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Proteomic analysis of the venom of the social wasp *Apoica pallens* (Hymenoptera: Vespidae)

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ABSTRACT

Wasps are a diverse group of insects that possess a sting apparatus associated with a venom gland, which is used for predation and colony defense. The biochemistry of Hymenoptera venom has been evaluated in relation to allergy and immunology, and proteomics has been shown to be a powerful tool for the identification of compounds with pharmacological potential. Data on wasps venom the of genus *Apoica* are scarce, so the objective of the present work was to identify the venom proteins of the eusocial wasp *Apoica pallens*, as a first step towards further investigation of applied uses of the venom and its protein constituents. The venom proteins were separated by two-dimensional gel electrophoresis, followed by MALDI-TOF/TOF mass spectrometry. A total of 259 spots were detected, with molecular weights from 4.9 to 141 kDa. Thirty of these proteins were identified and classified into eight functional categories: allergen, enzyme, metabolism, structural, environmental response, proteoglycan, active in DNA and RNA, and unknown function. Due to the few available proteomic data for wasp venom, many proteins could not be identified, which makes studies with proteomic analysis of Hymenoptera venom even more important.

Introduction

The Hymenoptera represents a diverse group of insects, with an estimated 120,000 species distributed among wasps, bees, and ants (Van Emden, 2013). Within the group, the development of a sting apparatus and venom glands, used as a mechanism to aid in the capture of prey and/or defense of the colony, has been one of the key elements for the evolutionary success of these insects (Wilson, 1971; Macalintal and Starr, 1996).

Venom is defined as a secretion produced by specialized glands, which is capable of altering or disrupting the normal biological or physiological processes of the target organism (Casewell et al., 2013). In addition to the offensive and defensive functions, volatile compounds present in glandular venom can act as an alarm pheromones (Schmidt, 1982; Orivel et al., 2001), and the venom can have antiseptic activities against bacteria, fungi, protozoa, and viruses (Orivel et al., 2001; Baracchi and Tragust, 2017).

The composition of the Hymenoptera venom is the result of an evolutionary process over millions of years, during which the development and incorporation of a great number of compounds occurred (Casewell et al., 2013). Despite the patterns found in the venom, its composition can vary even within a single species (Aili et al., 2017; Bernardi et al., 2017; Mendonça et al., 2017). In the wasps, the venom is composed of biologically active amines, mainly serotonin and histamine, which are responsible for pain, vasodilation, and increased permeability of the blood capillaries, facilitating the penetration of toxins into the tissue (Banks and Shipolini, 1986; Oliveira et al., 1999). Also present are volatile compounds, lipids, peptides, and proteins that act as toxins, enzymes, and allergens (Lima and Brochetto-Braga, 2003; Monteiro et al., 2009; Czaikoski et al., 2010).

In humans, a single wasp sting may cause local inflammation characterized by pain, redness, and swelling (Graaf et al., 2009). In general, the human population presents a type 1 hypersensitivity reaction to Hymenoptera venom (Nittner-Marszalska et al., 2004;

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Bilo et al., 2005). Multiple stings can be fatal, as a result of toxic action, when venom is injected in large quantities (Vetter et al., 1999), and the systemic reaction occurs in 5% of the population (Charpin et al., 1992). Consequently, the biochemical characterization of Hymenoptera venom has become a focus of research in the area of allergy and immunology, with the proteomics approach providing a tool for the identification of compounds in the diagnosis and treatment of hypersensitive persons (Santos et al., 2011b).

The antiseptic function of venom is part of the innate immunity of Hymenoptera and its initial evolutionary value is associated with the need to minimize potential contamination by prey (Orivel et al., 2001; Baracchi and Tragust, 2017). Specifically in eusocial insects, the marked genetic homogeneity and physical proximity among the individuals of the colony provide an ideal environment for the dissemination of infectious diseases. In addition to asepsis of the prey, venom has a prophylactic function in the hygiene of the individuals of the colony and the structures of the nest. The evolution of sociality was accompanied by the evolution of agents associated with social immunity in Hymenoptera (Stow et al., 2007; Hoggard et al., 2011). Hence, the antiseptic characteristic of the venom has further increased interest in the compounds present in this glandular material, in terms of understanding the evolution of these compounds and their contribution to innate immunity and eusociality (Stow et al., 2007; Hoggard et al., 2011). Furthermore, these compounds have attracted attention as models for the development of new therapeutic products (Touchard et al., 2006), which has been explored in recent decades (Wang et al., 2011; Yang et al., 2013; Čeřovský et al., 2007; Silva et al., 2017).

Some of the compounds present provide the venom with pharmacological potential, an example being the venom of the *Polybia paulista* wasp, which forms pores in the plasma membranes of cancer cells, without damaging the healthy cells (Leite et al., 2015). However, little is known about the effective action of compounds found in the venom of social Hymenoptera, due mainly to the limited amount that can be obtained from a single individual (Aili et al., 2017; Lima and Brochetto-Braga, 2003), together with the difficulty involved in dissection and extraction of this glandular material (Fox et al., 2015). Nonetheless, new isolation tools and advances in mass spectrometry and compound databases have enabled progress in research with the venom of the group, allowing even small amounts of sample to be studied (Aili et al., 2014, 2017).

Thus, proteomic analyzes are extremely important, since they allow structural and functional characterization of proteins, allowing the isolation, sequencing and molecular cloning producing recombinant allergens, which are standardized and purified, unlike the raw venom with variable composition (Perez-Riverol et al., 2017). Recombinant compounds are also useful in the diagnosis of allergy and immunotherapy. Thus, the use of recombinant compounds could help solve the problem of the limited amount of venom obtained for analyzes, since the commercial form could be used to study their applications.

Despite the interest in insect venom, little is known about its chemical composition in social wasps (Mendes et al., 2004; Souza et al., 2004, 2005; Santos et al., 2010; Baptista-Saidemberg et al., 2011; Santos et al., 2011a; Souza et al., 2019). Among the social wasps, we highlight the genus *Apoica*, composed of swarming and nocturnal wasps that have specific adaptations for this way of life (Schremmer, 1972). *Apoica pallens* (Fabricius, 1804) is the species within the genus *Apoica* with the largest geographical distribution and contains hundreds to thousands of individuals, found from Mexico to northeastern Argentina (Richards, 1978). In Brazil, this species has wide distribution and because of its size and aggressiveness can cause accidents, so the study of its venom composition is extremely relevant from a medical point of view.

Therefore, the objective of this study was to identify the proteins present in the venom of the *Apoica pallens* Neotropical wasp, as a first step towards investigating the applied use of the crude venom and/or its isolated compounds.

Materials and methods

Collection and preparation of samples

A colony of *A. pallens* was collected in Dourados (22°14′S, 54°49′W), State of Mato Grosso do Sul, Brazil, in February 2015, and 1,600 females were used for the study. We used females from a single colony to standardize the results of our samples, since according to the literature; the venom may vary due to genetic or environmental factors (Badhe et al., 2006; Cologna et al., 2013), and also to minimize impacts on their population, since a single colony is sufficiently populous to provide enough samples for analysis.

The wasps were dissected in ultrapure water and the venom reservoirs were transferred to microtubes with water, for maceration of the secretory part of the gland, followed by centrifugation of the samples at 12,000 x *g* for 20 min at 4 °C. The supernatant was then stored at - 20 °C for further analysis.

The concentration of total proteins present in the venom was determined by the Bradford method (Bradford, 1976), using bovine serum albumin as standard.

A triplicate composed of three extracts from a colony (methodological repetition) were used for proteomic analysis of the venom. The reagents used were analytical grade and were supplied by Sigma-Aldrich (USA), Bio-Rad (USA), Dynamics (Brazil), Labsynth (Brazil), Merck (Germany), or Oxoid (Brazil). The buffers used were prepared with ultrapure water (Master All 2000 ultra-purifier, Gehaka, Brazil).

Two-dimensional gel electrophoresis

Immobiline DryStrip gels (7 cm, pH 3–10; GE Healthcare) were rehydrated for 10 h with 125 μL of Destreak (GE Healthcare) containing 100 μg of *A. pallens* venom protein, 2.5 μL of immobilized pH gradient (IPG) buffer (pH 3–10, 2% v/v) to assist in electrical conductivity during focusing, 5 μL of 40 mM dithiothreitol (DTT) to break down the spatial conformation of the protein, and 96.6 μL of Destreak solution (GE Healthcare) to decrease drag in the second phase of the separation and reduce oxidation of the thiol groups of the protein.

Isoelectric focusing was performed using an Ettan IPGphor 3 system at 20 °C, with the following program: 300 V for 12 h, 1000 V for 30 min, 5000 V for 2 h, 5000 V for 1 h, and 200 V for 1 h. After focusing, the immobilized pH gradient tapes were equilibrated in equilibration buffer solution containing 6 M urea, 75 mM hydroxymethyl aminomethane hydrochloride (Tris-HCl, pH 8.8), 29% (v/v) glycerol, 2% (w/v) sodium dodecyl sulfate (SDS), and 0.002% bromophenol blue. The triplicates were placed in two equilibrium solutions, firstly in 1% DTT, and then in 2.5% iodoacetamide (IAA), for 15 min each. The tapes were then transferred to 14% SDS-polyacrylamide separation gel (Laemmli, 1970) for analysis using a Mini-PROTEAN II system (Bio-Rad).

The electrophoresis was performed at 100 V for 40 min and at 60 V until the dye reached the base of the gel. The gels were fixed in 40% (v/v) methanol with 10% (v/v) acetic acid for 30 min, washed in water, and stained for 24 h in Coomassie Brilliant Blue G-250 solution (Görg et al., 2000). Subsequently, the gels were stored at 21 °C in 5% (v/v) acetic acid solution.

The gels were scanned using an ImageScanner III system (GE Healthcare) in transparency mode (16 bit, 600 dpi, colors red-blue and green-blue). The images were analyzed using ImageMaster Platinum v. 7 (GE Healthcare).

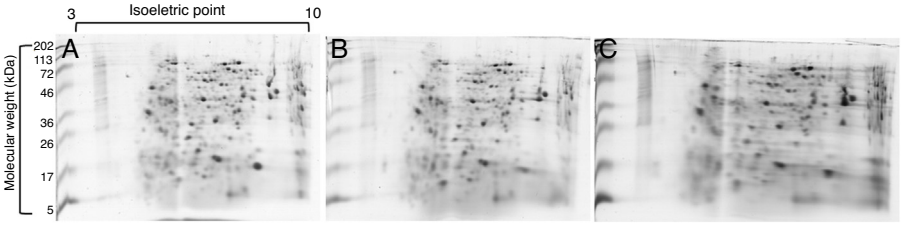

Fig. 1. Two-dimensional gel (14%) in triplicate (three extracts from a colony), with the three gels (A, B, and C) of the *Apoica pallens* wasp venom.

In-gel extraction and digestion of proteins by trypsin

Following the protocol of Shevchenko et al. (1996) for sample digestion, the spots were excised and transferred to microtubes containing 100% acetonitrile (ACN) for 5 min. Subsequently, they were transferred to 10 mM DTT for 30 min at 56 °C, and then to 55 mM iodoacetamide solution for 20 min, followed by washing in acetonitrile. Gel fragments were incubated for 16 h with 100 mM ammonium bicarbonate solution containing 25 ng/μL trypsin (Sequencing Grade Modified Trypsin, Promega), at 37 °C. Extraction of the peptides from the gel fragments was performed with 5% formic acid in 50% acetonitrile. The extract obtained was lyophilized using a SpeedVac and resuspended in 10 μL of 0.1% trifluoroacetic acid. The samples were desalted with ZipTip tips with C18 resin (Millipore), vacuum-dried, and prepared for analysis by matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF/TOF MS).

Mass spectrometric analysis (MALDI-TOF/TOF MS)

The samples obtained from the gel digestion were solubilized in 10 μL of 0.1% (v/v) trifluoroacetic acid (TFA) and mixed with 2.5 mg/mL of α-cyano-4-hydroxycinnamic acid in 50% (v/v) ACN and 0.1% (v/v) TFA, using a ratio of 1:1 (sample:matrix). Aliquots of these samples were transferred to an MTP AnchorChip 600/384 TF steel plate (Bruker Daltonics) and analyzed by MALDI-TOF/TOF MS (Ultraflex III, Bruker Daltonics).

In the first peptide fragmentation (MS), the spectra were obtained in reflector mode (LPPepMix), with a detection range from 500 to 5000 Da, corresponding to the linear detection mode for positive ions. The second peptide fragmentation (MS/MS) was performed in LIFT mode, with a range from 40 to 1878 Da.

Identification of proteins

All the MALDI-TOF/TOF mass spectra were processed using FlexAnalysis v. 3.3 software (Bruker Daltonics) to remove contaminants and obtain a list of masses that were then subjected to two software searches for protein identification: MASCOT (Peptide Mass Fingerprint and MS/MS Ion Search, Matrix Science Ltd.) and PEAKS DB v. 7.0 (Bioinformatics Solutions Inc., Canada). The identifications obtained with the MASCOT software were validated using Scaffold v. 4.0 software (Proteome Software Inc., USA). The identifications obtained from PEAKS DB v. 7.0 were validated assuming the FDR (False Discovery Rate) to be equal to 0.0%.

Identification of the proteins in MASCOT used the publicly available deposited protein sequences in NCBI-nr and SwissProt, at the levels of 0.5 and 0.1 kDa, with 50 ppm of error. The reactions of carbamidomethylation of cysteine and oxidation of methionine were used as fixed and variable modifications, respectively. Metazoa (animals), Insecta, and proteins from animal venom (mollusk, snake, insect, arachnid, and amphibian) were selected as taxa for entry into the databases. The results were considered significant when the score of the suggested protein was higher than the score calculated by the MASCOT software, assuming $p < 0.05$ and $p < 0.01$.

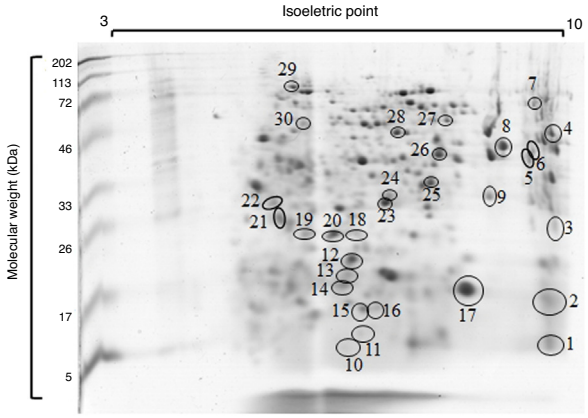

Fig. 2. Two-dimensional reference gel (14%) of the venom of the social wasp *Apoica pallens*, showing the proteins identified by MALDI-TOF/TOF analysis.

After analysis by MASCOT, the MS/MS spectra were also submitted to evaluation using the PEAKS Studio v. 7.0 program. The protein sequences suggested by this program were submitted to the UniProt 2017 database (http://www.uniprot.org/) (UniProt Database, 2017), while those with average local confidence (ALC) of at least 70% were submitted to MS-BLAST (http://genetics.bwh.harvard.edu/msblast/). Positive results suggested by the research banks and the animal venom database were accepted as positive identification.

Results

The concentration of total proteins present in the *A. pallens* crude venom was 19.79 ± 5.88 μg/μL. The electrophoresis gels analyzed in triplicate presented 92% similarity (Fig. 1). A total of 259 spots were detected, with molecular weights ranging from 4.9 to 141 kDa and isoelectric points between 3.81 and 9.75 (Figs. 1 and 2). The results showed that 50.65% of the volume of these spots had pI lower than 6.9, while 20.82% of the volume had pI between 7 and 7.9, and 28.53% of the volume had pI higher than 8.

A total of 180 of the more significant spots that represented 69.5% of the total and were present in the three gels were excised from the gel. Of these 180 spots, 30 were identified (Table 1), totaling 16.7% of proteins identified in the *A. pallens* venom. These identified proteins corresponded to a total volume of 24.2% of all the spots found in the analysis of the two-dimensional electrophoresis.

The proteins identified by homology in this study were classified into eight categories: allergenic (spot 4), enzymatic (spots 1, 2, 3, 10, 14, 20, and 23), metabolic (8, 9, 16, 19, 24, 25, 26, 27 and 30), structural (spots 7, 12, 13 and 22), environmental response (spots 21, 28, and 29), proteoglycan (spot 15), DNA and RNA active agents (spots 5, 11 and 17), and unknown function (spots 6 and 18) (Fig. 3). In addition, we present in Fig. 4 a functional picture of the described proteins according to their functions divided into:

Table 1
Proteins identified in the venom of the *Apoica pallens* wasp, after 2D gel fractionation and MALDI-TOF/TOF MS analysis.

Categories	Spot	pI	MM	Accession Code	Protein	Peptides sequences	Organism
Allergenic	4+ ¢$	9.46	52734	ADL09135.1	Hyaluronidase		*Polybia paulista*
Enzymatic	1+ ¢	9.42	8596	U3T755_PROFL	Nucleoside diphosphate kinase		*Protobothrops flavoviridis*
	2+ ¢	9.38	18206	NXAH1_MICCO	Alpha-neurotoxin homolog 1		*Micrurus corallinus*
	3+ ¢	9.51	28637	SL6_CROAD	C-type lectin 6		*Crotalus adamanteus*
	10+ ¢	6.28	7014	T1DLR5_CROHD	Proteasome subunit beta type		*Crotalus horridus*
Metabolic	14*#	6.26	19811	G7YVQ7_CLOSI	Activated CDC42 kinase 1	TPSPTHGHHRR	*Clonorchis sinensis*
	20*#$	6.10	27162	E2BMB4	Probable adenylate kinase isoenzyme F38B2.4	TGFLIDGYPR	*Harpegnathos saltator*
	23*#	6.91	32781	Q2ABP1_DANRE	Metalloendopeptidase	LLFDVFETEGNDVSTRFLPLR	*Danio rerio*
	8*#$	8.72	46238	A0A026WWN8_CERBI	Fructose-bisphosphate aldolase	IVPIVEPEILPDGDH DLARGVVPLFGTDNEC (+57.02)TTQGLDDLQAR	*Cerapachys biroi*
	9*#	8.50	33759	K7IV97_NASVI	Phosphoglycerate mutase 2	YAAEPKPEEFPK YGEEQVQIWR	*Nasonia vitripennis*
	16+ ¢	6.77	16602	V8NPR9_OPHHA	CD40 ligand		*Ophiophagus hannah*
	19*#	5.69	27242	Q28943	Dihydropyrimidine dehydrogenase [NADP(+)]	C(+57.02)PIIDC(+57.02)IR	*Sus scrofa*
	24+ ¢	6.91	32781	ETE67223.1	Calcium-binding protein 1		*Ophiophagus hannah*
Structural	25*#$	7.60	36861	V9IA48_APICE	Calcium-transporting ATPase	GSTYEPVGEIFLR C(+57.02)N(-17.03)DSAIDFNEFK EFTLEFSR TYEPVGEIFLR	*Apis cerana*
	26*#$	7.72	43829	F4WH18_ACREC	Glyceraldehyde-3-phosphate dehydrogenase	LISWYDNEFGYSSR	*Acromyrmex echinatior*
	27*#	7.85	60913	A0A093HYS1_STRCA	Calcium-binding protein 4	LKIAFREFDVNGDGEISSAEMR	*Struthio camelus australis*
	30+ ¢	5.64	59247	V8NNP4_OPHHA	Serum response factor-binding protein 1		*Ophiophagus hannah*
	7*#	8.92	94274	G5AYM2_HETGA	Myomesin-2	APTDVHASEISR	*Heterocephalus glaber*
	12*#$	6.39	23313	E2BL16_HARSA	Cofilin/actin-depolymerizing factor-like protein	Q(-17.03)IDVEVIGPR YGLFDFEYTHQ QIDVEVIGPR ATDLSEASEEAVEEK DAAYDAFLQ(sub E)DLQK	*Harpegnathos saltator*
	13*#$	6.33	21157	A0A087ZNF1_APIME	Transgelin	KFPPGELFEDVIK	*Apis melifera*
	22*#$	5.18	32589	G9C5F3_SCHGR	Rho GDP dissociation inhibitor	TIEELLEADKEDESLR	*Schistocerca gregaria*
Proteoglycan	15+ ¢	6.56	16442	V8P0M2_OPHHA	Glypican-6		*Ophiophagus hannah*
Environmental response	21*#	5.28	29752	G0T332_SPAAU	Peroxiredoxin 1	GLFIIDDKGILR	*Sparus aurata*
	28*#	7.08	53322	Q0QWE4_FUNHE	Heat shock cognate 70	VEIIANDQGNR	*Fundulus heteroclitus macrolepidotus*
	29*#	5.49	103126	A0A034VDA3_BACDO	Heat shock 70 kDa protein cognate 3	DVHEIVLVGGSTRVTHA VVTVPAYFNDAQRAKFEELNM (+15.99)DLFRN(+.98)GDTHLGG EDFDQRATN(+.98)GDTHLGGE DFDQRAKFEELNMDLFR	*Bactrocera dorsalis*
DNA and RNA active agents	5+ ¢	9.18	44947	A9QQ88_LYCSI	rRNA processing protein RRP7		*Lycosa singoriensis*
	11+ ¢	6.52	10035	J3S0P4_CROAD	U2 small nuclear ribonucleoprotein B"-like protein		*Crotalus adamanteus*
	17+ ¢	8.15	19201	V8NR38_OPHHA	DNA repair protein XRCC3		*Ophiophagus hannah*
Unknown function	6*#$	9.12	42739	E9INY0_SOLIN	Putative uncharacterized protein	LISWYDNEFGYSSR	*Solenopsis invicta*
	18*#	6.41	27162	A0A087ZUB8_APIME	Uncharacterized protein	TGFLIDGYPR	*Apis melifera*

+ Identified by Mascot software, ¢ Validated by Scaffold 4.0, * Identified by Peak DB 7.0 software, Taxon for database entry: # Metazoa, $ Insecta.

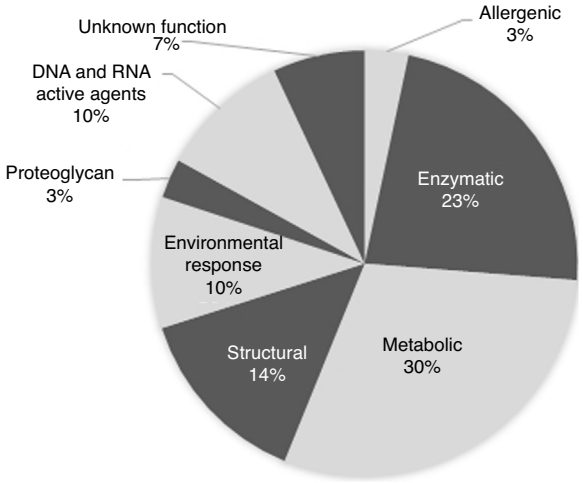

Fig. 3. Classification of proteins according to the representativity in number of proteins identified in the *Apoica pallens* venom.

allergenic, inflammatory, neurotoxin, self-protection venom activity, associated with the muscle/cytoskeleton, provides energy, DNA and RNA actuators, against pathogens, degradation and cytolytic action, and unknown.

Discussion

The electrophoresis gel data indicated that the *A. pallens* wasp venom had a relatively acidic character, with 50.65% of the volume of all the proteins detected on the gel having pI 3-6.9. The molecular weight of the proteins detected in the venom of this species varied from 4.9 to 141 kDa.

The molecular weight variation of the proteins of *Polybia paulista* was previously found to be lower, with values between 8 and 96 kDa (Santos et al., 2010). In addition, Santos et al. (2010) detected 237 spots on *P. paulista* venom gels, compared to 259 detected in the case of the *A. pallens* samples, suggesting that each wasp species synthesizes different numbers and/or concentrations of proteins in the venom. Furthermore, in this same study, the authors describe that *P paulista* venom has a higher number of allergens (antigen-5, hyaluronidase, PLAs, serine proteinases) compared to *A. pallens* which has only hyaluronidase. However, in both studies the venom collection method used leaves the possibility that some cellular proteins may have been mixed with the actual venom components, and although there are protein differences between these species, which belong to the same tribe (Epiponini), we found that these wasps have some proteins in common, which will be discussed throughout our study.

The most representative functional class in the *A. pallens* venom, in terms of the number of proteins identified, was that of the energy metabolism proteins. Fructose-bisphosphate aldolase catalyzes the cleavage of fructose 1,6-bisphosphate into dihydroxyacetone phosphate and glyceraldehyde 3-phosphate during glycolysis (Midelfort et al., 1976). Phosphoglycerate mutase converts 3-phosphoglycerates to 2-phosphoglycerates in gluconeogenesis, and glyceraldehyde-3-phosphate dehydrogenase converts glyceraldehyde 3-phosphate to 3-phosphoglycerol phosphate (Boiteux and Hess, 1974, 1981). All these enzymes are involved in the oxidation of glucose (Nelson and Cox, 2014), which is the main energy substrate for tissues (Gmeinbauer and Crailsheim, 1993). Elias-Santos et al. (2013) reported the presence of these three proteins in the salivary glands of the head of the honey bee *Melipona quadrifasciata anthidioides*, while two (glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate mutase) were present in the salivary glands of the thorax. Teixeira et al. (2017) identified phosphoglycerate mutase in the Dufour's gland of *Apis mellifera*, suggesting that this occurrence was due to the high metabolism of these glands. Therefore, the presence of these proteins in the venom of *A. pallens* was suggestive of a high metabolic rate of the venom gland.

Dihydropyrimidine dehydrogenase (NADP+) is involved in amino acid biosynthesis (UniProt Database, 2017) and the CD40 ligand is responsible for binding to the tumor necrosis factor receptor and is involved in biological immune response processes

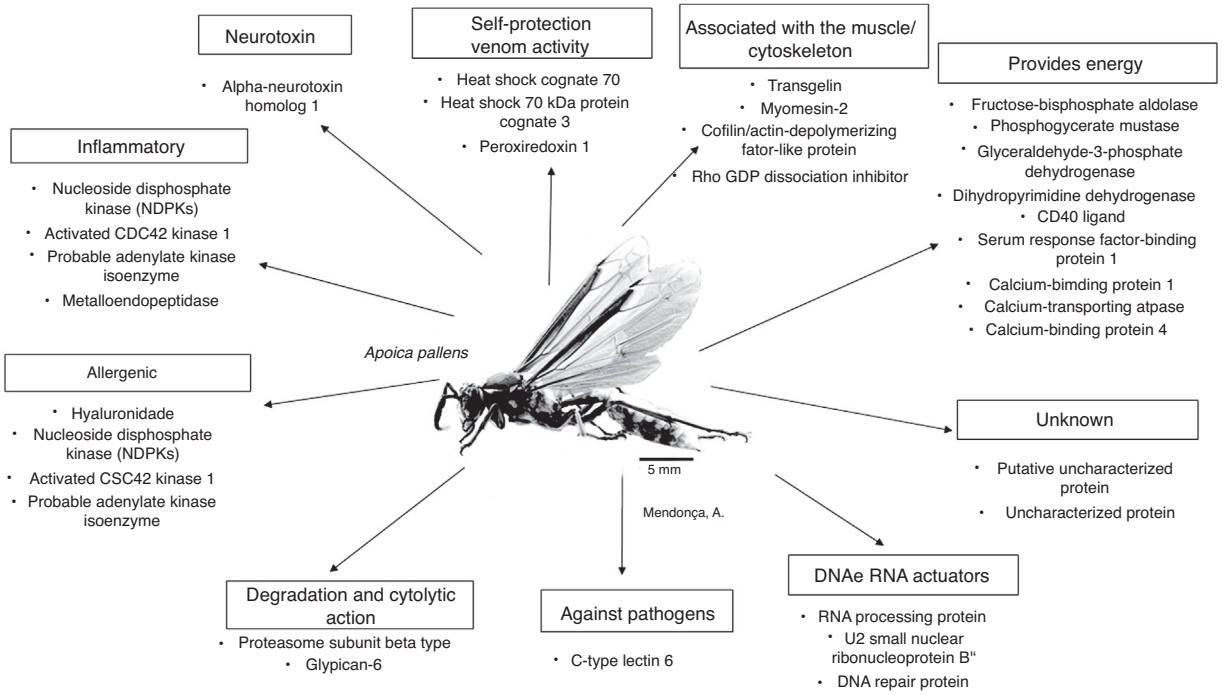

Fig. 4. Identified proteins of social wasp *Apoica pallens* venom and their functions according to the literature.

(UniProt Database, 2017). Serum response factor-binding protein 1, calcium-binding protein 1, calcium-transporting ATPase, and calcium-binding protein 4 are also metabolic proteins that probably originate from venom secreting cells, but do not constitute true toxins of the venom. They perform metabolic functions in venom secretory cells, but have no functional roles in the poisoning process (Santos et al., 2010).

The class with the second highest number of proteins included the enzymatic nucleoside disphosphate kinase (NDPK), activated CDC42 kinase 1, and probable adenylate kinase isoenzyme F38B2.4, which are kinases with allergenic, inflammatory, and immunity activities. The kinase enzymes catalyze the transfer of phosphate among their substrates (Cheng et al., 2011). The NDPKs act in the synthesis of macromolecules (Francois-Moutal et al., 2013), besides presenting activities for cell proliferation, differentiation, and development, signal transduction, G protein coupled receptor, endocytosis, and gene expression. The main role of NDPKs is to maintain the set of different nucleotides within the cell by catalyzing the reversible transfer of the terminal phosphoryl group from a nucleoside triphosphate to a nucleoside diphosphate (Lascu and Gonin, 2000).

Alpha-neurotoxin homolog 1 can be classified as both type I and type II alpha neurotoxin and acts by binding to the nicotinic acetylcholine receptor for postsynaptic block in neuromuscular junction transmission (Oliveira et al., 2000). It is responsible for causing paralysis in the victims and prey, potentially culminating in respiratory failure and death (Wang et al., 2014; Nirthanan and Gwee, 2004). Lauridsen et al. (2017) evaluated the action of *Naja melanoleuca* venom and observed that most of the fractions with alpha neurotoxin I and II induced lethality within 24 h. Hence, the alpha-neurotoxin homolog 1 found in *A. pallens* venom, is probable has a similar function.

C-type lectin 6, which has an enzymatic function and recognizes carbohydrates, is found in a variety of plants and animals (Castanheira et al., 2017). Lectins act in the protection of plants and invertebrates against pathogens, induction (or inhibition) of blood coagulation, stimulation of an immune response, cell-cell adhesion, and cytotoxicity. They are used in cancer therapy and diagnosis (Peumans and Van Damme, 1995; Sartim and Sampaio, 2015; Yau et al., 2015), and studies with snake venoms have demonstrated the effects of lectins in different tumor cell lines (Calderon et al., 2014; Dhananjaya and Sivashankari, 2015). Therefore, *A. pallens* venom containing type C lectin might act against pathogens and tumor cells, although this would need to be confirmed in antimicrobial and antitumor tests.

The beta type proteasome subunit is involved in the proteolytic degradation of most intracellular proteins, playing a key role in maintaining protein homeostasis by removing damaged proteins that could impair cellular functions, as well as proteins whose functions are no longer necessary (UniProt Database, 2017).

Metalloendopeptidase is a protease responsible for moderate necrosis in some tissues (Touchard et al., 2006). Metalloproteinases are found in the venoms of the wasp *P. paulista* (Santos et al., 2010) and the ants *Solenopsis invicta* (Kang et al., 1998) and *Tetramorium bicarinatum* (Hoffman, 2006). In the case of wasps, metalloproteinases are associated with inflammation, necrosis, edema, and skin damage after seizures (Pinto et al., 2012), while in ants they may be involved in disruption of the coagulation cascade (Bouzid et al., 2013).

Transgelin is recognized as a structural protein, although its function has not been fully elucidated (UniProt Database, 2017). It has been associated with functions in the cytoskeleton (Lawson et al., 1997) and could act in muscle contraction (Assinder et al., 2009). It is possible that the presence of this protein in *A. pallens* venom could be associated with contraction of the sting apparatus muscles, rather than with the venom itself. In the *P. paulista* sting apparatus, the calponin protein of the same transgelin family has the function of structuring the musculature of the sting apparatus (Santos et al., 2010). The other structural proteins identified in the *A. pallens* venom, such as myomesin-2, cofilin/actin-depolymerizing factor-like protein, and rho GDP dissociation inhibitor, are also not components of venom and could be associated with the muscle of the sting apparatus.

The proteins heat shock cognate 70 and heat shock 70 kDa protein cognate 3 were identified in the venom of *A. pallens*. These proteins also occur in the venom glands of bees (Peiren et al., 2008) and the *P. paulista* wasp (Santos et al., 2010), acting as chaperones that facilitate the folding of other proteins. Thermal shock proteins also occur in the Dufour's gland of the bee *A. mellifera* (Teixeira et al., 2017). These proteins have roles in cellular protection, anti-apoptosis, development, regulation, and signal transduction (Peiren et al., 2010). According to Snutch et al. (1988) the shock proteins can be expressed according to the different sources of stress, protecting the cells and proteins against molecular damage. Santos et al. (2010) suggested that the exposure of venom toxins to high temperatures, especially in tropical regions, might lead to thermal denaturation and loss of their biological activity, and that the shock proteins in *P. paulista* venom could be important for protection of the venom in order to preserve its activity.

Another protein identified in the *A. pallens* venom, involved in the environmental response that acts according to fluctuations in oxidative and osmotic conditions, thermal shock, and pH variations, is peroxiredoxin 1, which preserves the cellular structure and is therefore involved in protection of the venom against oxidative stress. This protein, which has been identified in the venom of *A. mellifera* (Peiren et al., 2008), also catalyzes the reduction of hydrogen peroxide and organic hydroperoxides to water and alcohols. It may participate in signaling cascades of growth factors and tumor necrosis factor alpha, regulating the intracellular concentrations of hydrogen peroxide (Kang et al., 1998).

In the class of allergenic proteins, the venom of *A. pallens* contained hyaluronidase, which is an enzyme of the glycosyl hydrolase family, found in the venom of *P. paulista* (Santo et al., 2010). According to Hoffmam (2006), this protein acts in dispersion of the venom, causing increased tissue permeability and facilitating the diffusion of toxic substances. Hyaluronidase is commonly found in the venoms of wasps and bees, and in addition to potentiating the effect of toxins, it causes inflammation at the site of the sting (Santos et al., 2010; Kemparaju and Girish, 2006). It also acts as an important cross-reactive allergen (Lu et al., 1995). Hyaluronidase activity is also found in the venoms of the ants *Paraponera clavata*, *Ectatomma tuberculatum* (Schmidt et al., 1986), *Myrmecia pyriformis* (Wanstall and De La Lande, 1974), *Solenopsis invicta* (Baer et al., 1979), *Pseudomyrmex triplarinus* (Hink et al., 1994), and *Pachycondyla striata* (Santos et al., 2017). Touchard et al. (2006) reported that the activity of this protein was higher for wasp venom than for ant venom.

Glypican-6 is a cell surface heparan sulfate proteoglycan that acts as a co-receptor protein and selectively interacts with heparan sulfate glycosaminoglycans, which are potent blocking proteins for the cytotoxic action of venom (Lomonte et al., 1994).

Some proteins identified in the *A. pallens* venom are active in DNA and RNA, such as rRNA-processing protein 7 and U2 small nuclear ribonucleoprotein B, which are repair proteins. These proteins, which act on DNA and RNA, but do not have direct action in the venom, are common in cells, so it is also possible that this protein originated from some type of contamination during the extraction, despite all the care taken. Aili et al. (2016) reported that the method of venom extraction could lead to the possibility of mixing of cellular proteins with the true components of the venom.

In this sense, the venom gland has an ectodermal origin with a muscular-chitinous structure that ends in a venom sac that

opens at the base of the sting apparatus and allows venom release instantaneously with a sting (Abdalla and Cruz-Landim, 2001; Britto and Caetano, 2005). They are constituted by type III cells, according to the Noirot and Quennedey (1974) classification, described as secretory cells with canaliculi responsible for collecting and conducting the secretion until its destination. Thus, it is possible that soluble cellular proteins from the venom reservoir wall and/or convoluted gland present inside the reservoir (Ortiz and Camargo-Mathias, 2006; Schoeters and Johan, 1998) have ruptured during venom extraction contaminating the samples.

Two proteins did not have their function characterized and were classified as putative uncharacterized proteins. The remaining spots had no similarity to any known sequences, indicating the difficulty in identifying the venom proteins of the wasps by homology, due to the absence of a database for these insects.

This is the first protein characterization of the venom of the eusocial wasp *A. pallens*, and since there is little information about the biochemistry of its venom, the proteomic analysis presented here contributes significantly to knowledge of its biology and physiology.

Thus, due to the absence of a database for wasp venom, there is a large number of proteins that do not have known functions and, consequently, are not identifiable, which makes studies with proteomic analysis of venom even more important. Therefore, this study will pave the way for future pharmacological characterization of each compound and antimicrobial potential studies, contributing to a better understanding of the venom action and its biotechnological applications.

Conflicts of interest

The authors declare no conflicts of interest.

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