^{13}C-Enriched Global Metabolomics Approach Using Inadequate

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Introduction

Usually with NMR-based metabolomics, 1D and 2D ^{1}H-detected methods are used and though useful, 1D ^{1}H NMR of mixtures can suffer from heavy peak overlap as well as problems with signals that arise from solvents. The limited use of ^{13}C-detected methods stems from the difficulty of acquiring spectra due to the low sensitivity of ^{13}C at natural abundance. 2D techniques such as ^{13}C-^{13}C 2D-INADEQUATE mixture analyses are made possible with enrichment. We are developing a method for analyzing 2D-INADEQUATE data using the ^{13}C-enriched exo- and endometabolomes of *C. elegans* under stressful conditions.

Experimental

Concentrated ^{13}C labeled worm endo- and exudates were placed in 1.5 mm tubes. Using the 1.5 mm cold probe at the Amris facility at the University of Florida, we collected INADEQUATE spectra of the endo- and exometabolomes. Exometabolome data were collected with a 3 second relaxation delay, 2048 t1 increments, 0.134s acquisition time, and 4 scans. This experiment took approximately 14 hours. We wrote a script called INADEQUATE NETwork Analyzer (INETA) in order to annotate our INADEQUATE data in an automated fashion.

Results and Discussion

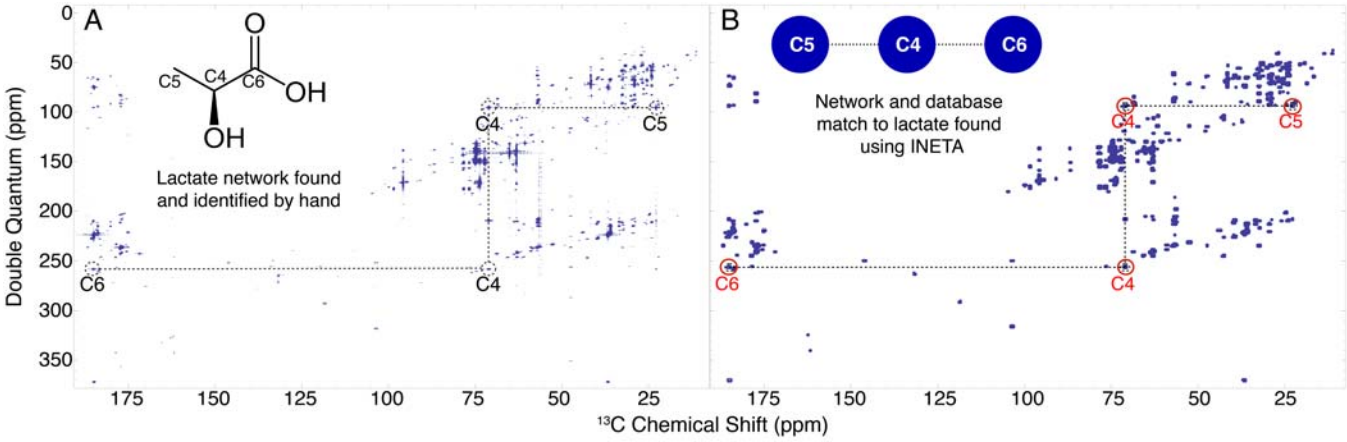

Figure A displays a network that was identified by hand and Figure B displays the network outputted and identified by INETA. A red circle above the carbon numbers in Figure B indicates the area in which the peak for lactate should be found. The steps to INETA include peak picking, network finding, and matching to database that was computationally derived from 1D ^{13}C spectra from the BMRB. We were able to annotate loadings spectra from PCA of the endo and exometabolome to determine the differences in the metabolites between the exo and the endometabolome.

Conclusions

We were able to successfully label *C. elegans* and collect INADEQUATE spectra of *C. elegans* endo and exometabolites. We can see clear differences between the endo and exometabolome particularly in the sugar regions and chemical shifts indicative of unsaturated fatty acids exist. We have successfully developed an automated means of metabolite identification in INADEQUATE spectra of mixtures.

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References

[1] Ramaswamy,V. *et al.* eMagRes. 2013