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Quantitative proteomic analysis of the fall armyworm saliva

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ABSTRACT

Lepidopteran larvae secrete saliva on plant tissues during feeding. Components in the saliva may aid in food digestion, whereas other components are recognized by plants as cues to elicit defense responses. Despite the ecological and economical importance of these plant-feeding insects, knowledge of their saliva composition is limited to a few species. In this study, we identified the salivary proteins of larvae of the fall armyworm (FAW), *Spodoptera frugiperda*; determined qualitative and quantitative differences in the salivary proteome of the two host races—corn and rice strains—of this insect; and identified changes in total protein concentration and relative protein abundance in the saliva of FAW larvae associated with different host plants. Quantitative proteomic analyses were performed using labeling with isobaric tags for relative and absolute quantification followed by liquid chromatography-tandem mass spectrometry. In total, 98 proteins were identified (>99% confidence) in the FAW saliva. These proteins were further categorized into five functional groups: proteins potentially involved in (1) plant defense regulation, (2) herbivore offense, (3) insect immunity, (4) detoxification, (5) digestion, and (6) other functions. Moreover, there were differences in the salivary proteome between the FAW strains that were identified by label-free proteomic analyses. Thirteen differentially identified proteins were present in each strain. There were also differences in the relative abundance of eleven salivary proteins between the two FAW host strains as well as differences within each strain associated with different diets. The total salivary protein concentration was also different for the two strains reared on different host plants. Based on these results, we conclude that the FAW saliva contains a complex mixture of proteins involved in different functions that are specific for each strain and its composition can change plastically in response to diet type.

1. Introduction

The composition and secretion of saliva are of paramount importance to the nutrition and health of higher animals (Lamy and Mau, 2012). For arthropod parasites, their saliva is essential not only for food digestion, but as a mechanism to overcome host immunity. This mechanism is best understood for blood-feeding insects whose saliva contains anticoagulants, vasodilators, and antiplatelet compounds to facilitate the ingestion of blood meals (Ribeiro and Francischetti, 2003). Similar to hematophagous arthropods, insect herbivores also need to overcome immune responses of their plant hosts to successfully grow and develop. Insect feeding triggers the production of a wide array of plant physical and chemical defenses that can be toxic, reduce food digestibility, or recruit insect natural enemies (Howe and Jander, 2008). Both mechanical injury and insect-derived cues are recognized by plants to activate production of specific defense responses (Howe and Jander, 2008). For example, plants recognize insect attack by their damage pattern and by perceiving insect-derived chemical cues in their saliva, such as herbivore-associated elicitors or herbivore-associated molecular patterns (HAMPS) and effectors (Felton et al., 2014; Kaloshian and Walling, 2015; Schmelz, 2015; Stuart, 2015). However, despite the important role of insect saliva in plant defense regulation, the salivary composition in lepidopterans is known in only a few species (Afshar et al., 2013; Celorio-Mancera

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Abbreviations

FAW	fall armyworm
iTRAQ	isobaric tags for relative and absolute quantification
HAMPS	herbivore associated molecules patterns
GOX	glucose oxidase
USDA-ARS	United States Department of Agriculture, Agricultural Research Service
SDS	sodium dodecyl sulfate
PAGE	polyacrylamide gel electrophoresis
LC-MS/MS	liquid chromatography-tandem mass spectrometry
MQ	Milli-Q
SCX	strong cation-exchange chromatography
HPLC	high-performance liquid chromatography
FDR	false discovery rate
PSPEP	Proteomics System Performance Evaluation Pipeline
PSUTraq	Penn State University software for the analysis of iTRAQ data
WHATraq	software for the analysis of iTRAQ data
qLFDR	local false discovery rate calculation
CAN	acetonitrile
TFA	trifluoroacetic acid
FAC	fatty acid-amino acid conjugate
HSP	heat shock protein
JA	jasmonic acid
POX	peroxinectin
REPAT	proteins expressed in response to pathogen attack
proPO	prophenoloxidase
PBAN	pheromone biosynthesis activating neuropeptide
5-HT	5-hydroxytryptamine
SNMP	sensory neuron membrane protein
CAP	cardioactive peptide

et al., 2015, 2012, 2011; Dong et al., 2013; Harpel et al., 2015; Koenig et al., 2015; Margam et al., 2011; Tian et al., 2012).

Although the salivary proteome seems to be species specific, a few studies have found variation in the protein composition among populations of the same insect species. In insect biotypes, the salivary proteome seems to rapidly change in response to host plant chemistry. For example, quantitative changes in 14 salivary proteins were identified when comparing the salivary proteome of four biotypes of the Russian wheat aphid, *Diuraphis noxia* (Nicholson et al., 2012). Likewise, six proteins were quantitatively different among four biotypes of the green bug, *Schizaphis graminum* (Nicholson and Puterka, 2014). Because these biotypes have variable virulence to resistant wheat (*Triticum* spp.) varieties, it is possible that changes in their salivary composition are part of the mechanisms to overcome host resistance (Cui et al., 2012; Nicholson and Puterka, 2014; Nicholson et al., 2012). Similarly, populations of the brown planthopper *Nilaparvata lugens*, with different virulence to rice (*Oryza sativa*), had differences in the transcript accumulation of 67 genes encoding secretory proteins in their salivary glands (Ye et al., 2013). These studies suggest that the salivary composition of these sucking insects is likely to play an important role in plant colonization and therefore is under strong selection pressure.

Furthermore, quantitative differences in protein activity or gene expression have been found within a given insect genotype feeding on different host plants. Larvae of the tomato fruitworm, *Helicoverpa zea*, have glucose oxidase (GOX) in their saliva; this enzyme catalyzes the reaction of glucose into gluconic acid and hydrogen peroxide, which regulates defense responses in a variety of plants (Eichenseer et al., 1999). Both the activity and amount of GOX secreted by this insect changes when feeding on different host plants (Peiffer and Felton, 2005). Likewise, in fourth-instar larvae of the beet armyworm, *Spodoptera exigua*, the activity of GOX in the salivary glands was higher in caterpillars fed on artificial diet compared with those fed on barrelclover, *Medicago truncatula* (Merkx-Jacques and Bede, 2005). Subsequent experiments found that the activity of GOX in insect salivary glands was positively associated with the amount of glucose and protein present in their diet (Babic et al., 2008; Hu et al., 2008). Similar variation has been found in other salivary enzymes. For example, the transcript accumulation of a lysozyme-encoding gene in the salivary glands of *H. zea* was higher when caterpillars fed on tomato (*Lycopersicon* spp.) and cotton (*Gossypium* spp.) compared with tobacco (*Nicotiana* spp.) plants (Liu et al., 2004). Finally, salivary protein secretion pathways in *S. exigua* caterpillars were influenced by the nutritional quality of their diet (Afshar et al., 2013). Together, these studies suggest plastic variations in the biochemical composition and secretion of insect saliva associated with diet.

In this study, we investigated the protein composition of secreted saliva from fall armyworm (FAW) (*Spodoptera frugiperda*) caterpillars. The FAW is a polyphagous lepidopteran insect comprising two host strains that show different plant preferences: in field, the “corn strain” is mainly associated with maize (*Zea mays*), sorghum (*Sorghum bicolor*), and cotton, whereas the “rice strain” is mainly associated with forage grasses and rice (Machado et al., 2008; Pashley, 1986; Whitford et al., 1988). Studies aiming to understand their differential host plant adaptation have found differences in detoxification enzymes, oviposition preferences, and host-associated differences in larvae growth and development (Groot et al., 2010; Hay-Roe et al., 2011; Meagher and Nagoshi, 2012; Meagher et al., 2011; Veenstra et al., 1995); however, differences in the composition of plant defense elicitors in oral secretions and saliva have yet to be explored. Here, we identified the salivary protein composition of FAW caterpillars and determined qualitative and quantitative differences in the salivary proteome of the two host races of this insect. We also quantified differences in the total protein concentration and relative protein abundance in the FAW saliva associated with different host plants.

2. Materials and methods

2.1. Insects

The FAW strains were obtained from a laboratory colony maintained at the United States Department of Agriculture, Agricultural Research Service (USDA-ARS) in Gainesville, Florida. The rice strain colony was composed of individuals collected from a Tifton 85 Bermuda grass (*Cynodon dactylon*) field in Chiefland (Levy Co.), Florida, and from pastures in Jacksonville, Florida. The corn strain was initiated from sweet corn fields in Hendy and Palm Beach counties in South Florida. For each strain, the field-collected insects were pair mated to select the F1 individuals containing the corresponding mitochondrial marker that identifies each strain (Nagoshi and Meagher, 2003).

2.2. Plants

Maize (inbred line B73) was grown in Hagerstown loam soil