Journal of Proteomics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

Combined transcriptomic/proteomic analysis of salivary gland and secreted saliva in three planthopper species

Hai-Jian Huang[a,b], Jia-Bao Lu[b], Qiao Li[b], Yan-Yuan Bao[b], Chuan-Xi Zhang[b,*]

[a] Department of Entomology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China
[b] Institute of Insect Science, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Keywords:
Planthopper
Transcriptome
Proteome
Salivary glands
Saliva
Comparison

ABSTRACT

The planthoppers are piercing-sucking pests that continuously inject saliva into host plants using specialized stylets. However, knowledge on the constituent and function of planthopper saliva proteins was still limited. In this study, the transcriptomic and proteomic approach were adopted to characterize the composition of salivary glands and their secreted saliva in three planthoppers, respectively. Gene repertoires of salivary glands in brown planthopper (*Nilaparvata lugens*, BPH), white-backed planthopper (*Sogatella furcifera*, WBPH) and small brown planthopper (*Laodelphax striatellus*, SBPH) were very similar, which actively involved in protein synthesis and energy metabolism. Comparative analysis of saliva proteome was performed among three planthoppers and other reported insect species. The saliva composition in three planthoppers was diverse, with 55 saliva proteins commonly identified in more than two species. A few proteins, including serine protease, carboxylesterase, aminopeptidase N, lipophorin, elongation factor, carbonic anhydrase, and calcium binding protein were ubiquitous distributed in different insects, indicating conserved function of saliva. While, the majority of saliva proteins were specifically identified in planthoppers, which might be the evolutional adaptation of insects to different hosts. Our work gained insight into the interaction between insect and host plant through salivary approach, and provided a good resource for functional characterization of effectors.

Biological significance: Secreted saliva from insects is attracting immense research interest on the global level due to the crucial roles in determining the compatibility between the insects and their hosts. The three planthoppers: brown planthopper (*Nilaparvata lugens*, BPH), small brown planthopper (*Laodelphax striatellus*, SBPH), and white-backed planthopper (*Sogatella furcifera*, WBPH) caused serious damage to rice plants throughout Asia. However, knowledge on the composition and function of their secreted saliva proteins was limited. Our study characterizes the global gene expression of salivary glands and their secreted saliva by Illumina sequencing technology and LC–MS/MS analysis, respectively. By comparative analysis, the ubiquitous and specific saliva compounds in different insects were unveiled.

1. Introduction

Saliva, an oral secretion predominantly produced from salivary glands, mediate the intimate interaction between insect and host plant during all feeding stages, especially the probing and ingestion process [1]. It was the mixture of bioactive compounds that played multiplied roles in lubrication, digestion, penetration, and handling plant defenses [2–5]. Recently, with the development of sequencing technology, the composition of salivary glands and secreted saliva in several herbivorous insects have been unveiled [6–12]. Saliva proteins such as SHP, C002, and NlShp were indispensable for insect feeding [5,13,14]. However, current knowledge on saliva was still limited, especially the saliva composition that ubiquitously or specifically existed among insect species.

The brown planthopper (*Nilaparvata lugens*, BPH), small brown planthopper (*Laodelphax striatellus*, SBPH), and white-backed planthopper (*Sogatella furcifera*, WBPH) are most destructive insect pests that belong to Delphacidae [15]. Although all three species use rice as their main food source, their host range was a bit different. The BPHs were extremely monophagous that restricted to rice plant, while the WBPHs and SBPHs were oligophagous that can survived on several Poaceae plants including rice, wheat and maize [16,17]. As an important tissue for insect feeding, the salivary glands played critical roles in host selection. It was of great interest to unveil the different gene repertoires of this secretory tissue. Moreover, saliva directly mediate the plant and insect interaction. The larger complement of saliva

* Corresponding author.
E-mail address: chxzhang@zju.edu.cn (C.-X. Zhang).

https://doi.org/10.1016/j.jprot.2017.11.003
Received 30 August 2017; Received in revised form 30 September 2017; Accepted 1 November 2017

proteins was essential for insect to combat a greater diversity of plant defenses [18]. Only a few effector proteins were reported in aphid [19–21]. The potential effector molecules that associated with different host adaption in planthoppers were still unknown.

In this study, we characterize the global gene expression of salivary glands and their secreted saliva by Illumina sequencing technology and Liquid Chromatography–Mass Spectrometry/Mass Spectrometry (LC–MS/MS) analysis, respectively. By parallel comparison, we found that the gene repertoires of three species were very similar, but their saliva secretion varied. In addition, we compared the saliva proteins of planthoppers with other insect species. The ubiquitous and specific saliva compounds were unveiled. Our result provided a deeper understanding on plant-insect interaction.

2. Material and methods

2.1. Insect strains

The BPH, SBPH, and WBPH population were originally collected from a rice field in Huajiachi Campus of Zhejiang University, Hangzhou, China. The insects were reared at 26 ± 0.5 °C on rice seeding (*Oryza sativa* strain Xiushui 134) with 50 ± 5% humidity under a 16:8 h light:dark photoperiod.

2.2. Collection of salivary glands and cDNA library preparation

The fifth-instar insect of BPHs, SBPHs, and WBPHs were anesthetized on ice and their salivary glands were dissected as described previously (Fig. S1) [22]. Total RNA were extracted from 200 salivary glands using RNAiso plus (TaKaRa, Dalian, China). After determination of RNA integrity and quantity, the poly $(A)^+$ RNA was purified from 20 μg pooled total RNA using oligo (dT) magnetic beads. Fragmentation was conducted in the presence of divalent cations at 94 °C for 5 min and the cleaved RNA was transcribed according to manufacturer's instructions. After end-repairing and adaptor ligation, the products were PCR-amplified and purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany) to create a cDNA library.

2.3. Illumina sequencing and transcriptome assembly

The cDNA library was performed using Illumina sequencing platform and the raw data from the images were generated using Solexa GA pipeline 1.6. After removal of low quality reads, processed reads were assembled using Short Oligonucleotide Analysis Package (SOAP) de novo software and clustered with TIGR Gene Indices (TGI) Clustering tools.

2.4. Unigene annotation and peptide prediction

The assembled unigenes were analyzed by searching the GenBank databases using BLASTX algorithm (http://www.ncbi.nlm.nih.gov/). Gene Orthology (GO) and KEGG Orthology (KO) annotations of the unigenes were determined using Blast2go (http://www.blast2go.org/) and InterProScan software (http://www.ebi.ac.uk/Tools/pfa/iprscan/). In addition, the unigenes were subjected to the Clusters of Orthologous Groups (COG) database (http://www.ncbi.nlm.nih.gov/COG/).

The coding sequence (CDS) of each unigene was analyzed using blastx and Estscan software 3.03 [23]. The generated peptide database was used to support the proteomic analysis.

2.5. BLAST score ratio (BSR) test

BSR tests [24] were used to global visualize the degree of proteome similarity between BPH, WBPH, and SBPH. BLAST software (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for the reference comparison. The BSR index was calculated by dividing the BLAST query score by the reference score and normalized from 0 to 1. BPH, WBPH, and SBPH were used as reference library, respectively. Four quadrants were derived from a threshold value of 0.4, which empirically represented a commonly used threshold for peptide similarity as follows: approximately 30% amino acid identity over approximately 30% of the peptide length. BSR of X-axis < 0.4 and Y-axis < 0.4 represents no BLAST match in reference; X-axis < 0.4 and Y-axis > 0.4 represents conserved peptides in reference and query species peptides on the Y-axis; X-axis > 0.4 and Y-axis > 0.4 represents conserved peptides in all species; X-axis > 0.4 and Y-axis < 0.4 represents conserved peptides in reference and query peptides on the X-axis.

2.6. Collection of SBPH and WBPH saliva

The planthoppers were removed from rice plants by gentle shaking, and the fifth instar nymphs were transferred to sterile diets with 2.5% sucrose. The diet was prepared under aseptic conditions and filtered through a 0.22 μm syringe filter (Millipore, MA, USA). Appropriately 100 planthoppers were trapped in each glass tube with 1 mL diet provided between two layers of Parafilm (Neenah, WI, USA). After 24 h feeding, the diets were collected from the space between the two layers of Parafilm with a pipet. Ultrafiltration was conducted using a 3-kDa molecular-weight cutoff Amicon Ultra-4 Centrifugal Filter Device (Millipore) at 5000*g* at 4 °C for 30 min. The concentrated samples were precipitated using a trichloro-acetic acid protein precipitation kit (Sangon, Shanghai, China). The pellets were solubilized in 200 μL of SDT buffer (4% sodium dodecyl sulfate (SDS; Sigma, St. Louis, MO, USA), 100 mM dithiothreitol (DTT; Sigma), 150 mM Tris-HCl pH 8.0) and incubated in hot water for 15 min, and then centrifuged at 14,000*g* for 45 min.

2.7. In-solution digestion

Protein digestion was conducted according to Yu et al. recommendation [25]. Briefly, the soluble samples were concentrated with a 3-kDa filtration unit and alkylated with 200 mM IAA for 1 h. To remove DTT and other low-molecular-weight components, the samples were washed by UA buffer (8 M Urea, 150 mM Tris-HCl pH 8.0) twice, and 25 mM NH_4HCO_3 (Sigma) twice. Digestion was performed using the trypsin in NH_4HCO_3 buffer overnight at 37 °C. After centrifugation, the digested peptides were desalted using C18 pipette tip (Thermo Scientific, Rockford, IL61101, USA) according to manufacturer's protocol. Then, the samples were concentrated by vacuum drying and dissolve in 30 μL of 0.1% (v/v) formic acid.

2.8. LC–MS/MS analysis

LC–MS/MS analysis was performed as follow: The digested peptides (20 μL) were loaded onto the trap column at a flow rate of 10 μL/min by Thermo Scientific Easy nanoLC 1000 (Thermo Fisher Scientific, MA, USA). After trap equilibration, the samples were eluted with a linear gradient of buffer A (0.1% formic acid) and buffer B (84% acetonitrile and 0.1% formic acid) at a flow rate of 250 nL/min. The chromatographic system includes a trapping column (75 μm × 2 cm, nanoviper, C18, 3 μM, 100 Å) and an analytical column (50 μm × 15 cm, nanoviper, C18, 2 μM, 100 Å). Separated MS data were analyzed using Thermo LTQ-Orbitrap Velos Pro (Thermo Fisher Scientific) equipped Nanospray Flex ionization source and FTMS (Fourier transform ion cyclotron resonance mass analyzer) combined with Thermo LTQ-Orbitrap Elite equipped Ion Trap analyzer. The top 20 ions were chosen from a full mass scan (300–2000 *m/z*) by collision induced decomposition (1.0 m/z isolation width, 35% collision energy, 0.25 activation Q, 10 ms activation time). Dynamic exclusion duration was 60 s. Survey scans for MS1 were acquired at a resolution of 30,000 at *m/z* 400.

The MS/MS spectra were searched against the three peptide databases of salivary glands as we generated above using SEQUEST HT