Identification of Pheromones from Entomopathogenic Nematodes

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Introduction

Entomopathogenic nematodes, such as *Heterorhabditis spp.* and *Steinernema spp.*, are lethal parasites of insects and have shown promise as biological control agents.[1] These nematodes survive in the soil as stress-resistant infective juveniles (IJs) that seek out and infect insect hosts. Upon sensing internal host cues, the IJ regurgitates a bacterial pathogen from its gut that ultimately kills the host.[2] Inside the host, the nematode develops into a reproductive adult and reproduces until unknown cues ultimately trigger IJ formation. In past work, we have shown that *Heterorhabditis bacteriophora* secretes an ascaroside pheromone to trigger IJ formation.[3] In our current work, we are isolating and characterizing the structures of pheromones produced by *Steinernema carpocapsae*. In the long-term, we would like to study cross-species specificity of these pheromones.

Experimental

dqf-COSY, HSQC, HMBC, and NOESY experiments were performed on the AMRIS Bruker Avance 600 MHz spectrometer with a 5mm cryoprobe on purified secreted secondary metabolites from *S. carpocapsae*.

Results and Discussion

We have purified IJ pheromone molecules from *S. carpocapsae*. The nematode was cultivated on nutrient agar with its bacterial symbiont until IJs developed. Crude pheromone was obtained by extracting the nutrient agar with organic solvent. We fractionated the crude pheromone using solvent partitioning, followed by C_{18} chromatography, then silica gel chromatography, and then HPLC. At each fractionation step, we followed the activity of the pheromone using a bioassay (IJ recovery assay). Additionally, using LC-MS/MS, we determined whether each fraction contained known ascarosides and structurally related novel compounds (Fig. 1). We purified and characterized these structurally related novel compounds using NMR spectroscopy (dqf-COSY, HSQC, HMBC, and NOESY experiments). We have fully eludicated their structures and shown that they are novel glucosides. Based in part on this preliminary data, we have recently been awarded (with PI S. Patricia Stock, Univ. of Arizona, and co-PI Fatma Kaplan) a USDA NIFA grant, entitled "Role of *Xenorhabdus* bacteria on pheromone production by *Steinernema* nematodes: Impact on nematode fitness and formulation."

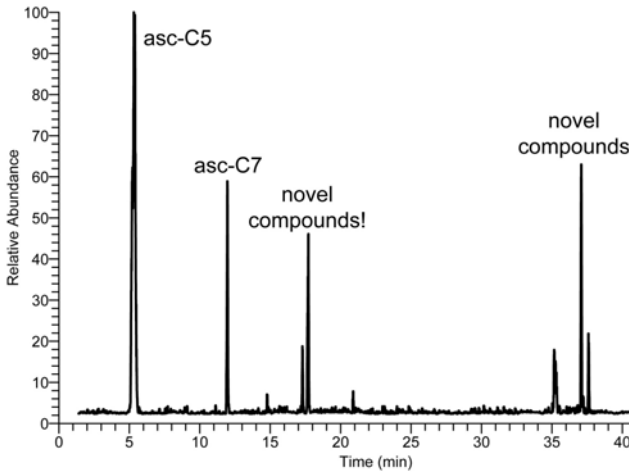

Figure 1. Known ascarosides (asc-C5, asc-C7) and novel ascarosides and other types of compounds in an active pheromone fraction.

Conclusions

We have elucidated the structures of two novel glucosides, the structures of which will be published in the coming year. We are now synthesizing these novel glucosides to confirm their structures. In addition, we are continuing to purify novel ascarosides and other types of compounds from active pheromone fractions and are characterizing them using 2D NMR spectroscopy.

Acknowledgements

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References

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[3] Noguez, J.H., *et al.*, ACS Chem Biol, **7**, 961-966 (2012).