCATCAATGAAAAGCCCGAAGACTACAA-Eclipse	273–302
OBP5-Forward	CCAGTGCTATTACCAAAG	50–67
OBP5-Reverse	GTCAGCCATCAGATTCATC	216–234
OBP5-Probe	FAM-AATCATACACTTGCCATCATCGGTT-Eclipse	174–198
18S-Forward	GGCGACTTATCCTTCAAATGC	162–182
18S-Reverse	GATGTGGTAGCCGTTTCTCAG	272–292
18S-Probe	FAM-CATGGTTGTTACGGGTAACGGGGAA-Eclipse	221–245

distributed on multiple body parts including the proboscis, wing margins, legs, and ovipositor (Montell, 2009).

Host selection in herbivorous insects consists of a sequence of behavioral responses to an array of stimuli associated with host and non host plants: plant stimuli involved include in varying proportions visual, mechanical, gustatory, and olfactory characteristics (Visser, 1986). The taste of host plant substances can stimulate or inhibit feeding or oviposition. For example, tobacco hornworm feeding can be stimulated by polysaccharide of the host. Bitter compounds such as caffeine and aristolochine can inhibit feeding (Glendinning et al., 2002). Polysaccharides and amino acids can stimulate specific gustatory sensilla in caterpillars. There are also feeding inhibitory cells that specifically sense alkaloids and plant phenolic compounds in caterpillars (Schoonhoven et al., 2005).

To elucidate the molecular mechanism of *A. lucorum* gustation and design novel intervention strategies against these plant bugs based on gustation, we constructed a cDNA library from the legs of *A. lucorum* and successfully identified one OBP and three CSP genes. The expression pattern was used to locate the genes by a real-time qPCR method. Furthermore, volatile chemicals and cotton secondary metabolites were used to study the binding characteristics of the *A. lucorum* OBPs and CSP.

2. Materials and methods

2.1. Insects and tissue collection

Specimens of *A. luorum* were obtained from the Chinese Academy of Agriculture science, and raised indoors with fresh green beans and 10% honey in plastic boxes. Temperature was kept at 29 ± 1 °C, with relative humidity 60 ± 5%. And 14L:10D (Lu et al., 2007). Three days after metamorphosis, legs were dissected from 150 bugs, using an ophthalmologic scalpel, snap frozen in liquid nitrogen, transferred to −80 °C until use.

A legs cDNA library was created from the legs of approximately 150 3-day-old adult *A. lucorum*. Real-time quantitative polymerase chain reaction (qPCR) was performed using the reverse transcribed RNA isolated from heads (without antennae and proboscises), thoraxes, abdomens, legs, wings, antennae and proboscises of 3-day-old adult *A. lucorum*.

2.2. RNA extraction and cDNA library construction

Total RNA was extracted with Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA), then assessed under UV light with 2% agarose. First strand cDNA was synthesized with SMART PCR Synthesis Kit (Clontech, Cambridge, UK), using (1 μl) 3′ SMART CDS PrimerIIA (12 μM) and (1 μl) SMARTer IIoligonucleotide (12 μM) as primers, with 1 μg total RNA as template. Using the first strand cDNA as the template, the second was prepared with 5, PCR PrimerIIA (12 μM) and 3′ SMART CDS PrimerIIA (12 μM) for LD-PCR (Long-Distance PCR). The cDNA was assessed with 1.2% agarose. The synthesized products were separated by electrophoresis on a 1.2% agarose gel and purified with a CHROMA SPIN-400column, then ligated into the vector and transformed into DH5α *Escherichia coli* cells (TaKaRa, Dalian, Liaoning, China). Transformed colonies were selected on ampicillin plates.

For qPCR, total RNA was isolated using the SV Total RNA Isolation System kit (Promega, Madison, WI, USA). The PrimeScript® RT reagent kit (Perfect Real Time) (TaKaRa) was used for reverse transcription.

2.3. Sequencing of the legs cDNA library

Positive clones containing a cDNA insert greater than 400 bp were sequenced using an ABI3730 sequencer. All high quality EST sequences were assembled as unigenes using Vector NTI. Unigene sequences were subjected to BLASTX, TBLASTX, and BLASTN searches (Altschul et al., 1998), with a cut-off *E*-value of 1.0E−5 (Anderson and Brass, 1998). Finally, the Blast2 GO program was used to classify EST sequences by cellular component, function, metabolic process and species similarity (http://www.geneontology.org).

2.4. Tissue specific expression of OBP and CSPs

TaqMan probes were used for qRT-PCR experiments. TaqMan probes and primers were designed and synthesized by Takara from the sequences of OBPs and CSP (Table 1). A 20 μL qPCR mix

Table 2
Oligonucleotide primers used for bacterial expression of chemosensory protein and odorant binding proteins from *Apolygus lucorum*.

Primer name	Sequence (5′–3′)
CSP2-Forward	cgggatccGCTGATAAGTACACGGAT[a]
CSP2-Reverse	ggaattcTTAATATTCGACGGGTTT
CSP3-Forward	cgggatccGCTGCCACGTACACG
CSP3-Reverse	ggaattcTCAGTGGCTTCCGGG
CSP4-Forward	cgggatccTCGGAGCTGTACACGGACAAATA
CSP4-Reverse	ggaattcTTAGTATTCAATAGGTTTTCCTT
OBP5-Forward	cgggatccATTACCAAAGAGTACCACGA
OBP5-Reverse	ggaattcTCAGCGTCTTTGTTCTTCGT

[a] Restriction enzyme sites for *Bam*HI(ggattc) and *Eco*RI (gaatcc) are underlined.