**EVALUATIONAL METABOLOMICS IN TOLYPOCLADIUM FUNGI TO GUIDE NATURAL PRODUCTS DISCOVERY**

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**ABSTRACT**

The fungal genus *Tolypocladium* contains several species known to produce biologically active peptide, polyketide, and alkaloid secondary metabolites (SMs). Ongoing genomic analyses of 14 different strains in *T*. *spatula* lab indicate *Tolypocladium* as a potentially rich source of new SMs of various classes. LC/MS-based metabolomics tools were used to survey the secondary metabolites of 11 *Tolypocladium* species, guide the discovery of new SMs, and contribute to the understanding of the evolution of SMs in *Tolypocladium*. Peptide tandem mass spectrometry data evinced several unique peptidic metabolites in the extracts of most species analyzed. Unique amino acid losses in the tandem mass spectra suggest that many of these metabolites are likely to be members of the peptidic family of non-ribosomal peptides. These and other peptidic metabolites are being targeted for isolation, structure elucidation, and pairing with their biosynthetic gene clusters (BGCs), as well as biological characterization.

**INTRODUCTION**

Ecological Diversification in Order Hypocreales

**Phylogenetic Analysis**

*Hypocreales* is comprised of seven major clades, uniquely defined by their ecologies. Knowledge of the phylogeny and evolution of ecologies in *Hypocreales* provides a predictive framework for natural product drug discovery. Shifts in nutritional mode are associated with diversification of secondary metabolism.

**Secondary Metabolites Facilitate Nutritional Mode**

- Can tracing ecological diversification guide the discovery of novel NPs with specific biological activities?
- Can secondary metabolomic profiling inform the evolutionary biology of *Tolypocladium*?

**RESULTS**

**Molecular Networking for Natural Product Discovery**

- 14 strains from 11 spp.
- 12 media conditions in triplicate (total = 1512 fractions)
- 3 fractions/sample
- Compound prioritization, isolation, structure elucidation, biological characterization

**Pursuit of New Peptaibiotics**

*Peptaibiotics from *Tolypocladium*

A new peptide in the MeOH fraction of *T*. *cylindrosporum*. Molecular networking (Figure 5D) revealed its presence in four *Tolypocladium* spp. Tandem MS shows α-aminoisobutyric acid (Aib) monomers, evident from losses of 85 amu. (Aib) is characteristic of peptaibiotics, linear peptides that contain several non-proteinogenic amino acids and function as antibiotic and antifungal agents. Efrapeptides are a family of peptaibiotics previously isolated from *Tolypocladium* spp. Figure reproduced from Hayakawa et al.

**ACKNOWLEDGEMENTS**

Undergraduate workers and members of the McPhail and Spatafora Labs are gratefully acknowledged for fungal extraction and fractionation assistance. Funding is provided by National Science Foundation grant number DEB1354944.

**REFERENCES**