

Quorum: untargeted metabolomics using tandem mass spectrometry and molecular networking

Anand Patel¹, Stefano Bonissone¹, Natalie Castellana¹

¹Digital Proteomics LLC, La Jolla, CA



INTRODUCTION

Discovery of unknown metabolites is challenging, but fast and highly accurate mass spectrometers are enabling more comprehensive analysis of metabolomic samples. In a typical tandem mass spectrometry experiment, metabolites from a crude sample are:

1. separated by liquid chromatography (retention time)
2. ionized and the mass-to-charge ratio of metabolites is measured (MS1)
3. the metabolites undergo dissociation in a collision cell and the mass-to-charge ratio of fragments is measured (MS2)

To identify metabolites, the MS1 mass or fingerprint of MS2 masses are matched against spectra from a library of known metabolites. **In a recent study, Wang et al. found that less than 2% of experimental MS2 spectra contributed to the GNPS resource can be annotated by spectrum library search.**

INFERENCE FROM MOLECULAR NETWORKS

Figure: Represent each compound with an MS2 spectrum as a node.

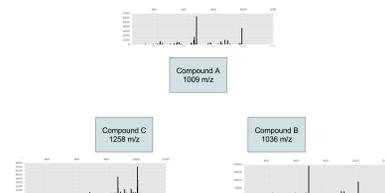


Figure: Pairs of MS2 spectra can be compared for matching peaks. Cosine score is used to measure similarity.

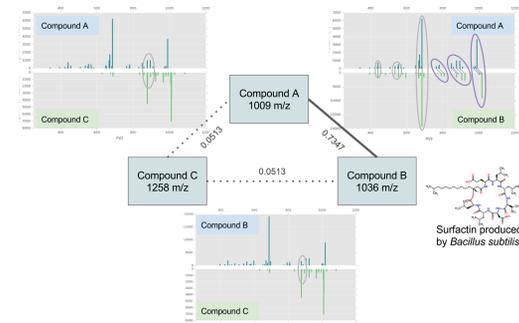
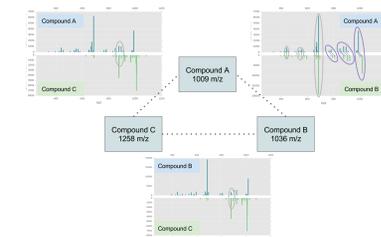


Figure: Only Compound B matches exactly to a Surfactin library spectrum. A high scoring relationship shows Compound A is likely an analog of Surfactin.

DATASETS:

Title	Samples	MS Analysis	Data
Indexing of specialized metabolites from <i>Pseudomonas</i> strains	Cultured 260 strains from around the world and different environments, and an additional 370 wheat-associated strains from the UK.	electrospray ionization (ESI) source in positive ion mode on Bruker Daltonics microOTOF-Q II	MassIVE (UCSD) MSV000079450
Identification of metabolites to stratify Cystic Fibrosis (CF) patients from non-Cystic Fibrosis patients	7 CF and 6 non-CF sputa by direct untargeted LC-MS/MS, microbiome sequence profiling, and culturing <i>Pseudomonas aeruginosa</i>	ESI source in positive ion mode on Bruker Daltonics Maxis qTOF	MassIVE (UCSD) MSV000078586
Identification of gestation associated metabolites	Maternal plasma of 180 women between months 3-8 of their pregnancy	ESI in positive and negative ion mode on Thermo Fisher LTQ Orbitrap Velos	MetaboLights (EMBL-EBI) MTBLS146
Metabolism of anti-hypertensive drugs	Urine samples from 26 elderly patients diagnosed with hypertension	ESI in positive negative combined fragmentation mode on a Thermo Fisher Q-Exactive Orbitrap	MetaboLights (EMBL-EBI) MTBLS307

MS/MS DATA ANALYSIS

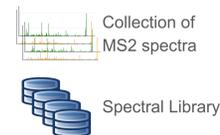


Natural Product Discovery

Biomarker Discovery

Pharmacokinetics

Input:



Analysis:

1. Cluster identical spectra
2. Match spectra against library
3. Construct network
4. Statistical analysis

Output:



NATURAL PRODUCT DISCOVERY

Natural products observed from *Pseudomonas* strains Microbes are bountiful sources of natural products with diverse chemical activities, including anti-infective and anti-fungal agents used to fight both plant and human infectious diseases.

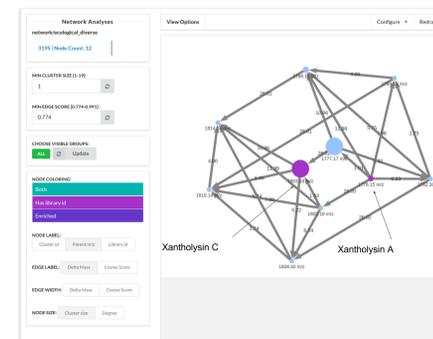


Figure: Shadow network of Xantholysin A and C from *Pseudomonad* strains

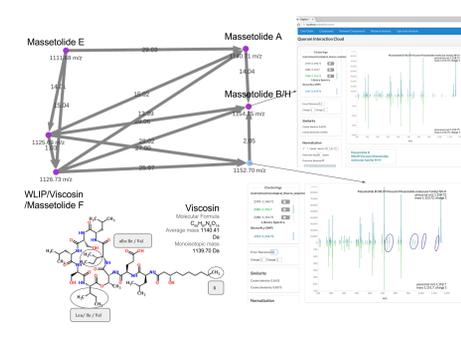


Figure: Network component of Massetolides from *Pseudomonad* strains

Pseudomonas strains are found in diverse environments produce diverse specialized metabolites contributing to encouraging growth or toxicity and pathogenicity to plants or animals depending on the environment. Nguyen et al. (2016) discovered a new poaeamide analogue, and a new subfamily of cyclic lipopeptides, the bananamides.

REFERENCES

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- Nguyen, D. D., et al. (2016). Indexing the *Pseudomonas* specialized metabolome enabled the discovery of poaeamide B and the bananamides. *Nature Microbiology*, 2, 16197.
- Quinn, R. A., (2016). Microbial, host and xenobiotic diversity in the cystic fibrosis sputum metabolome. *The ISME Journal*, 10(6), 14831498.
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BIOMARKER DISCOVERY

Case-control screening of Cystic Fibrosis (CF) sputa. Networking on CF patients shows the structurally similar sphingolipids enriched. Sphingolipid homeostasis is typically lost in CF patients, have been proposed as pharmacological targets to reduce inflammation, and recover ability to fight infection.

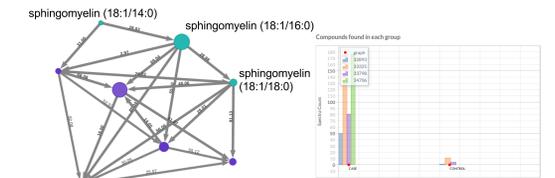


Figure: Sphingolipid cluster with 3 annotated sphingomyelins (green) and 5 unannotated nodes (purple). Every compound is significantly enriched in the CF sputa versus control.

Screening maternal plasma of healthy mothers. Networking on 180 healthy mothers groups carnitines. 4 carnitines have matches to library spectra (green). Annotations for remaining nodes are inferred matching to other known carnitines.

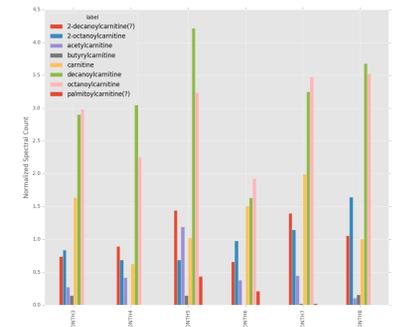
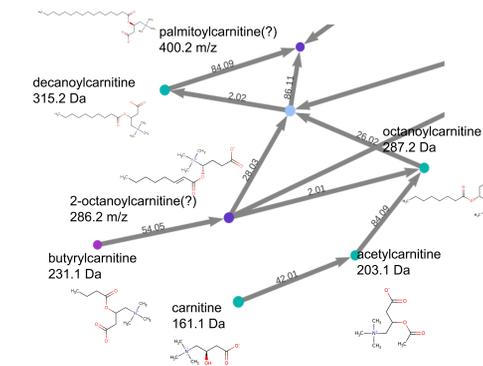


Figure: Spectral counts are misleading for quantifying metabolite abundance. Extracted ion current shows long-chain acyl-carnitines are elevated in third trimester, while short-chain acyl-carnitines peak at the second trimester and decrease going into late stage

Figure: Carnitines are involved in transporting fatty acids across the mitochondrial membrane. Free carnitine binds to fatty acids producing acyl-carnitines with varying lengths.

PHARMACOKINETICS

Measuring drug metabolism from urine. Networking on urine samples from elderly patients with hypertension revealed presence of commonly used drugs to treat hypertension and heart disease: losartan, irbesartan, and candesartan.

- Irbesartan circulating in plasma:
- 80% of irbesartan is unchanged
- 6% inactive irbesartan glucuronide conjugate
- 14% to oxidative metabolites.

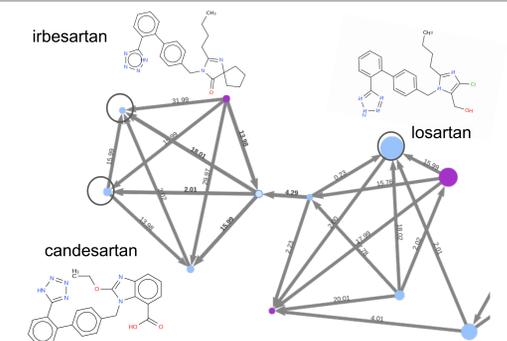


Figure: 3 members of the sartan family of drugs were annotated by library search (purple). Oxidative metabolites (circled) did not match the library.