Identification of Pheromones from Entomopathogenic Nematodes

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Introduction

Entomopathogenic nematodes, such as *Heterorhabditis bacteriophora* and *Steinernema carpocapsae*, 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 *H. bacteriophora* secretes an ascaroside pheromone to trigger IJ formation (**Figure 1**). In our current work, we are isolating and characterizing the structures of additional pheromones produced by *H. bacteriophora*, as well as pheromones from *S. carpocapsae*. In the long-term, we would like to study cross-species specificity of these pheromones.

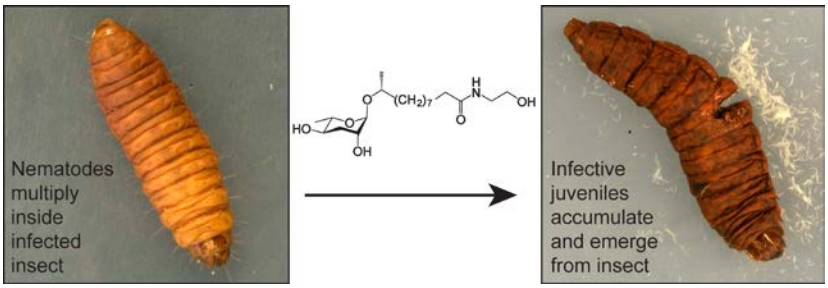

Figure 1. Ascaroside pheromone that induces IJ accumulation

Experimental

dqf-COSY, HSQC, and HMBC experiments were performed on a Bruker Advance 600 MHz NMR with a 1 mm microcryoprobe.

Results and Discussion

Using activity-guided fractionation, we have purified several additional components of the IJ pheromone from *H. bacteriophora*. Several of these appear to be similar to the previously identified pheromone but with different side-chain lengths. One appears to be similar to the previously identified pheromone, but contains an indole moiety. To characterize this structurally novel ascaroside, we have performed dqf-COSY, HSQC, and HMBC experiments and are currently analyzing this data.

Concurrently, we are purifying IJ pheromone molecules from *S. carpocapsae*. The nematode was cultivated on nutrient agar spotted with *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, and then silica gel chromatography. At each fractionation step, we followed the activity of the pheromone using an IJ recovery assay. Once active compounds are purified, we will perform NMR experiments to characterize their structures.

Conclusions

We are currently analyzing the NMR data for a novel ascaroside from *H. bacteriophora*. Once we have identified the structure, we will synthesize the molecule to verify our structural assignment and to perform further biological characterization of the molecule.

Acknowledgements

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References

[1] Ciche, T., WormBook, 1-9 (2007).
[2] Hallem, E. A., *et al.*, Curr Biol, **17**, 898-904 (2007).
[3] Ciche, T. A., *et al.*, Appl Environ Microbiol, **74**, 2275-2287 (2008).