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Cowpea aphid–plant interactions: endosymbionts and related salivary protein patterns

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Abstract

The specificity of plant use by aphids is related to symbiont diversity of some aphid models. *Aphis craccivora* Koch (Hemiptera: Aphididae, Aphidini) is a well-known aphid that feeds on species of Fabaceae, but has also been recorded recently on *Amaranthus* species (Amaranthaceae) in Gabon (Africa). *Aphis craccivora* strains used in this study were originally collected from crop *Vigna unguiculata* L. Walp. (Fabaceae) from Togba in Benin (Africa) and *Amaranthus hybridus* L. from Libreville in Gabon, for a comparative study of symbionts. Saliva composition, potentially including bacterial proteins, also contributes to the phytotoxic effect of aphid attacks. Both, endosymbiont bacteria and saliva protein diversity should be targeted to investigate the feeding behavior of aphids and to explain plant–aphid interactions. Bacteria-targeted PCR was conducted on six symbionts in *A. craccivora*. The obligate symbiont *Buchnera aphidicola* Munson et al. (Enterobacteriaceae) was identified in all aphids collected. In comparison, the facultative symbiont *Serratia symbiotica* Moran et al. (Enterobacteriaceae) was only found in *A. craccivora* from Gabon, whereas *Rickettsia* sp. (Rickettsiaceae) was only found in aphids from Benin. Using nano-LC-MS/MS (liquid chromatography-tandem mass spectrometry), some proteins were only found in solid or soluble saliva, whereas others originated from *S. symbiotica*. Two of the identified proteins are involved in plant–pathogen interactions: calmodulin and elongation factor Tu. This information on endosymbionts and related salivary proteomes from *A. craccivora* in Gabon helps improve our understanding of aphid–plant interactions.

Introduction

Vigna unguiculata L. Walp. (cowpea) (Fabaceae) and *Amaranthus hybridus* L. (amaranth, smooth pigweed) (Amaranthaceae) are considered as indigenous vegetables in urban agriculture in Africa. Crops of these two species provide the most important sources of leafy vegetable in the continent; however, some communities also grow cowpeas to use their seeds or as fodder (Shackleton et al., 2009). Both plant species are subject to pest pressure, particularly from aphids. *Aphis craccivora* Koch (Hemiptera: Aphididae, Aphidini) is one of the most important pests in vegetable crops in tropical Africa (Singh & Allen, 1979; Jackai & Daoust, 1986). This cosmopolitan pest is polyphagous, with a clear preference for Fabaceae species (Stoetzel & Miller, 2001). Aphids cause damage by removing plant sap, which weakens aerial parts (leaves, pods, seeds, and others). Plant growth is stunted, leading to distortion and necrosis of leaves, followed by premature defoliation and death of seedlings. All steps result in yield losses. *Aphis craccivora* is a vector of 51 plant viruses (Chan et al., 1991). The most important viruses are *Groundnut rosette virus* (GRV), *Subterranean clover stunt virus* (SCSV), *Bean common mosaic virus* (BCMV), and *Cucumber mosaic virus* (CMV) – these viruses causes economic damage to vegetables (Borowiak-Sobkowiak et al., 2017). In Africa, *Cowpea aphid-borne mosaic virus* (CABMV) and *Blackeye cowpea mosaic virus* (BICMV) are essential in cowpea (Agunbiade et al., 2013), and *Amaranthus mosaic virus* (AMV) is important in amaranth (Kareem et al., 2011).

Hemipteran insects use their stylet-shaped mouthparts to pierce the plants and suck phloem (Powell et al., 2006; Fereres & Moreno, 2009), with pests having the ability to

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modulate the defensive responses of plants (Hogenhout & Bos, 2011). For instance, while feeding, aphids inject two types of saliva into host plant tissues: solid and soluble (Miles, 1959). Both types of saliva function in aphid–plant interactions, and have a major impact on plant defense and physiology (Miles, 1999; Will et al., 2007, 2009; Mutti et al., 2008). There has been an increase in the number of studies focusing on the composition of aphid saliva since the paper by Miles (1999). As a result, we now know the salivary proteins of 12 aphid species; however, many proteins of other species have yet to be identified.

Aphids are also classified according to the type of damage that they cause to plants. For instance, phytotoxic and non-phytotoxic aphids are distinguished as they act directly and indirectly, respectively, on plant tissues by altering plant defenses or other physiological parameters (Miles, 1999; Goggin, 2007; Will et al., 2009). Nicholson et al. (2012) suggested that aphid–plant interactions are regulated by general proteins fed by aphids. Some aphid species, like *Acyrthosiphon pisum* Harris and *Myzus persicae* Sulzer, do not induce direct or immediate phytotoxic effects on plants (Nicholson & Puterka, 2014). The salivary proteins of these non-phytotoxic aphids mainly serve to destabilize general plant defense responses (Miles, 1999; Cherqui & Tjallingii, 2000; Tjallingii, 2006; Will et al., 2009). In contrast, during feeding, phytotoxic aphids secrete proteins that strongly interact with the plant by inducing rapid changes to the shape and composition of plant organs, leading to stronger symptoms of aphid occurrence (Burd, 2002). Based on our personal observations, some damage to amaranth is related to *A. craccivora* phytotoxicity. *Schizaphis graminum* Rondani and *Diuraphis noxia* Kurdjumov are phytotoxic aphids that are also pests of wheat and other cereals that cause major economic damage (Nicholson et al., 2012; Nicholson & Puterka, 2014).

Almost all aphids contain the obligate endosymbiont *Buchnera aphidicola* Munson et al. (Enterobacteriaceae), whereas facultative endosymbionts are also found in some aphids. *Buchnera* provides the host with essential amino acids that are lacking in the host diet. This kind of symbiosis is obligate, with both partners being mutualistically dependent on the other (Oliver et al., 2010; Simon et al., 2011). Facultative symbionts have been extensively studied in the pea aphid *A. pisum* (Simon et al., 2003). This aphid hosts at least eight species of facultative symbionts, which often vary in frequency between locations and host plants. Most aphid clones are infected with one or more facultative symbionts (Enterobacteriaceae), including *Hamiltonella defensa* Moran et al., *Regiella insecticola* Moran et al., *Serratia symbiotica* Moran et al., *Spiroplasma* sp., *Rickettsia* sp., and *Rickettsiella* sp. (Enterobacteriaceae) (Simon et al., 2011). Other aphid species are also infected with similar bacterial symbionts; however, the diversity of symbionts varies across aphid species. For instance, large symbiont diversity was detected in *Sitobion avenae* (Fabricius) (Yu et al., 2013). Six facultative symbionts have been detected in the polyphagous and cosmopolitan aphid *A. craccivora*, and were correlated with host plant use (Brady et al., 2014). In particular, *Regiella* and *Hamiltonella* symbionts were only found in aphids collected from alfalfa (*Medicago sativa* L). In comparison, *Rickettsia*, *Spiroplasma*, *Arsenophonus*, and *Serratia* symbionts were associated with aphids feeding on two or more host plants. Aphid symbiont identities are associated with degree of host plant specificity. Finally, facultative symbionts modify aphid dietary breadth (Wagner et al., 2015).

This study aimed to investigate two aspects of aphid–plant interactions in relation to microbiome patterns: (1) identification of the endosymbiont diversity in *A. craccivora* clones, and (2) identification of the protein composition of soluble and solid saliva of this aphid in relation to symbiont patterns. Complementary biological and 'omics' approaches were developed. Our results are expected to provide novel insights on plant–aphid–microbiome interactions and adaptive mechanisms.

Materials and methods

Rearing of aphids and plants

Aphis craccivora was originally collected from crop (1) *V. unguiculata* during May 2015 from Togba in Benin (6°26′58.46″N, 2°20′50.24″E) and (2) *A. hybridus* during August 2015 from Libreville in Gabon (0°24′39″N, 9°29′26″E) and kept in micro centrifuge tubes in 70% ethanol. *Aphis craccivora* collected from *A. hybridus* since August 2013 in Libreville (0°27′30.46″N, 9°25′6.30″E) was reared on amaranth plants (*A. hybridus*) at 24 ± 1 °C, 60–70% r.h., and L16:D8 photoperiod. *Myzus persica*e and *A. fabae* clones were collected from beans in a crop field in Gembloux (Belgium) and reared on *V. fabae* beans under similar conditions, but at a lower temperature (22 ± 1 °C) in dedicated environmental chambers in the laboratory (Functional & Evolutionary Entomology, Gembloux Agro-bio Tech, University of Liege). The aphids were reared on plants grown in loamy soil (VP113BIO; Greenyard Horticulture, Ghent, Belgium) and were placed in 45 × 45 × 45 cm cages with 96 × 26 mesh (BugDorm; MegaView Science, Taichung, Taiwan).

Detection of symbiotic bacteria in aphids

Five aphids from the three species and clones from each location were used (with five replicates) for DNA extraction following the protocol of Sunnucks & Hales (1996).

Myzus persicae and *A. fabae* were used to compare symbiont composition with *A. craccivora*, because the three aphids are amaranth pests. Diagnostic polymerase chain reactions (PCR) were conducted using species-specific primers (Table 1) to detect endosymbiotic bacteria. Each facultative symbiont (*Arsenophonus*, *Hamiltonella*, *Regiella*, *Rickettsia*, *Serratia*, and *Spiroplasma*) was screened for, as they are the most common facultative bacteria associated with aphids (Oliver et al., 2010). For each specific primer, specific symbiont was used as positive control and also a negative control was used without DNA template.

To detect *Buchnera*, the PCR mix consisted of 5 µl buffer (products from Promega, Madison, WI, USA), 1 µl dNTP, 1 µl of each primer (10 µM), 2 µl DNA, 0.5 µl taq polymerase (0.4 U), and water to form a final volume of 25 µl. The mixture was subjected to 5 min of initial DNA denaturation at 94 °C, followed by 30 cycles consisting of 30 s denaturation at 94 °C, 30 s annealing at 58 °C, and 90 s of elongation at 72 °C (Peccoud et al., 2014). For the other endosymbionts, the PCR mixture consisted of 2.5 µl buffer, 0.5 µl dNTP, 0.5 µl of each primer (10 µM), 2 µl DNA, and 0.25 µl of taq polymerase (0.4 U) to form a final volume of 25 µl. The mixture was subjected to 90 s of initial DNA denaturation at 94 °C, followed by 30 cycles consisting of 30 s denaturation at 94 °C, 30 s annealing at a specific temperature related to the targeted symbiont (Table 1), and 75 s of elongation at 72 °C. Amplification was repeated in at least five replicates. After electrophoresis on 1% agarose gel, amplicons were visualized using a protocol for loading GeneRuler 100 bp Plus DNA Ladder #SM0321 under UV light. After DNA was extracted from agarose gels using a PCR Clean-up Gel kit (Macherey-Nagel, Düren, Germany; Anonymous, 2014), the samples were sent for sequencing to Germany (GATC Biotech, Konstanz). The sequence of each sample was matched using the NCBI blastn database (https://blast.ncbi.nlm.nih.gov). Sequences with >96% identity and query cover were kept.

Saliva collection and concentration

Saliva was collected from ca. 50 000 aphids of *A. craccivora* from Gabon (reared in the laboratory). Diet of 15% sucrose (wt/vol) was prepared under aseptic conditions with Milli-Q water, filtered through 0.45-µm filters (Millipore, Billerica, MA, USA), and sealed between two layers of stretched Parafilm (SERVA Electrophoresis, Heidelberg, Germany) on the bottoms of 27-mm-diameter cylinders (PVC tube); microbial filtered Parafilm sheets were used to avoid bacterial and fungal contamination (Vandermoten et al., 2014). One hundred aphids (fourth instars or apterae adults) were gently removed from plants (*A. hybridus*) using a fine brush and were placed on white paper, cleaned of debris before placing them in the PVC tube (20 tubes per set-up, 500 tubes totally). These aphids were kept in a feeding chamber for 48 h at a constant 20 ± 1 °C. This method was previously described by Cherqui & Tjallingii (2000) and Harmel et al. (2008). Saliva was collected from fluid diets (liquid fraction). The soluble fraction was placed between two membranes of Parafilm. The sheath materials (solid fraction) were collected after washing membranes with a water solution of 1% triton. All soluble and solid saliva were managed separately. The samples were concentrated by centrifugation (15 000 ***g***) for 10 min at 4 °C. The saliva extracts (solid

Table 1 Primers and PCR cycling conditions used to detect the secondary symbionts of aphids

Target	Target gene	Primer name	Primer sequence (5′-3′)	Expected product size (bases)	Annealing temperature (°C)	References
Buchnera	16S	16SA1	AGAGTTTGATCMTGGCTCAG	~270	58	Fukatsu & Nikoh (1998)
		Buch270R	TGCCTTGGTAGGCTATTACTC			Peccoud et al. (2014)
Arsenophonus	23S	Ars23sF	CGTTTGATGAATTCATAGTCAAA	~550	60	Thao & Baumann (2004)
		Ars23sR	GGTCCTCCAGTTAGTGTTACCCAAC			Thao & Baumann (2004)
Hamiltonella	16S	Ham1F	TGAGTAAAGTCTGGAATCTGG	~700	55	Chiel et al. (2007)
		Ham1R	AGTTCAAGACCGCAACCTC			Chiel et al. (2007)
Regiella	16S	U1279F	CGAACGTAAGCGAACCTCAT	~700	58	Russell et al. (2003)
		35R	CCTTCATCGCCTCTGACTGC			Russell & Moran (2005)
Rickettsia	16S	16SA1	AGAGTTTGATCMTGGCTCAG	~200	60	Fukatsu & Nikoh (1998)
		Rick16sR	CATCCATCAGCGATAAATCTTTC			Fukatsu (2001)
Serratia	16S	R1279F	CGAGAGCAAGCGGACCTCAC	~700	56	Russell et al. (2003)
		35R	CCTTCATCGCCTCTGACTGC			Russell & Moran (2005)
Spiroplasma	16S	16SA1	AGAGTTTGATCMTGGCTCAG	~350	55	Fukatsu & Nikoh (1998)
		TKSSspF	AAGCCTGATGGAGCAATGC	~100	62	Toju & Fukatsu (2011)
		TKSSspR	TAGCCGTGGCTTTCTGGTAA			Fukatsu & Nikoh (2000)

and soluble) were treated with a two-dimensional clean-up kit, according to the manufacturer's instructions. The extracts were subsequently resuspended in trypsin (Roche, porcine, proteomics grade) for further digestion. Proteins were quantified using the RCDC (reducing agent and detergent compatible) quantification kit from Bio-Rad.

Protein identification

Peptide separation was performed on a nano-UPLC (nanoAcquity; Waters, Bremen, Germany)-ESI-Q-Orbitrap (Q Exactive; Thermo, Bremen, Germany) in positive ion mode. Each sample (10 μg) was resuspended in 50 mM ammonium bicarbonate and then reduced (DTT), alkylated (iodoacetamide), and digested using trypsin (protein concentration 0.5 mg ml^{-1}). For each sample, a quantity of 3.5 μg digested protein was purified on a Ziptip C18, and then dried and resuspended in 100 mM ammonium formiate (pH 10) at 0.333 μg μl^{-1}. A volume of 9 μl per sample, corresponding to 3 μg of digested proteins, was injected on the nano 2D UPLC-Orbitrap mass spectrometer (MS) system. An internal standard sample 'MPDSMIX' (Waters) containing four digested proteins was spiked in each sample, at a quantity of 150 fmoles of ADH digest per injection.

The liquid chromatography method was a 2D LC with three steps of 180 min. The three steps were performed on the column at high pH, with increasing percentages of acetonitrile and the peptides eluted from the column at high pH being loaded after dilution on the low pH column. Each step consisted of a gradient of 5 min from 99% of A (A = water and 0.1% formic acid, B = acetonitrile) to 93% of A, followed by a gradient of 135 min from 93 to 65% of A. The MS acquired one full spectrum, from which the 12 most intense peaks were selected (singly charged precursors were excluded). Then, a full MS2 spectrum of each of these 12 compounds was completed. The parameters for MS spectrum acquisition were: mass range from 400 to 1 750 m/z, resolution of 70 000, AGC target of le6, and maximum injection time of 200 ms. The parameters for MS2 spectrum acquisition were: isolation window of 1.6 m/z, collision energy (NCE) of 25, resolution of 17 500, AGC target of le5, and maximum injection time of 50 ms.

Data analysis

The MS/MS spectra were performed using the software Proteome Discoverer v.1.4 and the search engine Sequest HT on the FASTA NCBI non-redundant with parameters set for Insecta (TaxID50557). Results of these BLASTs were treated with: (1) MEGAN 6 (Huson et al., 2016), to clustered proteins in functional groups with KEGG pathway, and (2) proteins who have the individual score of low-scoring peptides were not taken into account in the hit score. The significance threshold (α) used was 0.05, testing the null hypothesis that an observed match is a random event. Results were processed first by accepting all proteins with at least three peptides scoring above 15. Subsequently, all redundant queries and corresponding peptides were eliminated. Taxonomic affiliation was assigned according to sequence identity results.

Results

Bacterial proteins

The primary symbiont *B. aphidicola* was identified from all the samples, whereas two facultative symbionts, *Serratia* and *Rickettsia*, were also detected by diagnostic PCR (Table 2). Six facultative symbionts were examined in this study. *Serratia* was present in *A. craccivora* from Gabon and in *M. persicae*. *Rickettsia* was found in *A. craccivora* from Benin. No secondary symbionts were identified in *A. fabae.*

Aphis craccivora salivary proteins

A KEGG analysis of peptides (Figure 1) showed the different pathways of *A. craccivora* salivary proteins. Five of them are common to the two types of saliva. The most represented group was the carbohydrate metabolism pathway, some proteins are unclassified (no functional identification). Two pathways were found solely in solid saliva (energy metabolism and metabolism of other amino acids). Lipid metabolism was identified only in the soluble saliva.

Another proteomic approach by NCBI database was developed to characterize the composition of the saliva in *A. craccivora* (Table 3). The most common enzymes to both types of saliva were glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ribosomal protein, enolase, and tubulin. Some of the identified proteins were associated with bacterial organisms. ATP synthase subunit alpha, phosphopyruvate hydratase, and peroxiredoxin have originated from *Serratia* sp., whereas multispecies cold shock protein associated with enterobacteriaceae was extracted from the solid saliva of *A. craccivora*. Other proteins common to the two types of saliva were also associated with *Serratia* sp. (elongation factor G, GAPDH), one was associated with Enterobacteriaceae (30 S ribosomal protein S1). A large number of identified proteins were related to general functions.

Discussion

Bacterial proteins in aphids

Our cowpea aphid clones only supported a small diversity of facultative symbionts. Only one facultative symbiont, *Rickettsia*, was found in *A. craccivora* from Benin (Brady

Table 2 Aphids examined in this study and the diversity of their symbionts

Locality	Host plant	Aphid	Symbiont	Accession number	Query score (%)	Identity (%)
Benin (Togba)	*Vigna unigulata* (cowpea)	*Aphis craccivora*	*Buchnera aphidicola* (*A. craccivora*) clone 969 16S ribosomal RNA gene	JX629767.1	98	98
			Rickettsia endosymbiont of *A. craccivora* haplotype 2 16S ribosomal RNA gene	KF362029.1	100	99
Gabon (Libreville)	*Amaranthus hybridus* (amaranth)	*A. craccivora*	*B. aphidicola* (*A. craccivora*) clone 880 16S ribosomal RNA gene	JX629768.1	96	96
			Serratia symbiotica SCt-VLC genomic scaffold 01	FR904230.1	99	97
Gabon (Libreville)	*A. hybridus*	*A. craccivora*	*B. aphidicola* (*A. craccivora*) 16S ribosomal RNA gene	EF614236.1	99	99
			S. symbiotica SCt-VLC genomic scaffold 01	FR904230.1	100	98
Belgium (Gembloux)	*Vicia fabae* (fava bean)	*Myzus persicae*	*B. aphidicola* (*M. persicae*) clone SP-GPA-Buch 16S ribosomal RNA gene	KM577346.1	99	98
		M. persicae	*S. marcescens* strain SADAAB_25 16S ribosomal RNA gene	KX908027.1	96	95
		A. fabae	*B. aphidicola* (*A. fabae fabae*) clone AFFBNS2 16S ribosomal RNA gene	KT175936.1	100	99

et al., 2014). This symbiont has also been found in other aphid species, including *A. pisum* (Chen et al., 1996; Simon et al., 2011). It might be an obligate symbiont with *B. aphidicola* (Manzano-Marin & Latorre, 2014). More generally, *Serratia* has been reported to enhance the heat tolerance of aphids (Chen et al., 2000; Montllor et al., 2002; Russell & Moran, 2006). Thus, *A. craccivora* infected with *Serratia* might give aphids a fitness advantage, especially under higher temperatures, which occur when aphids occupy amaranth under hot African climatic conditions.

Co-infection with two symbionts at once has been previously detected in cowpea aphids. Brady et al. (2014) suggested that some *A. craccivora* symbionts are associated with particular host plants. For instance, *Regiella* and *Hamiltonella* are strongly correlated with *A. craccivora* feeding on alfalfa in certain geographical areas. Brady & White (2013) found that populations of *A. craccivora* on

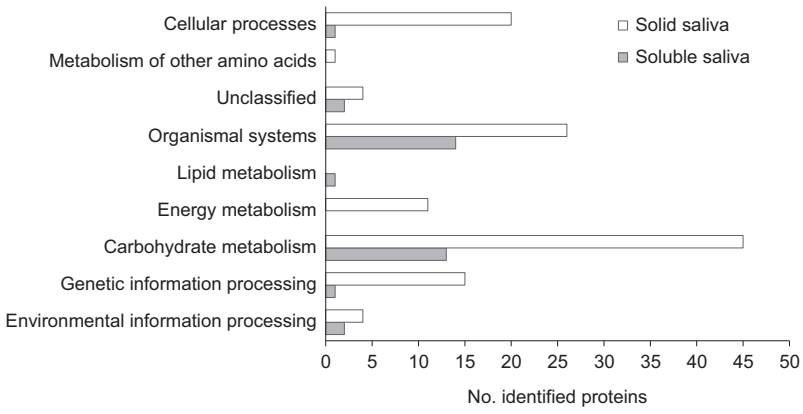

Figure 1 Distribution of identified salivary proteins of *Aphis craccivora* over functional groups based on Megan6 (KEGG analysis).

Robinia pseudoacacia L. (locust) contained a high abundance of *Arsenophonus*. *Arsenophonus* promotes specialization on locusts instead of alfalfa.

Coeur d'Acier et al. (2007) showed evidence of genetic differentiation in aphid races associated with host plants. The genome evolution of aphid species might propagate the diversity of symbionts in aphids across host plants. Polyphagous insects exhibit genetic differentiation in host races that are specialized on herbaceous legumes (Simon et al., 2003; Peccoud et al., 2009). As found for *A. pisum*, our study showed different lineages of *A. craccivora* to be associated with different host plants and different strains of facultative symbionts (Brady et al., 2014). However, additional studies are required to determine how plants–aphids–endosymbionts interact, along with identifying the role of *Arsenophonus* in aphids.

Aphis craccivora salivary proteins and functions

Protein metabolism is prevalent in solid saliva, more peptides are related to carbohydrate and energy metabolism. Lipase protein detected in *A. craccivora* soluble saliva breaks down lipids and fatty acids may function as virulence factors to promote aphid colonization (van Bel & Will, 2016). Aphid-secreted lipases may also trigger plant jasmonic acid-induced defense responses as in a grasshopper (Chaudhary et al., 2015). Vitellogenin protein in this study is assimilated to lipase protein, as in female ticks where it occurs only after mating and feeding (Donohue et al., 2008). Chitinase in aphids plays an important role in penetrating insect cuticles to improve the insecticidal activity of fungal isolates such as *Beauveria bassiana* SFB-205 in *Aphis gossypii* Glover (Kim et al., 2010).

Oxidoreductase is an enzyme that facilitates electron transfer, is involved in sugar metabolism, and potentially detoxifies plant defense compounds (Miles & Oertli, 1993). Three separate glucose dehydrogenases and two trehalases were identified in the saliva of *A. craccivora*. Glucose dehydrogenase was previously detected in the saliva of *A. pisum* (Carolan et al., 2009, 2011; Vandermoten et al., 2014) and *M. persicae* (Harmel et al., 2008; Vandermoten et al., 2014). Trehalase and glucose dehydrogenase have been found in the saliva of *D. noxia*. The action of these two enzymes affects the defensive responses of plants to aphids (Nicholson et al., 2012).

Calmodulin is an essential protein that is present in all organisms, and is responsible for the regulation of a variety of target enzymes (O'Neil & DeGrado, 1990). Apolipophorin is important for lipoprotein metabolism and lipid transport. Vandermoten et al. (2014) has been detected in the salivary proteomes of *M. persicae* and *A. pisum*. This lipid-binding protein might interfere with signaling defense responses of plants. A lipoprotein lipase was also identified in the soluble saliva of *A. craccivora*.

Aphis craccivora salivary proteins and bacterial origin

The results indicate that some protein belongs to aphid endosymbionts. The protein chaperonin GroEl is detected in aphid saliva (Vandermoten et al., 2014), and is particularly abundant in the solid saliva of *Macrosiphum euphorbiae* (Thomas) (Chaudhary et al., 2014) and in the obligate endosymbiont *B. aphidicola*. Yet, this endosymbiont is only found inside aphids. Of note, chaperonin GroEl is secreted into the saliva of aphids and activates plant defenses. When GroEL is absent in plants, the fertility of aphids is reduced (Chaudhary et al., 2014; Elzinga et al., 2014). Some proteins that were identified in *A. craccivora* saliva might contribute to plant–aphid interactions. Indeed, elongation factor Tu (EF-Tu) and the bacterial cold shock protein induce defensive responses to environmental stresses in many plant species (Zipfel et al., 2006). EF-Tu is the most abundant bacterial protein in Brassicaceae (Kunze et al., 2004) and has also been detected in aphid honeydew (Sabri et al., 2013). Peroxiredoxin was identified in *A. pisum* and *Megoura viciae* Buckton. This protein belongs to the peroxidase family, providing protection against oxidative stress. Peroxiredoxin was rarely found in aphid saliva (Vandermoten et al., 2014), whereas peroxidase was identified in the saliva of *S. avenae* (Rao et al., 2013).

Aphid salivary proteins previously identified

Some of the proteins isolated in our study were detected in earlier studies. A carbonic anhydrase was found in three aphid species, including *M. persicae* feeding on celery (Giordanengo et al., 2010), the cereal pest *S. avenae* (Rao et al., 2013), and *S. graminum* (Nicholson & Puterka, 2014). This protein might regulate the pH of plant tissue and the phloem (Rao et al., 2013). One uncharacterized protein (LOC100159632 isoform X1) was also identified by Vandermoten et al. (2014). Another protein, C002, facilities aphid infestation, and is present in the salivary glands of *A. pisum*. This protein allows aphids to feed continuously on plants (Mutti et al., 2008). When this protein is suppressed, the fecundity of *M. persicae* is significantly reduced (Pitino et al., 2011). C002 in *M. persicae* and *A. pisum* has essential functions in aphid–plant interactions (Mutti et al., 2008).

In conclusion, this study was the first to investigate the diversity of endosymbionts and saliva proteomes in *A. craccivora* from Gabon reared on amaranth. This study identified the symbionts of *A. craccivora* after rearing it on *A. hybridus*. The proteomic results showed that proteins in the two types of saliva of *A. craccivora* are diversified in

Table 3 Peptide matches and identified proteins in the saliva of *Aphis craccivora*

Protein identification		Organism	NCBI Accession	MW	E value	Peptides
Bacterial proteins	30S ribosomal protein S1	Enterobacteriaceae	gi|498975437	132483	3.70E-04[a] 1.70E-06[b]	KGDEIAAVVLQVDAER[a,b], VVNVGDVVEVMVLDIDEER[b], QLGEDPWVAIAK[a,b], DVVLVDAGLK[b], NAAGELEIQVGDEVDVALDAVEDGFGETLLSR[b], SESAIPAEQFK[a,b], AFLPGSLVDVRPVR[b]
	Multispecies: cold shock protein	Enterobacteriaceae	gi|546686220	7414	4.60E-07[b]	TLAEGQNVEFEIQDGQK[b], DVFVHFSAIQGNGFK[b], GFGFITPADGSK[b]
	ATP synthase subunit alpha	*Serratia* sp. M24T3	gi|546686306	104025	1.60E-04[b]	ELAAFSQFASDLDEATR[b], EAYPGDVFYLHSR[b], DRGEDALIVYDDLSK[b], VVNTLGAPIDGK[b], DIIAILGMDELAEEDK[b]
	Elongation factor G	*Serratia* sp. M24T3	gi|546687042	120873	4.00E-05[a] 3.90E-06[b]	VEVETPEENTGDVIGDLSR[a,b], TTLTAAITTVLAK[a,b], AIDKPFLLPIEDVFSISGR[a,b], VGEEVEIVGIK[a,b], GQYGHVVIDMYPLEPGSNPK[a], IIELAGYLDSYIPEPER[b], QVGVPFIIVFMNK[b], FFGGEELTEEEIK[b], EHILLGR[b], DVTTGDTLCDPDNVIILER[b], CDMVDDEELLELVEMEVR[b], IATDPFVGNLTFFR[a,b]
	Glyceraldehyde-3-phosphate dehydrogenase	*Serratia* sp. M24T3	gi|546670845	36087	1.10E-05[a] 1.10E-03[b]	LVSWYDNETGYSNK[b], GASQNIIPSSTGAAK[b], FGIVEALMTTVHATTATQK[b], VGINGFGR[a,b], VPTPNVSVVDLTAR[a,b]
	Peroxiredoxin[1,5]	*Serratia* sp. M24T3	gi|546686263	22256	3.90E-03[b]	HQVVNDLPLGR[b], AYGIEHPDAGVALR[b]
	Phosphopyruvate hydratase	*Serratia* sp. M24T3	gi|546671596	45468	2.90E-08[b]	SGETEDATIADLAVGTAAGQIK[b], IQLVGDDLFVTNTK[b], AYTSEEFTHFLEDLTK[b]
Carbohydrate metabolism	Endochitinase	*Acyrthosiphon pisum*	gi|641661172	61220	4.60E-04[a] 1.60E-04[b]	LVMGVPFYGR[a,b], LMEGYYVPGLCR[a,b], LNVADGMQLWVDLGCPPNK[b], NNFYYFVQELR[a,b], NYNIGTYINK[a,b], RPSDQYAYEK[a,b], YFMCDHGK[a,b], DGAYSDRNNFYYFVQELR[a,b], VVCYFSNWAIYRPGIGK[b]
	Enolase	*Oncometopia nigricans*	gi|53830714	347	4.91E+01[a]	YDLDFKNPNSDK[a], YDLDFK[a]
		A. pisum	gi|193669445	47492	1.10E-02[b]	AGLIVTEQR[b], GNPTVEVDLTIDNGPVFR[b], LIETAIEK[b]
		Pediculus humanus corporis	gi|212515287	55403	1.00E-05[a] 8.80E-05[b]	AAVPSGASTGIYEALELR[a,b], VNQIGTVTESIK[a,b]
		Ceratitis capitata	gi|498993219	65	1.12E+01[a]	SGETEDATIADLAVGTAAGQIK[a], DAGYTAVISHR[a]
		Panstrongylus megistus	gi|656772023	345	2.34E+01[a]	IQIIGDDLTVTNPK[a], DGKYDLDFK[a]
		Psorophora albipe	gi|520832780	46886	1.50E-06[b]	YDLDFKNPNSDKb, YDLDFK[b], DGKYDLDFK[b]
	Chain A, the crystal structure of fructose-1,6-bisphosphate aldolase	*Drosophila melanogaster* A	gi|253722156	39231	3.80E-06[b]	VTETVLAAVYK[b], IVPIVEPEVLPDGDHDLDR[b]

Table 3. Continued

Protein identification		Organism	NCBI Accession	MW	E value	Peptides
	Glucose-specific phosphotransferase enzyme IIA component	*C. capitata*	gi|498992360	17914	4.70E-04[b]	VGDPVIEFDLPLLEEK[b], MVAPVDGTIGK[b]
	Glyceraldehyde-3-phosphate dehydrogenase	*Glossina morsitans*	gi|64175015	35868	5.20E-07[a] 6.00E-06[b]	LISWYDNEFGYSNR[a,b], VPTPNVSVVDLTVR[a,b], VIPELNGK[a,b]
		Borkhausenia fuscescens	gi|343112941	50	1.11E+01[a]	IISNASCTTNCLAPLAK[a], LISWYDNEFGYSSR[a]
	Soluble trehalase	*Aphis glycines*	gi|386266701	68578	7.40E-08[a] 2.30E-08[b]	SQPPMVTLMVASYYK[a,b], SGAETGWDFSSR[a,b], SVASSVLGYLR[a,b], EYFYISNIVPLWTESYNMPK[a,b], YDVLASGETGGGGEYTPQTGFGWTNGVVFEFLNR[b], ATNDFDYVKK[a,b], NTYNGNVPPNDELTK[a,b], ATNDFDYVK[a,b], YSLIWVPNGFAIPGGR[b], GVIDNILYLVK[a,b], LAEVWLR[a,b,] KSVASSVLGYLR[b], YTESEILQK[a,b], LKYTESEILQK[a,b], GVIDNILYLVKLFGFMPNGAR[b], LFGFMPNGAR[a,b], SREYFYISNIVPLWTESYNMPK[b], VYYLNR[a,b], QWSLGLNK[a,b], GPRPESYR[b], QWSLGLNKVWK[b]
	Trehalase-like isoform X2	*A. pisum*	gi|193715980	68545	6.00E-06[b]	SQPPMVTLMVSSYYK[b], ELYYWDTYWIVNGMLLCDMSTTAR[b]
	Glycogen phosphorylase	*Bactrocera dorsalis*	gi|618047766	97686	8.70E-06[a] 4.30E-05[b]	VLYPNDNFFEGK[a,b], TCAYTNHTVLPEALER[a,b], VIFLENYR[a,b], VLYPNDNFFEGKELR[a], VAIQLNDTHPSLAIPELMR[a,b], DFYELEPHK[a,b], NLAENISR[a,b], YGNPWEK[a,b], DFYELEPHKFQNK[a], TIAEYAR[a,b], SLQNTMINLGIQSECEEAMYQLGLDIENLEDIEEDA GLGNGGLGR[b]
		Cerapachys biroi	gi|607354331	1095	1.94E+01[a]	TDFDAFPDK[a], IHSEILK[a]
		Tribolium castaneum	gi|546683562	97983	4.60E-05[b]	DYYFALAHTVR[b], GIAEVGDVTEIK[b]
Energy metabolism	ATP synthase subunit alpha	*P. h. corporis*	gi|212511246	59669	1.70E-02[b]	EVAAFAQFGSDLDAATQQLLNR[b], VVSIGDGIAR[b], QMSLLLR[b]
	ATP synthase subunit beta	*Musca domestica*	gi|557764177	56419	2.20E-02[b]	ILNVTGDPIDER[b], IGLFGGAGVGK[b]
	ACYPI23752 (carbonic anhydrase 7)[5]	*A. pisum*	gi|239789413	32705	3.30E-03[a]	LQSPVDINTK[a,b], YLPFLR[a,b], YEFEQMHFHWGK[b]
	Glucose dehydrogenase	*Nasonia vitripennis*	gi|345482856	74652	3.20E-03[b]	VIGIDGLR[b], TQPSETSCLAMKNHQCK[b]
	Glucose dehydrogenase[2,3,4]	*A. pisum*	gi|328709186	67398	2.40E-08[a] 1.30E-04[b]	WSLALNTEYDWK[a,b], GIEFVVEMCK[a,b], ASGNPDIEIMK[a,b,] RASGNPDIEIMK[a], ASGNPDIEIMKIR[a], GCMLGGSSSMNVMLQIR[a,b]

Table 3. Continued

Protein identification		Organism	NCBI Accession	MW	E value	Peptides
	Glucose dehydrogenase[1,2,3,4,6,7]	*A. pisum*	gi|193659536	80592	9.40E-11[a] 2.10E-09[b] 3.90E-03[b]	CFGGTTALNTMLYDR[a,b], YGYNVEGLYVVPEFLR[a,b], SYSISEMVFEYLMK[a,b], GKCFGGTTALNTMLYDR[a], WSWEDVLK[a,b], LCVDSFR[a,b], GIESDYTK[a,b], TVEIRQVYSK[a], DYSLSIK[a,b], FEMQPVK[a,b], LLHSLNR[a,b]
	Carbonic anhydrase	*A. pisum*	gi|193652561	36196	9.40E-06[a] 4.20E-05[b]	YQSPIDIEENLVTK[a,b], VNLPLLR[a,b]
Genetic information processing	Chaperonin groEL[1]	*Sitophilus oryzae*	gi|7443844	51	1.07E+01[a]	GVNVLADAVK[a], EIELEDKFENMGAQMVK[a], FENMGAQMVK[a], LAGGVAVIK[a]
	60 kDa chaperonin	*C. capitata*	gi|498994263	57505	9.00E-04[b]	ANDAAGDGTTTATVLAQSIITEGLK[b], QQIEEATSDYDR[b], GVNVLADAVK[b], AVAAGMNPMDLK[b], LAGGVAVIK[b]
	Chaperone protein DnaK	*C. capitata*	gi|498977694	134802	3.50E-04[b]	TTPSIIAYTQDGETLVGQPAK[b], FQDEEVQR[b], KFEELVQTR[b], DDDVVDAEFEEVK[b]
	Multifunctional chaperone	*Cimex lectularius*	gi|263173438	28258	2.30E-05[b]	QAFDDAIAELDTLNEDSYK[b], DSTLIMQLLR[b], AYQDAFEISK[b]
	30S ribosomal protein S10	*Bombus impatiens*	gi|350424445	11759	2.00E-07[b]	LIDQSTAEIVETAK[b], LVDIVEPTEK[b]
	30S ribosomal protein S4	*B. impatiens*	gi|350424472	23578	2.50E-03[a] 1.00E-02[b]	LDNVVYR[b], LSDYGVQLR[a,b], AALELAEQR[a,b]
	30S ribosomal protein S7	*B. impatiens*	gi|350425809	17485	3.60E-06[b]	LANELSDAAENK[b], FGSELLAK[b], WIVEAAR[b]
	50S ribosomal protein L2	*B. impatiens*	gi|350424454	30282	1.00E-03[b]	SANIALVLYK[b], LEYDPNR[b]
	Ribosomal protein S14e	*Diaphorina citri*	gi|110671466	16283	3.60E-02[b]	TPGPGAQSALR[b], ITGGMKVK[b]
	Elongation factor 1-alpha 1	*T. castaneum*, *N. vitripennis*	gi|478257096	49	1.68E+00[a]	QTVAVGVIK[a], YYVTIIDAPGHR[a], QLIVGVNK[a]
	Elongation factor-1 alpha	*B. dorsalis*	gi|300952938	50730	1.10E-04[b]	GITIDIALWK[b], VHTNIVVIGHVDSGK[b], LPLQDVYK[b], EHALLAFTLGVK[b], YYVTIIDAPGHR[b], QTVAVGVIK[b]
Environmental information processing	Calmodulin	*D. citri*	gi|662200666	31075	5.10E-04[b]	LTDEEVDEMIR[b], HVMTNLGEK[b], EAFSLFDK[b], EAFSLFDKDGDGTITTK[b]
	60 kDa heat shock protein,	*Camponotus floridanus*	gi|307173631	168027	2.50E-07[b]	TALTDAAGVASLLTTAEAVVTELPK[b], ALMLQGVDILADAVAVTMGPK[b], VGGSSEVEVNEK[b]
Organismal systems	Actin	*D. melanogaster*	gi|156750	356	6.81E+01[a]	SYELPDGQVITIGNER[a], DLTDYLMK[a], DSYVGDEAQSK[a], GYSFTTTAER[a], AGFAGDDAPR[a], AVFPSIVGRPR[a], QEYDESGPSIVHR[a], EITALAPSTMK[a], RGILTLK[a], VAPEEHPVLLTEAPLNPK[a]
	Actin 5	*Aedes aegypti*	gi|67782283	42194	8.70E-09[b]	SYELPDGQVITIGNER[b], DSYVGDEAQSK[b], QEYDESGPSIVHR[b], VAPEEHPVLLTEAPLNPK[b], TTGIVLDSGDGVSHTVPIYEGYALPHAILR[b], GYSFTTTAER[b],

Table 3. Continued

Protein identification		Organism	NCBI Accession	MW	E value	Peptides
						DLTDYLMK[b], AVFPSIVGRPR[b], AGFAGDDAPR[b], YPIEHGIITNWDDMEK[b], EITALAPSTMK[b]
	Elongation factor Tu	*B. impatiens*	gi|350425826	47341	1.70E-07[a] 2.40E-04[b]	GITINTSHVEYDTPTR[a,b], ELLSQYDFPGDDTPVIR[a], QVGVPYIIVFLNK[b], EHILLGR[a], TTDVTGTIELPEGVEMVMPGDNIK[a]
Cellular processes	Tubulin	*Agasicles hygrophila*	gi|576098237	47	9.27E+00[a]	LAVNMVPFPR[a], AILVDLEPGTMDSVR[a]
	Tubulin beta-3	*D. melanogaster*	gi|158749	51438	6.10E-06[b]	GHYTEGAELVDNVLDVVR[b], LAVNMVPFPR[b], NSSYFVEWIPNNVK[b]
	Tubulin alpha chain	*Zootermopsis nevadensis*	gi|646711831	51118	3.60E-04[b]	LIGQVVSSITASLR[b], VGINYQPPTVVPGGDLAK[b]
	Tubulin alpha-1 chain	*D. citri*	gi|662213931	108	1.87E+01[a]	AVFVDLEPTVVDEVR[a], EIIDLVLDR[a]
	Tubulin alpha-1 chain-like isoform X1	*Solenopsis invicta*	gi|322778876	53246	3.70E-02[b]	LIGQIVSSITASLR[b], EIIDLTLDR[b]
	Tubulin beta-4 chain	*Megachile rotundata*	gi|383861412	65357	7.30E-03[b]	INVYYNEASGGK[b], GHYTEGAELVDSVLDVVR[b], AILVDLEPGTMDSVR[b], ISEQFTAMFR[b]
Unclassified	LOC100159632 isoform X1[1]	*A. pisum*	gi|193676365	128504	3.80E-10[a] 8.70E-09[b]	YMVSTTSSTAGSCR[a,b], LEDIDLDGCAK[a,b], DIEVGFVSMQGIIK[a,b], KHLSEMEVPVVK[a,b], HLSEMEVPVVK[a,b], TNEHWECLLK[a,b], ESDINEKDIEVGFVSMQGIIK[a,b], CGLIPVSK[a,b], LQTFVHSR[a,b], ESDINEKDIEVGFVSMQGIIKNGR[a], FNVLNSK[a,b], SFLEAIK[a,b], TETNTTIYGK[a,b], IIGSAMTR[a,b], KLQTFVHSR[a,b], DIEVGFVSMQGIIKNGR[a], KESDINEKDIEVGFVSMQGIIK[a]
	LOC103519435	*D. citri*	gi|662217522	104480	2.00E-02[b]	STELLIR[b], EIAQDFK[b], YRPGTVALR[b]
	LOC101744625 isoform X1	*Bombyx mori*	gi|357626708	35	1.93E+00[a]	LVYYSPK[a], RTDVLELK[a]
	LOC100166244	*A. pisum*	gi|328712999	55421	2.00E-03[b]	FTDFLETEASR[b], ILDLLITSLSDTLTEHPELMAAAK[b], NYESSDFNDIDGTLTELK[b], VQLSVDIPSK[b]
	GF22288	*D. ananassae*	gi|190619734	101366	8.50E-03[a] 4.10E-02[b]	LQDWDYKK[a,b], LQDWDYK[a,b]
Lipid metabolism	Apolipophorin	*A. pisum*	gi|641667063	331110	7.40E-06[a]	IENLEPLLEK[a,b], FILIGYSGK[a,b], FVALLYK[a,b], EGYLGVGALLR[a], LFGPEGYFAK[a,b], YSPIFEAK[a,b], SLNDILR[a,b], IENLEPLLEKLFGPEGYFAK[b],
	ACYPI008569 (apolipophorin-3)	*A. pisum*	gi|239789136	23670	1.10E-07[b]	DIEEIILALIPDK[b], LLPDKPDSPFDELFR[b], YFDHLK[b]

Table 3. Continued

Protein identification		Organism	NCBI Accession	MW	E value	Peptides
Other insect proteins	A-agglutinin anchorage subunit	*A. pisum*	gi|328713741	84	2.37E+00[a]	ALNFNPDR[a], ALNFNPDRSMIK[a]
	Adenosylhomocysteinase	*D. citri*	gi|662203320	120	1.98E+01[a]	VAVVAGYGDVGK[a], SKFDNLYGCR[a], FDNLYGCR[a]
		Z. nevadensis	gi|646703582	48491	6.00E-04[b]	VAVVAGYGDVGK[b]
	Alcohol dehydrogenase 4	*Anoplophora glabripennis*	gi|550249790	30049	9.50E-04[a]	YVVDTSK[a,b], EAIDFFAR[a,b], ADTREAIDFFAR[a]
	Histone H2A	*Chironomus thummi*	gi|7085	13415	7.30E-07[b]	VGAGAPVYLAAVMEYLAAEVLELAGNAAR[b], LLSGVTIAQGGVLPNIQAVLLPK[b]
	Histone H2A,V	*T. castaneum*	gi|91076988	13448	5.40E-05[b]	VGATAAVYSAAILEYLTAEVLELAGNASK[b], GDEELDSLIK[b]
	Hypothetical protein G5I_04458	*Acromyrmex echinatior*	gi|332026925	106253	1.90E-02[b]	INRIVRER[b], WTAPSMISLIK[b]
	Lysine-arginine-ornithine-binding periplasmic protein	*C. capitata*	gi|498989859	28290	1.70E-06[b]	LDAAFQDEVAASEGFLK[b], KIDAIISSLSITEK[b]
	Outer membrane protein A	*C. capitata*	gi|498975325	41974	1.40E-04[a] 5.20E-04[b]	AALIDCLAPDR[a,b], DGSVVVLGFTDR[a,b], SDVLFNFNK[a,b], AQSVVDYLVSK[a,b]
	Phosphoglucomutase isoform X3	*Apis mellifera*	gi|66561330	94	2.56E+01[a]	LSGTGSSGATIR[a], TIPDISIDISK[a]
	Gtp-binding adp-ribosylation factor arf1	*Aedes albopictus*	gi|604772541	20714	2.70E-02[b]	QDLPNAMNAAEITDK[b], ILMVGLDAAGK[b]
	Tropomyosin invertebrate	*Corethrella appendiculata*	gi|545914646	58	8.84E+00[a]	IVELEEELR[a], LQCEVMRR[a]
	Thioredoxin peroxidase 1	*D. melanogaster*	gi|7230426	65	6.80E+00[a]	GLFIIDDK[a], QITVNDLPVGR[a]
	Vitellogenin	*Pteromalus puparum*	gi|134290336	128	3.02E+01[a]	YTIQSSVTTNK[a], TNPPASMLQR[a]
		Lethocerus deyrollei	gi|169219461	213698	5.70E-05[b]	YTIQSSVTTNK[b], GLCGTFDGEK[b]
	Titin-like isoform X1	*A. pisum*	gi|641658040	412655	3.40E-04[a] 9.60E-04[b]	MLAVAHNLNHETK[a,b], LAATNLQK[a], SVLDTSEMR[a,b], QYDPNQYILK[a,b], SNMLATIK[a,b]
	Armadillo/beta-catenin/plakoglobin	*G. morsitans*	gi|289739777	71	3.08E+01[a]	LVQNCLWTLR[a], AFQDIER[a]
	Ubiquitin	*Z. nevadensis*	gi|646722712	51	1.07E+01[a]	TITLEVEPSDTVENLK[a], TLTGKTITLEVEPSDTVENLK[a]
	Ubiquitin, partial	*Schizaphis graminum*	gi|326698725	8552	1.20E-07[b]	TITLEVESSDTIDNVK[b], ESTLHLVLR[b], TLSDYNIQK[b]
	Polyubiquitin	*D. melanogaster*	gi|361584491	26278	3.00E-05[a] 2.50E-04[b]	TITLEVEPSDTIENVK[a,b], TLTGKTITLEVEPSDTIENVK[a], TITLEVEPSDSIENVK[a,b]

Table 3. Continued

Protein identification	Organism	NCBI Accession	MW	E value	Peptides
WASH complex subunit strumpellin	*Papilio polytes*	gi|357619265	67	2.24E+01[a]	YIELITR[a], ITEVPTR[a]
Translation initiation factor IF-2	*C. capitata*	gi|498978463	97828	4.80E-03[b]	DNVVIYEGELESLR[b], GPVATVLVR[b]
ACYPI000294	*A. pisum*	gi|239799135	19337	1.20E-04[b]	QIENLIGQGK[b], NNLNAFAESLK[b]
ACYPI005249	*A. pisum*	gi|239793648	21000	2.10E-03[b]	LSEDIIK[b], YVLDADK[b], SFELTNDYVK[b], YIADDPDKYSVDLNALYK[b], KFETIVLEHLPK[b]
COO2	*A. glycines*	gi|359801951	24589	3.60E-07[a] 3.40E-07[b]	ELGTNDVCSDTIR[a,b], MLAFIAR[a,b]
GF20391	*Drosophila ananassae*	gi|190631520	21231	1.60E-03[a] 2.50E-08[b]	AMSIMNSFVNDIFER[b], TVTAMDVVYALK[b], VFLENVIR[a,b], ISGLIYEETR[a,b], DNIQGITKPAIR[b], LLLPGELAK[a,b]

[a]Soluble saliva.
[b]Solid saliva.
Previously reported by: [1]Vandermoten et al. (2014); [2]Carolan et al. (2011); [3]Nicholson et al. (2012); [4]Carolan et al. (2009); [5]Rao et al. (2013); [6]Cherqui & Tjallingii (2000); [7]Harmel et al. (2008).

the aphid and in some of the endosymbiotic bacteria. *Serratia* proteins were present in both types of saliva. Fifteen percent of the proteins detected in aphid saliva was of bacterial origin. This result confirmed that bacteria contribute to plant–aphid interactions, influencing the adaptive capacity of aphids and plant responses to aphid feeding. Several proteins from aphids were identified, which interact with plant defense mechanisms. Some proteins of symbiotic bacteria were also identified in aphid saliva. It is important to resolve how these bacterial proteins affect the metabolism of the host plant. Thus, future studies should focus on determining the functional role of aphids and associated bacterial proteins found in saliva on plant–insect interactions.

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