Identification of Novel Pheromones from *Caenorhabditis elegans* using Comparative Metabolomics and Multi-dimensional NMR Spectroscopy

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Introduction

The nematode *C. elegans* secretes pheromones into its environment and uses them in chemical communication. The ascaroside pheromones are derivatives of the 3,6-dideoxysugar ascarylose, modified with different fatty acid-derived side chains. *C. elegans* uses specific ascarosides, which are together known as the dauer pheromone, to trigger entry into the stress-resistant dauer larval stage.[1] In addition, *C. elegans* uses specific ascarosides to control certain behaviors, including mating attraction, aggregation, and avoidance.[2, 3] In this project, we are using LC-MS-based metabolomics to identify compounds that are present in wild-type worm extracts, but missing in the extracts of ascaroside biosynthetic mutants. We are then purifying novel ascarosides and using NMR spectroscopy to elucidate their chemical structures.

Experimental

dqf-COSY, HSQC, and HMBC experiments were performed on the AMRIS Bruker Avance 600 MHz NMR with a 5 mm cryoprobe.

Results and Discussion

In order to identify novel ascarosides produced by *C. elegans*, we have pursued a metabolomic approach in which we have compared the metabolites produced by wild-type worms and mutant worms (*daf-22*)[4], which do not produce short-chain ascarosides, using LC-MS. We have focused on several fractions from wild-type worms containing candidate novel ascarosides and purified these ascarosides. dqf-COSY, HSQC, and HMBC experiments enabled the chemical structure of one of these ascarosides to be elucidated (**Fig. 1A**). We are further purifying additional compounds for characterization by NMR spectroscopy (**Fig. 1B**).

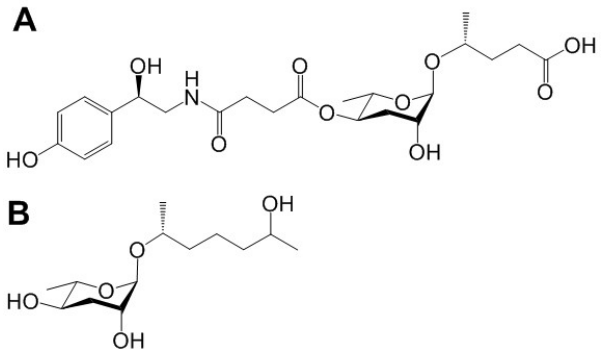

Figure 1. Structures of novel ascarosides. (**A**) One of the ascarosides identified using comparative metabolomics and NMR spectroscopy. (**B**) Example of a proposed structure for a novel ascaroside that is currently being confirmed through purification and NMR spectroscopy.

Recently, we have started to do metabolomic comparisons between wild-type worms and mutants in the biosynthetic pathways for other types of metabolites. These comparisons have enabled us to focus on several candidate masses for novel compounds. We have successfully obtained ^{1}H and dqf-COSY spectra for these compounds and are now scaling up our purification to obtain enough material for 2D NMR experiments.

Conclusions

Our results suggest that LC-MS-based comparative metabolomics, followed by NMR characterization of purified compounds, is a viable approach for the identification of novel secondary metabolites in *C. elegans*. Further studies will be required to identify the biological function of molecules identified using this method.

Acknowledgements

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References

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