Characterisation of Ant Venom Peptides and Proteins

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Certificate of Authorship and Originality

I, Samira Ryma Aili, certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

This research is supported by an Australian Government Research Training Program Scholarship.

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List of Publications

1) **Aili, S. R.,** Touchard, A., Escoubas, P., Padula, M. P., Orivel, J., Dejean, A., & Nicholson, G. M. (2014). Diversity of peptide toxins from stinging ant venoms. *Toxicon*, *92*, 166-178.

2) Touchard, A., **Aili, S. R.,** Fox, E. G. P., Escoubas, P., Orivel, J., Nicholson, G. M., & Dejean, A. (2016). The biochemical toxin arsenal from ant venoms. *Toxins*, *8*(1), 30.

3) Touchard, A., Koh, J., **Aili, S. R.,** Dejean, A., Nicholson, G. M., Orivel, J., & Escoubas, P. (2015). The complexity and structural diversity of ant venom peptidomes is revealed by mass spectrometry profiling. *Rapid Communications in Mass Spectrometry*, *29*(5), 385-396.

4) **Aili, S. R.,** Touchard, A., Koh, J. M., Dejean, A., Orivel, J., Padula, M. P., & Nicholson, G. M. (2016). Comparisons of protein and peptide complexity in poneroid and formicoid ant venoms. *Journal of proteome research*, *15*(9), 3039-3054.

5) **Aili, S. R.,** Touchard, A., Petitclerc, F., Dejean, A., Orivel, J., Padula, M. P., & Nicholson, G. M. (2017). Combined peptidomic and proteomic analysis of electrically stimulated and manually dissected venom from the South American bullet ant *Paraponera clavata*. *Journal of proteome research*, *16*(3), 1339-1351.

Conference Proceedings

Venoms to Drugs Conference, Noosa, QLD, Australia 2017
Oral Presentation
"An integrated proteomic and transcriptomic analysis of venom gland toxins from the bullet ant *Paraponera clavata*"

TEDx Youth Sydney 2017
Oral Presentation, fast ideas session
Ant venom to the rescue

AMP Amplify Ignite PhD pitch competition 2017
Oral presentation
"Ants: the solution to world hunger"

XII Congress of the Pan American Section of the International Society on Toxinology, Miami, USA 2016
Oral Presentation
"Ant venom as a novel source of bioinsecticide leads"

Sydney Protein Group Thompson Prize finalist, Sydney 2016
Oral Presentation
"Ant venom as a source of bioinsecticides"

The 21st Lorne Proteomics Symposium, Lorne, Victoria 2016
Poster presentation
"Peptidomic and proteomic comparison of electrically stimulated and manually dissected venom of the bullet ant *Paraponera clavata*"

University of Technology Sydney 3-minute thesis competition 2015
Oral presentation
"Ant venom derived insecticides"

The 18th world congress of the International Society on Toxinology, Oxford, United Kingdom 2015
Oral presentation
"Ant venom as a source of bioinsecticide and antimicrobial drug leads"

The 20th Lorne Proteomics Symposium, Lorne, Victoria 2015
Lightning talk and oral presentation: awarded best poster prize
"Characterisation of the peptide and protein content of ant venoms for use as bioinsecticide and antimicrobial leads"

New horizons 31st Combined Health Science Conference, Kolling Institute RNSH 2014
Poster presentation
"Characterisation of the Antimicrobial and Insecticidal Peptides From Ant Venoms"

APAF 2nd Proteomics and Beyond Symposium, Sydney 2014
Oral presentation
"Characterisation of Poneroid and Formicoid ant venoms"

The 19th Lorne Proteomics Symposium, Lorne, Victoria 2014
Lightning talk and oral presentation: awarded best poster prize
"Characterising the peptide and protein diversity of Neotropical and Australian ant venoms and the identification of novel peptide toxins for bioinsecticide discovery"

New horizons 30th Combined Health Science Conference, Kolling Institute RNSH 2013
Oral presentation
"Characterising the diversity of Neotropical and Australian ant venoms: Novel peptide libraries for bioinsecticide discovery"

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Abbreviations

1,5-DAN	1,5-Diaminonaphtalene
1D-SDS-PAGE	One dimensional - sodium dodecyl sulphate - polyacrylamide gel electrophoresis
2D-SDS-PAGE	Two dimensional - sodium dodecyl sulphate - polyacrylamide gel electrophoresis
AA	Amino acid
AChE	Acetylcholinesterase
ACN	Acetonitrile
BCA	Bicinchoninic assay
BSA	Bovine serum albumin
Bt	*Bacillus thuringiensis*
BLAST	Basic local alignment search tool
CHCA	α-Cyano-4-hydroxycinnamic acid
CID	Collision induced dissociation
cDNA	Complementary deoxyribonucleic acid
Cys	Cysteine
DDT	Dichlorodiphenyltrichloroethane
DTT	Dithiothreitol
DNA	Deoxyribonucleic acid
EPA	Environmental Protection Agency
ESI-MS	Electrospray ionisation mass spectrometry
FA	Ferulic acid
FAO	Food and Agriculture Organisation
GABA	γ-Aminobutyric acid
GM	Genetically modified
Gln	Glutamine
Glu	Glutamic Acid

IEF	Isoelectric focussing
IPG	Immobilised pH gradient
ICK	Inhibitor cysteine knot
KD_{50}	Median knockdown dose
kDa	Kilodaltons
LD_{50}	Median lethal dose
LC-MS/MS	Liquid chromatography tandem mass spectrometry
m/*z*	Mass-to-charge ratio
NanoESI-QTOF MS	Nano electrospray ionisation quadrupole time-of-flight mass spectrometry
Lys	Lysine
MALDI-TOF MS	Matrix-assisted laser-desorption ionization/time-of-flight mass spectrometry
mRNA	Messenger ribonucleic acid
MS	Mass spectrometry
NIS	Normal insect saline
PAGE	Polyacrylamide gel electrophoresis
PI	Isoelectric point
PLA_2	Phospholipase A_2
RP-HPLC	Reversed-phase high-performance liquid chromatography
RPM	Revolutions per minute
SCX	Strong cation exchange
Ser	Serine
SDS	Sodium dodecyl sulphate
TFA	Trifluoroacetic acid
WHO	World Health Organisation

Abstract

Venom peptides are currently being developed as novel therapeutics and bioinsecticides. Given that ants use their venoms for predation and defence against insects, and other organisms, they are a potential source of these peptides. Although ants represent one of the largest groups of venomous animals, little is known about their venom composition. The present study therefore investigated the peptidome, proteome and transcriptome of a range of poneroid and formicoid ant venoms.

Initial experiments sought to confirm the insecticidal and antibacterial activity of whole ant venom using house crickets and minimum inhibitory concentration assays, respectively. Several ant venoms showed significant paralytic and insecticidal activity and others showed antibacterial activity peptides confirming the utility in studying ant venoms.

Subsequent experiments investigated the difference in venom composition obtained using differing venom collection methods: manual venom gland dissection or electrical stimulation. The peptide and protein components of the bullet ant (*Paraponera clavata*) were compared and revealed numerous proteins of which 96 could be assigned a biological function, and 70% of which were common to both collection methods. However, the peptidomic analysis revealed over 300 peptides of which only 30% were common to both collection methods. Therefore, each method reveals a unique set of peptides and proteins.

The peptide components of six different ant species were also characterised. The venoms were found to contain between 132–1032 peptides, but the large number of undescribed proteins and peptides highlighted the need for a transcriptomic investigation. Accordingly, an integrated approach using a combination of shotgun proteomics in parallel with Illumina sequencing of the venom gland transcriptome was used to identify toxins in the venom of *P. clavata*. A BLASTx search of the assembled contigs revealed 354 proteins with homology to existing toxins. Alignments of some of these toxins revealed novel insights into their role in ant venom.

A Tox|Note analysis revealed several predicted novel peptide toxins, with some conforming to the conotoxin cysteine frameworks VI/VII and framework I, both of which have yielded therapeutic drug and bioinsecticide leads. The translated transcriptome was then used as a database to query the MS/MS data obtained from the shotgun experiment which identified 44 toxins. Several of these were not identified in the transcriptome BLASTx search, such as δ-

paraponeritoxin-Pc1e (formerly poneratoxin). These results reveal the advantages of combining proteomic and transcriptomic methods, and further demonstrates the richness and diversity of ant venoms as potential sources of bioactive compounds.