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.[1,2] 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.[3] 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.[4] In our current work, we are isolating and characterizing the structures of additional pheromones produced by *H. bacteriophora*, as well as pheromones from *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 *H. bacteriophora* and *S. carpocapsae*.

Results and Discussion

We have purified IJ pheromone molecules from *S. carpocapsae*. The nematode was cultivated on nutrient agar spotted with its bacterial symbiont *Xenorhabdus nematophila* until IJs developed. We obtained crude pheromone 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 an IJ recovery assay. Additionally, using LC-MS/MS, we determined whether each fraction contained ascarosides. We have purified several ascaroside components of the IJ pheromone from *S. carpocapsae*. Several of these are ascarosides previously identified in other nematode species (**Fig. 1**). Others appear to be similar to known ascarosides but with novel modifications. To characterize these pheromone components, we have performed dqf-COSY, HSQC, HMBC, and NOESY experiments and are currently analyzing these data. In addition, we are in the process of purifying additional components of the *H. bacteriophora* IJ pheromone from previously unexplored fractions of the crude pheromone.

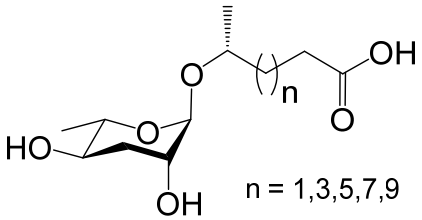

Figure 1. Ascarosides secreted *by S. carpocapsae* to induce IJ formation.

Conclusions

Once we have identified the structures of the novel components of the *S. carpocapsae* IJ pheromone, we will synthesize the molecules in order to verify our structural assignments and to perform further biological characterization.

Acknowledgements

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References

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