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SHORT COMMUNICATION

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Insight into watery saliva proteomes of the grain aphid, *Sitobion avenae*

Yong Zhang[1] | Yu Fu[1] | Frédéric Francis[2] | Xiaobei Liu[1] | Julian Chen[1]

[1]State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

[2]Functional and Evolutionary Entomology, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium

Correspondence
Julian Chen, State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, 100193 Beijing, China.
Email: chenjulian@caas.cn

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Abstract

The grain aphid, *Sitobion avenae*, is an economically important cereal pest worldwide. Aphid saliva plays an essential role in the interaction between aphids and their host plants. However, limited information is available regarding the proteins found in the saliva of *S. avenae*. Here, the watery saliva proteins from *S. avenae* were collected in an artificial diet and identified using a liquid chromatography–mass spectrometry/mass spectrometry analysis. A total of 114 proteins were identified in *S. avenae* saliva, including several enzymes, binding proteins, and putative effectors, as well as other proteins with unknown functions. In comparison with salivary proteins from nine other aphid species, the most striking feature of the salivary protein from *S. avenae* was the different patterns of protein functions. Several orthologous proteins secreted by other aphid species such as glucose dehydrogenase, elongation factors, and effector C002 were also detected in *S. avenae* saliva and speculated to play a significant role in aphid–plant interactions. These results provide further insight into the molecular basis between aphids and cereal plant interactions.

KEYWORDS

function, grain aphid, proteomics, salivary proteins, watery saliva

1 | INTRODUCTION

Aphids belong to the economically most important agricultural and silvicultural pests by directly drawing phloem sap from sieve tubes and indirectly transmitting various phytoviruses as vectors (Blackman & Eastop, 2000). Probing and feeding aphids secrete two types of saliva: Gelling saliva that hardens to form a sheath around the stylets, which is thought to have a protective function (Miles, 1999), and watery saliva that can be injected into plant mesophyll cells and vascular system (Tjallingii & Esch, 1993).

Aphid saliva is a complex mixture of enzymes and other components and plays an important role in the assistance of stylet penetration, digestion, detoxification, and the suppression or activation of plant defense responses (Elzinga & Jander, 2013; Miles, 1999). The grain aphid, *Sitobion avenae*, is an important agricultural pest of cereals that causes serious economic losses by directly drawing phloem sap and transmitting plant viruses, such as barley yellow dwarf virus (Blackman & Eastop, 2000). The watery saliva of *S. avenae* has been demonstrated to be involved in the induction of wheat resistance against aphids (Zhang, Fan, Francis, et al., 2017). However, limited studies have been undertaken to identify the precise components of *S. avenae* saliva. Here, the watery saliva was collected using a Parafilm™ diet sachet and the species of salivary proteins were investigated by one-dimensional (1D) gel electrophoresis and liquid chromatography–mass spectrometry/mass spectrometry (LC–MS/MS) analysis.

2 | MATERIALS AND METHODS

2.1 | Insects

Clones of *S. avenae* were initially established from an asexual reproducing single aphid collected from a wheat field in Langfang city, Hebei province, China, and maintained under laboratory conditions (16-h light photoperiod; 20 ± 1°C) on wheat plants (*Triticum aestivum* L. var. Zhongmai 175).

2.2 | Saliva collection and 1D gel electrophoresis of salivary proteins

Watery saliva was collected following the protocols described previously (Zhang, Fan, Francis, et al., 2017) (Figure 1). The collected diet (approximately 20,000 mixed developmental stages of aphids in total) was

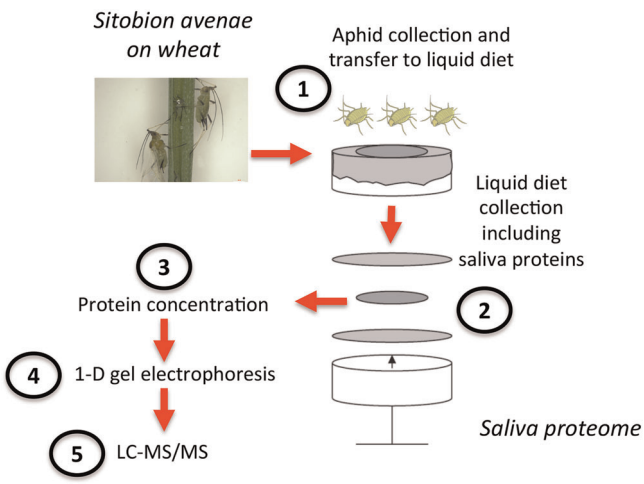

FIGURE 1 Experimental set up for saliva proteomics of *Sitobion avenae*. LC–MS/MS, liquid chromatography–mass spectrometry/mass spectrometry

concentrated using Vivaspin 6 centrifuge concentrator with a 3000 molecular weight cutoff PES membrane at 4°C and 15,000*g* for 2 h. The extracted protein sample was mixed with Laemmli buffer and boiled for 5 min at 100°C. Proteins were electrophoresed on a 12.5% acrylamide separating gel with a 4% stacking gel for 1.5 h at 120 v. Gels were silver-stained and digitalized using ImageScanner III.

2.3 | Identification of salivary proteins using LC–MS/MS

Protein bands were excised from the preparative 1D gels, and were then destained, reduced, alkylated, and trypsin-digested as described by Harmel et al. (2008). The shotgun analysis was conducted on an Easy nLC 1000 coupled with a Q Exactive mass spectrometer (Thermo Fisher Scientific). Peptide mixtures (10 μl) were separated with a custom-designed capillary C18 column by a 120-min gradient from 3% to 80% acetonitrile in 0.1% formic acid at a flow rate of 300 nl/min. The raw MS/MS data were analyzed using the MaxQuant software suite (version 1.5.2.8) and searched against the Uniprot—*Acyrthosiphon_pisum* and *S. avenae* salivary gland transcriptomic database (accession number SRR11476040) (Zhang, Fan, Sun, et al., 2017). Peptide mass tolerance was set at ±2 ppm. The search followed the enzymatic cleavage rule for trypsin, with a maximum of two missed cleavage sites and ±0.02 Da for the fragment ions tolerance. The cutoff for the global false discovery rate in the peptide-spectrum match and protein identification was below 0.1%. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the iProX partner repository (Ma et al., 2019) with the data set identifier PXD018645.

3 | RESULTS AND DISCUSSION

A total of 114 proteins were identified from the aphid watery saliva using LC–MS/MS in our study (Table S1). Among these, 90 proteins were successfully annotated, with 24 proteins uncharacterized. For the annotated proteins, gene ontology analysis was performed to provide an overview of the proteomes of the saliva samples. In the category of "biological process", proteins were mainly assigned to "response to oxidative stress" and "cell redox homeostasis" categories. In "molecular function" ontology, "ATP binding" with 26 proteins and "oxidoreductase activity" with nine proteins were the two most common categories. In the "cellular components" group, proteins were mainly distributed in two categories: "Cytoplasm" with eight proteins and "cell" with six proteins (Figure S1).

Consistent with a previous study (Rao et al., 2013), the presence of glucose dehydrogenase, peroxidase, carbonic anhydrase, and actin were also detected in *S. avenae* saliva in our study. However, some previously identified salivary proteins were not detected in the present study; for example, pectinase, a plant cell wall degradation enzyme, has been detected in the watery saliva of *S. avenae* before and was involved in the induction of wheat defense responses (Liu et al., 2009). The difference may be caused by the different composition of artificial diets which were used for the collection of aphid saliva.

In comparison with the salivary proteins obtained from nine other aphid species, 43 proteins were specifically detected in the *S. avenae* saliva, and 11 of which were uncharacterized (Table S2). *S. avenae* is oligophagous on grasses and cereals (Blackman & Easto, 2000). Several unique salivary proteins utilized by *S. avenae* may be the result of an adaptive strategy for aphids to their host plant. We also identified 23 salivary proteins in *S. avenae* that were detected in other more than four other aphid species, including glucose dehydrogenase, actin, apolipophorins, carbonic anhydrase 7, histone H4, ATP synthase, elongation factor 1, and peroxidase (Figure 2). This level of overlap in the identified salivary proteins for the different aphid species reflects their importance to aphid biology. Among these proteins, glucose dehydrogenase, apolipophorins, actin, and elongation factor 1-α were the four most conserved salivary proteins in the different aphid species. Although glucose dehydrogenase and apolipophorins

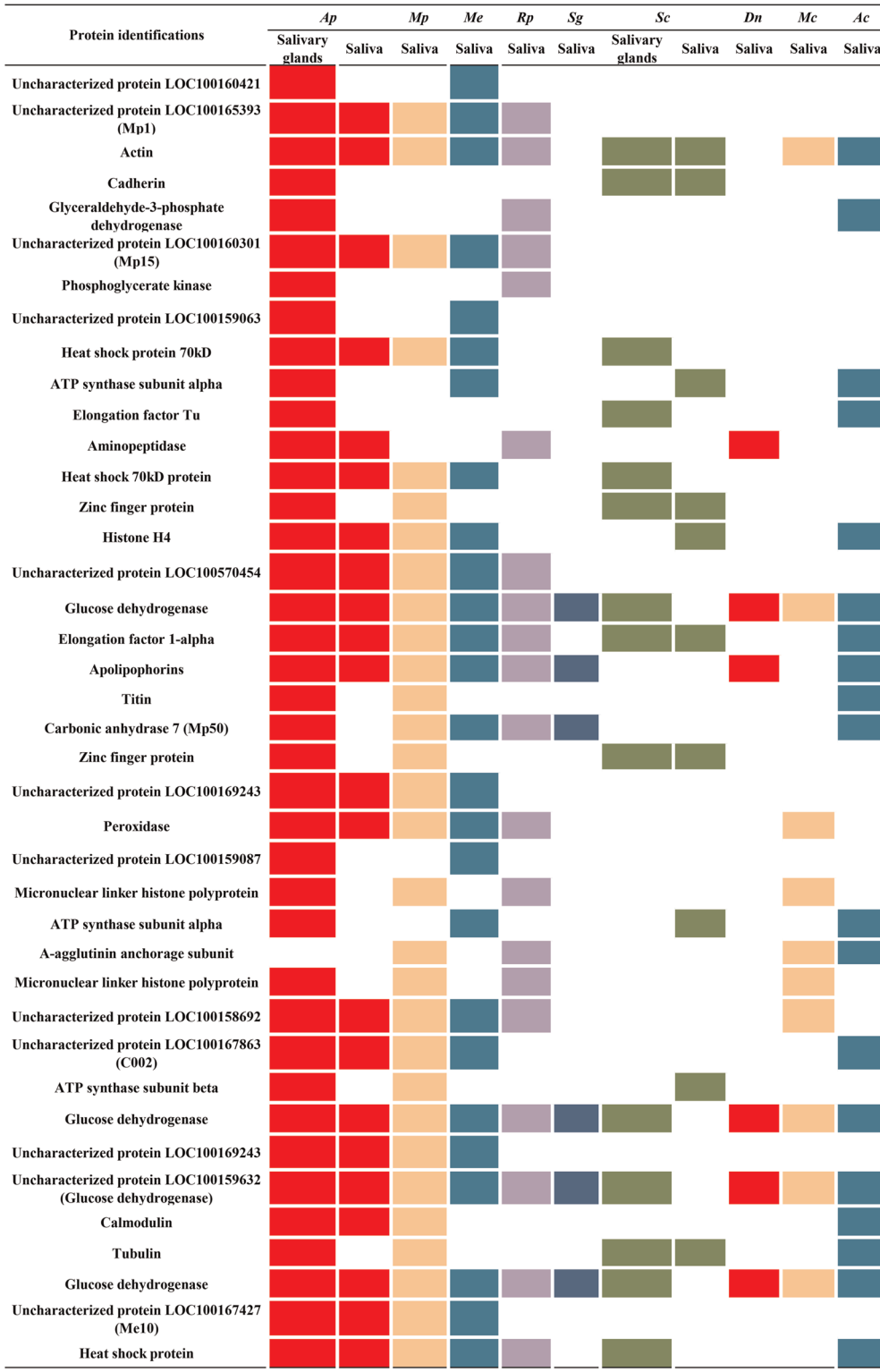

FIGURE 2 Comparative analysis of salivary proteins of grain aphid with other nine aphid species. Proteins identified in salivary gland and saliva proteomes of more than two aphid species were presented in this figure. Color areas indicate proteins were detected in the aphid species. Ac, *Aphis craccivora* (Loudit et al., 2018); Ap, *Acyrthosiphon pisum* (Boulain et al., 2018); Dn, *Diuraphis noxia* (Nicholson et al., 2012); Mc, *Myzus cerasi* (Thorpe et al., 2016); Me, *Macrosiphum euphorbiae* (Chaudhary et al., 2015); Mp, *Myzus persicae* (Thorpe et al., 2016); Rp, *Rhopalosiphum padi* (Escudero-Martinez et al., 2020); Sc, *Schlectendalia chinensis* (Yang et al., 2018); Sg, *Schizaphis graminum* (Nicholson & Puterka, 2014)

were proposed to be involved in modifying the plant defense response (Vandermoten et al., 2013), the functional mechanisms of these two proteins on aphid–plant interactions remain unknown.

We also identified several salivary proteins of *S. avenae* that had sequence similarity to several previously identified putative aphid effectors. For example, two salivary proteins with similarity to effector C002 from *Myzus persicae* and Me10 from *Macrosiphum euphorbiae* were detected in *S. avenae* saliva, which have previously been shown to suppress plant defense and promote aphid virulence (Atamian et al., 2013; Bos et al., 2010; Chaudhary et al., 2015). Whether these two candidate effectors of *S. avenae* display conserved functions in suppressing plant defense requires further study.

Proteins with a signal peptide in the secretome of pathogen and aphids are selected as candidate effectors for further functional analysis. However, some proteins lacking a signal peptide, such as actin, tubulin, and several ribosomal proteins, have also been identified in the watery saliva of *S. avenae* and other aphid species (Thorpe et al., 2016). The mechanisms of these proteins for being secreted in the saliva without a secretion signal peptide remain unclear. In addition, the potential roles of salivary proteins without a signal peptide in aphid–host plant interactions require complementary investigations.

Although many salivary proteins were identified in the present study, some proteins may still be missing because of the amounts of collected artificial diets. A significant variation in salivary secretion has also been observed in different artificial diets and different feeding stages of aphids (Bos et al., 2010; Miles, 1999). Some salivary proteins may only be expressed and secreted into plant cells when aphids feed on the host plants. Then, it is important to establish a more natural and stable approach for the collection of aphid saliva in the future.

Several salivary effectors from *Acyrthosiphon pisum*, *M. persicae*, and *M. euphorbiae* have been successfully identified using *Agrobacterium tumefaciens*-mediated transient transformation system in plants (Atamian et al., 2013; Bos et al., 2010; Cui et al., 2019). However, few effectors of cereal aphids have been identified because of the poor efficiency of *Agrobacterium*-mediated transformation in cereal plants. An alternative approach to screen the effectors of cereal aphids is to deliver proteins into cereal plant cells using a plant pathogen type III secretion system (T3SS), which is a method that has been successfully applied for the delivery of fungal effectors into wheat or barley (Upadhyaya et al., 2014; Xu et al., 2019). Several protein orthologs and candidate effectors, such as glucose dehydrogenase, C002, elongation factor 1-α, and calmodulin have been determined in *S. avenae* saliva; therefore, further studies on the functional analysis of effectors using T3SS in wheat are required.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Yong Zhang: Data curation (lead); Investigation (lead); Methodology (lead); Validation (lead); Writing-original draft (lead). **Yu Fu:** Investigation (supporting); Methodology (supporting); Validation (equal); Writing-original draft (supporting). **Frédéric Francis:** Investigation (supporting); Methodology (supporting); Validation (supporting); Writing-review & editing (supporting). **Xiaobei Liu:** Investigation (supporting); Writing-review & editing (supporting). **Julian Chen:** Conceptualization (lead); Funding acquisition (lead); Supervision (lead); Writing-review & editing (lead).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Yong Zhang https://orcid.org/0000-0003-4134-2405

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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