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Short communication

Analysis of venom sac constituents from the solitary, aculeate wasp *Cerceris rybyensis*

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ABSTRACT

Solitary aculeate wasps are abundant and diverse hymenopteran insects that disable prey using venom. The venom may possess neuromodulation, immunomodulatory, metabolic-modulatory and antimicrobial functions. Venom analysis of transcriptomes and proteomes has been previously performed in social and parasitoid wasp species. We develop methodologies including mass spectrometry-based shotgun proteomics to analyse the protein constituents from venom sacs of the solitary aculeate wasp *Cerceris rybyensis*. The venom sac constituents of *C. rybyensis* are discussed with respect to other wasp species.

1. Introduction

A huge number of insect species use venom to capture prey, deter predators and micro-organisms, or facilitate parasitism or extra-oral digestion (Walker et al., 2018). The bewildering diversity of insects and the multiple evolutionary origins of insect venoms represents an enormous and mostly untapped source of potentially useful therapeutic compounds, including peptides, proteins, alkaloids, and others (Walker et al., 2018). However, compared with vertebrate venoms, very little is known about those produced by insects. These venoms have been found to contain biogenic amines, peptides and proteins and are primarily for defence (Banks and Shipolini, 1986; Nakajima, 1986). These act as toxins, neuromodulators, immunomodulators, metabolic-modulators and antimicrobial agents (Konno et al., 2016).

A potentially rich hunting ground for interesting or novel venoms are the solitary aculeate wasps, of which there are around 20,000 known species (Konno et al., 2016). The ecology of these wasps is fascinating, so much so that they inspired many well-known biologists, such as Jean-Henri Fabre and Nikolaas Tinbergen (Fabre, 1916; Tinbergen, 1932). The prey is used to provision a nest, which vary in their complexity and are stored for different durations to serve as nutrition for the developing larvae. The few studies of solitary wasp venom indicate that in addition to neurotoxins they also contain antimicrobial and cytolytic peptides, bradykinin-related peptides and neuropeptides (Konno et al., 2016). To date, the study of insect venom has been hindered by the difficulty in collecting a sufficient quantity of the venom for analysis (Konno et al., 2016). In this study we exploit state of the art shotgun mass spectrometry-based shotgun proteomic analysis to determine to what depth the venom sac proteome can be defined from the solitary wasp, *C. rybyensis*. This wasp has a palearctic distribution and is a specialist predator of solitary bees (various genera). We selected this species on the basis that its venom is known to quickly paralyse its prey, which are subsequently deposited in the warm, humid, underground nest. Adult female *C. rybyensis* wasps (Fig. 1A) were collected from a site in central England, UK. The nesting sites of the wasps were located and they were caught in flight using a net when returning to their nests with prey. The wasps were kept individually in collecting tubes and stored in a cool box. Later that day,

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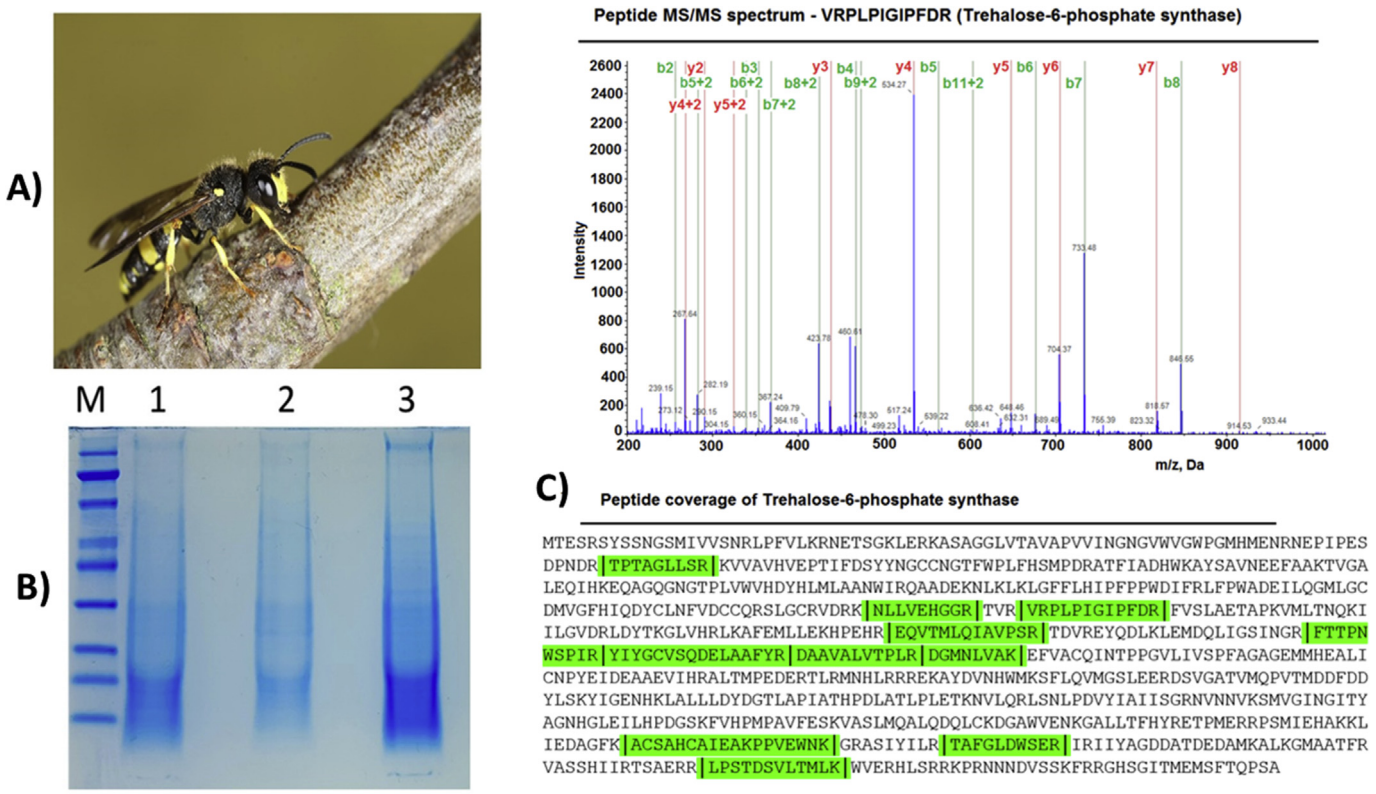


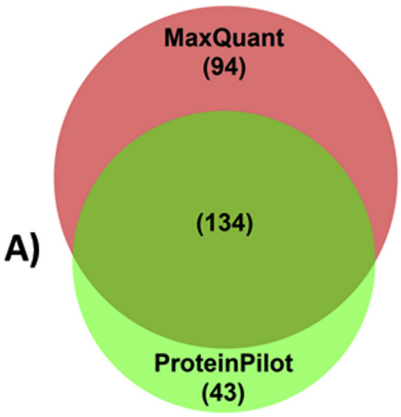
Fig. 1. A) *Cerceris rybyensis* female (Crabronidae: Philanthinae); B) SDS-gel stained with Coomassie Blue to qualitatively estimate the amount of recovered protein in each sample pool; C) A tandem mass spectrum (MS/MS) of high-confidence VRPLPIGIPFDR peptide from trehalose-6-phosphate synthase detected in *Cerceris rybyensis* venom sacs. The figure annotates the most intensive fragment ions corresponding to the peptide and peptide coverage of trehalose-6-phosphate synthase by high confidence tryptic peptides (highlighted in green) identified in the shotgun proteomic screen of *Cerceris rybyensis* venom sacs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the wasps were anaesthetised by placing them in a −20 °C freezer for 5 min. The venom sac was removed from each wasp under a dissecting microscope by gently pulling the sting with fine forceps while the body was held with a second pair of forceps. The extracted venom sacs were pooled into three sets of seven sacs and immediately snap frozen in microfuge tubes before storing at −80 °C. The recovery from the sacs can be seen in Fig. 1B. The amount of protein recovered from each pool was between 280 and 420 µg, which after extrapolation, equated to approximately 40–60 µg of protein per sac. Approximately 25–100 µg of protein is required for a typical shotgun proteomic experiment indicating that, in future, single animal (1 sac) experiments would be tolerated. Shotgun proteomic analysis of FASP-processed tryptic digests was performed with Eksigent Ekspert nanoLC 400 (SCIEX, CA, USA) liquid chromatograph coupled to quadrupole-time-of-flight mass spectrometer TripleTOF 5600+ (SCIEX, Canada) as reported previously (Way et al., 2016). The LC-MS profile of intact crude venom extracts (Supplementary Fig. 1), their annotation and deconvolution was performed using ProteinPilot 4.5 (SCIEX, Canada). Mass spectrometry (MS) and tandem MS (MS/MS) data were searched using the redundant Uniprot + swissprot database (02.2018, 19,479 entries) restricted to Hymenoptera.

An example tandem mass spectrum of a peptide from a key protein we identified is shown in Fig. 1C. This tandem mass spectrum was shown since it contains a novel protein of importance involved in trehalose production (See below). A summary of the total proteins identified using two distinct software packages (ProteinPilot and MaxQuant) that match tandem mass spectra to sequences in a database is shown in Fig. 2A (data available on request). As expected ProteinPilot identified targets not observed with MaxQuant leading us to use the best results, sum of both informatics tools to maximally cover protein discovery. This information was the source of the pathway characterisation described below (Fig. 2B).

Molecular chaperone proteins predominate in the venom sac, including; proteins HSP70 isoforms, HSP90, RuvBL2 (a subunit of the HSP90 containing R2TP chaperone), HSP10, BiP, HSP60, and stress-induced phospho protein-1 (Fig. 2B). Chaperones are generally involved in protein folding, protein assembly, and functional activation of protein pathways. The presence of molecular chaperones in insect venom sacs has been observed previously (Zhu et al., 2010; Colinet et al., 2014; Moreau and Asgari, 2015). Related to the maintenance of conformational integrity of proteins in the venom sac by chaperone is trehalose. Notably, our tandem mass spectra detected the enzyme trehalose-6 phosphate synthetase (Fig. 1C), which catalyses the first step in trehalose synthesis via glucose-6-phosphate. In insects, trehalose is the main storage sugar used to generate glucose for glycolysis and it is gaining interest in insect physiology as it regulates energy metabolism and glucose generation via trehalose catabolism (Nation, 2008; Shukla et al., 2015). Trehalose is known to be present in venom glands (Rivers and Denlinger, 1994a; Parkinson, 2003; Daneels et al., 2010). It has been hypothesised that venom sac trehalase converts the abundant host trehalose sugars into glucose (Rivers and Denlinger, 1994a; Parkinson, 2003), although more recent data do not support this hypothesis (Mrinalini et al., 2014). It is possible that trehalose serves as a protein stabiliser (Kaushik and Bhat, 2003; Liu et al., 2013), preserving the conformational integrity of proteins and peptides synthesised in the venom gland.

Presumably in nature, various adverse situations that protein molecules face in the venom, both within the cell and outside, can lead to protein aggregation and inactivation. This is especially true in species such as *C. rybyensis* that require high temperatures for foraging (Willmer, 1985). The use of trehalose as a natural biosynthetic agent capable of stabilizing protein molecules and helping them retain their



B)

A. Protein Translation	B. RNA splicing	C. GTP binding proteins	D. Biosynthesis machinery	E. Ubiquitination/Proteasome
40S ribosomal proteins	LSM14	Rab7a	V-type proton ATPase subunits	CatherpsinL2
Rps9	DDX39B	Rab1a	Transketolase	PSMC1
Rps5	hnRNPA1	Rab5c	Transaldolase	PSMC2
Rps25	DDX6	Rab2a	Triosphosphate isomerase	PSMC6
Rps16	DDX3X	GDI	Succinate co-A ligase	PSMC5
60S ribosomal proteins	SRSF4	Rab11a	Pyruvate kinase	Ubiquitin conjugating E2
Rpl15		RAN	Pyruvate carboxylase	UBE2
Rpl19		ERF3A	Phosphoglycerate mutase	UBE1
Rpl23		SAR1B	Phosphogylcerate kinase	
Rpl22			Malate dehydrogenase	
Translation initiation factors			Isocitrate dehydrogenase	
eIF4A			Glycerol 3-phosphate dehydrogenase	
eIF3A			Glutamate dehydrogenase	
eIF5A			Enolase	
eIF1A			Citrate synthetase	
Tu			Aconitate hydratase	

Fig. 2. A) Overview of WASP (*Cerceris rybyensis*) proteomics data analysed by MaxQuant and ProteinPilot. Quantitative Venn diagram shows, unique and common proteins between the two types of software. All the proteins were identified by shotgun proteomics approach and the dark green circle (134 proteins) indicates which are commonly identified by MaxQuant and ProteinPilot; B) *Cerceris rybyensis* venom proteins and the biochemical pathways they are involved in. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

functionally active forms under such conditions presumably has an adaptive role; if not a dual role as an energy source. For example, *Ag*-TreT1-silenced mosquitoes exhibit shorter survival under desiccation or elevated temperature (Liu et al., 2013). This latter work by Liu et al. is the first biological evidence that thermal stability can be maintained by trehalose in an animal and we would suggest this function might be important in preserving the bio-activity of venom components.

In conclusion, we report for the first time the venom sac protein constituents of the solitary aculeate wasp *C. rybyensis*. We define the yield of protein that can be recovered per gland of a solitary wasp species and the number of recovered proteins that can be detected by shotgun proteomics using two different types of discovery software. We also performed a detailed analysis of the dominant protein pathways that can be detected from these small samples. Apart from the common pathways observed in other wasp species, we also focus on the chitin biosynthetic pathway, molecular chaperone protein folding pathways, and trehalose biosynthetic pathways as dominating proteomes. We propose a novel function for trehalose production which has previously been suggested to provide a reservoir of glucose for metabolic demands. Emerging evidence suggests that trehalose can also function as an exceptional chemical chaperone and maintain the bioactive conformation of proteins. This is presumably critical for robust venom responses in host-prey interactions of *C. rybyensis.*

Ethical statement

Solitary wasps were the subject of this study, which are not currently covered by legislation on the protection of animals used for scientific purposes.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.toxicon.2019.07.012.

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