

Creating MALDI-TOF MS Software for Microbial Drug Discovery and Culturomics Characterization

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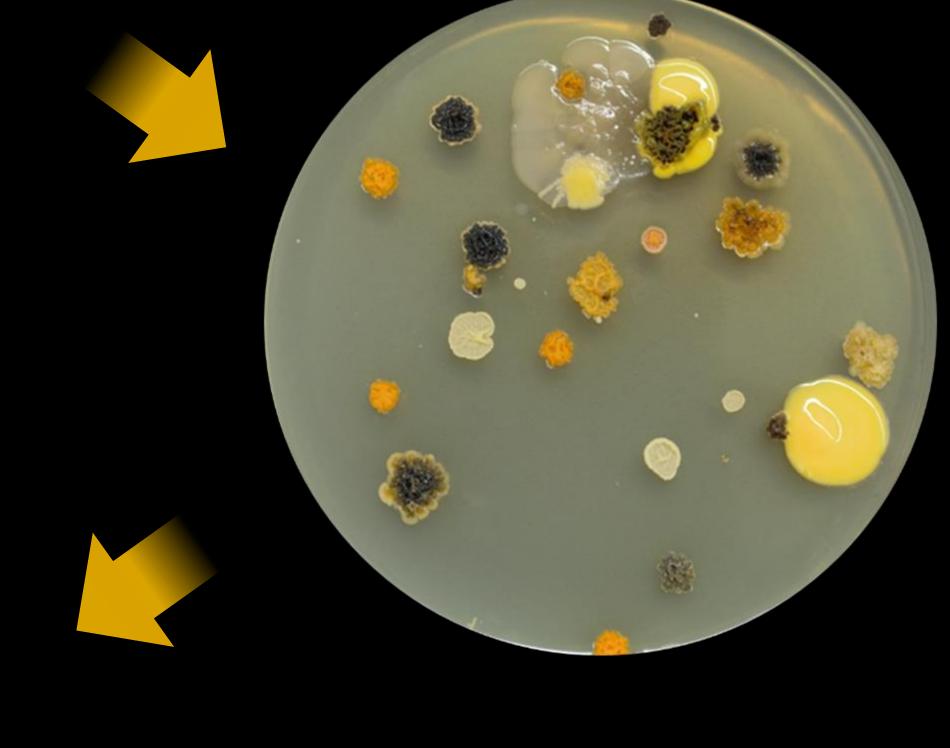


Sample Collection

Finding new microbial natural products begins with sample collection. Our lab has collected sediment and marine organisms from across the globe, including Iceland (shown here), the Great Lakes, and Vietnam.

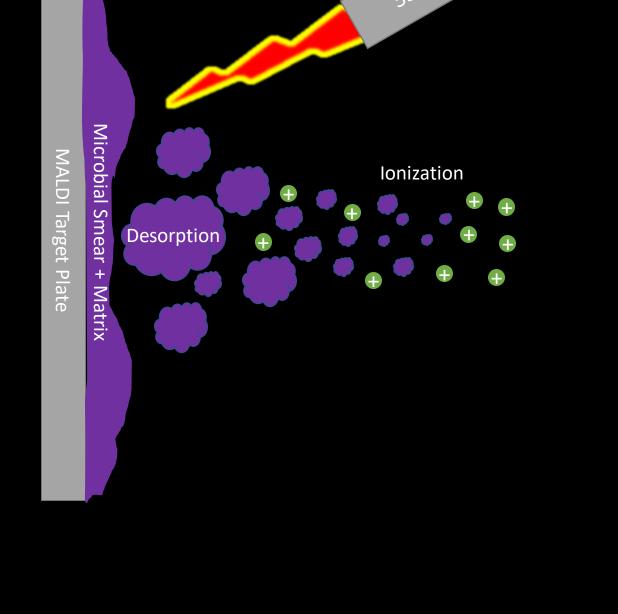


Back in lab, sediment, sponges, nudibranchs, etc. are processed and plated onto agar nutrient media promoting the growth and later isolation of microorganisms. A typical expedition generates 100's of plates with 0-100's of colonies.



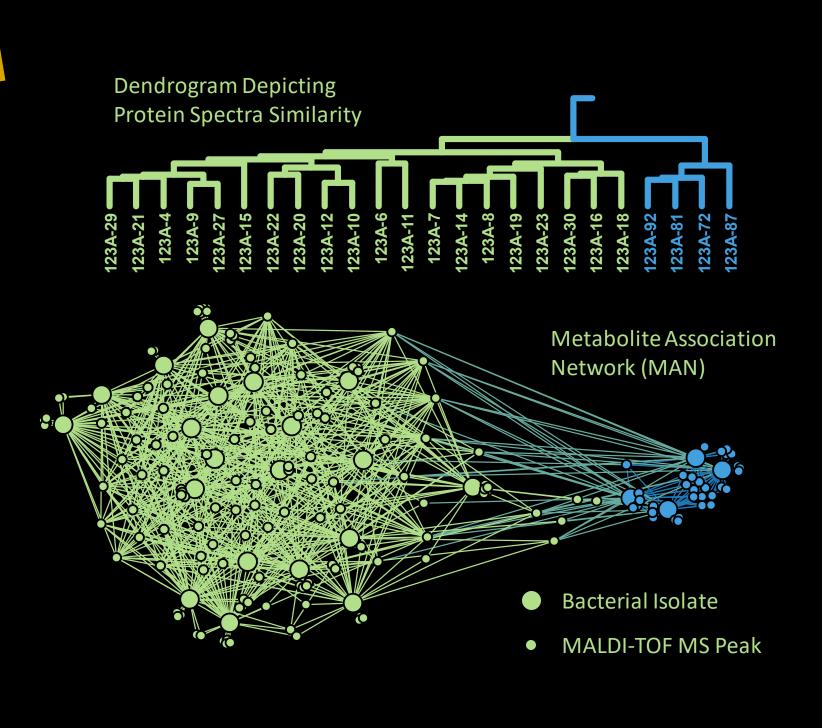
Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry

MALDI-TOF MS is a soft ionization technique that, with easy sample preparation, allows us to gather MS data on "protein" (2-20 kDa) and "small molecule" (0.2-2 kDa) mass regions.

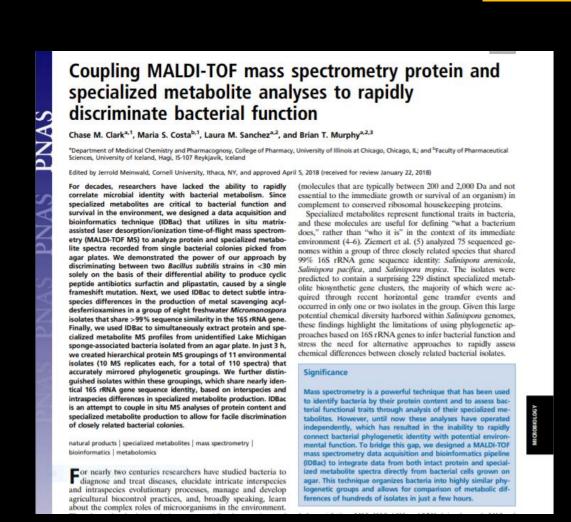


IDBac Software

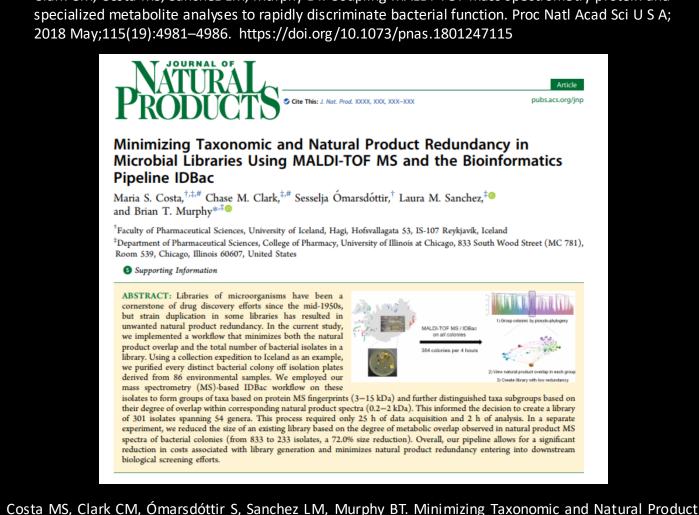
IDBac was originally developed as a GUI (R/Shiny) to help users analyze typical IDBac MALDI-TOF MS experiments that often generate thousands of protein and small molecule spectra. It is also an R package that was recently redesigned for easier programmatic use. For every IDBac "experiment" a SQLite database is created that contains metadata, mzML files, processed spectra and peaks- all contained within a single, shareable file.



IDBac Publications



Clark CM, Costa MS, Sanchez LM, Murphy BT. Coupling MALDI-TOF mass spectrometry protein and specialized metabolite analyses to rapidly discriminate bacterial function. Proc Natl Acad Sci U S A;



KEYWORDS MALDI-TOF MS, bioinformatics, dereplication, microbial ecology rapid and accurate techniques to differentiate, identify, and prioritize culturable microbial isolates. One such technique that continues to gain momentum among to rapidly identify microbial taxa and differentiate culturable microbes. This technology has become commonplace in clinical and veterinary laboratories where rigorousl validated methods are used in conjunction with commercially available reference databases to identify pathogenic microorganisms. However, the broader community especially laboratories working with environmental microbes, typically cannot access the expensive software and databases. It is our opinion that this community, which relies on free and open-source software, currently lacks a coherent set of accepted experimental practices, including employment of internal standard strains, statisticall driven determination of biological and technical replicates, and deposition of MS dat into open-access repositories. Establishing guidelines would enable researchers to better compare microbial typing methods and advance our ability to group and delineate environmental isolates in an effective manner, particularly at the subspecie Clark CM, Murphy BT, Sanchez LM. A Call to Action: the Need for Standardization in Developing Open-Source Mass Spectrometry-Based Methods for Microbial Subspecies Discrimination. mSystems. 2020;5(1):e00813-19. https://doi.org/ 10.1128/mSystems.00813-19 Search 10,747 video articles Advanced Q BIOPHARMA NEW ABOUT JoVE FOR LIBRA ABSTRACT INTRODUCTION PROTOCOL RESULTS DISCUSSION MATERIALS REFERENCES sing the Open-Source MALDI TOF-MS IDBac Pipeline for Analysis of Microbial Protein and Specialized ase M. Clark¹, Maria S. Costa^{1,2}, Erin Conley¹, Emma Li¹, Laura M. Sanchez¹, Brian T. Murph Preparation of MALDI Target Plates and Data Acquisition Installing the IDBac Software and Starting with Raw Data Analyzing Specialized Metabolite Data and Metabolite Association Networks (MANs)

Clark, C. M., Costa, M. S., Conley, E., Li, E., Sanchez, L. M., Murphy, B. T. Using the Open-Source

MALDI TOF-MS IDBac Pipeline for Analysis of Microbial Protein and Specialized Metabolite Data. J.

Vis. Exp. (147), e59219, (2019). https://doi.org/ 10.3791/59219

A Call to Action: the Need for Standardization in Developing

Subspecies Discrimination

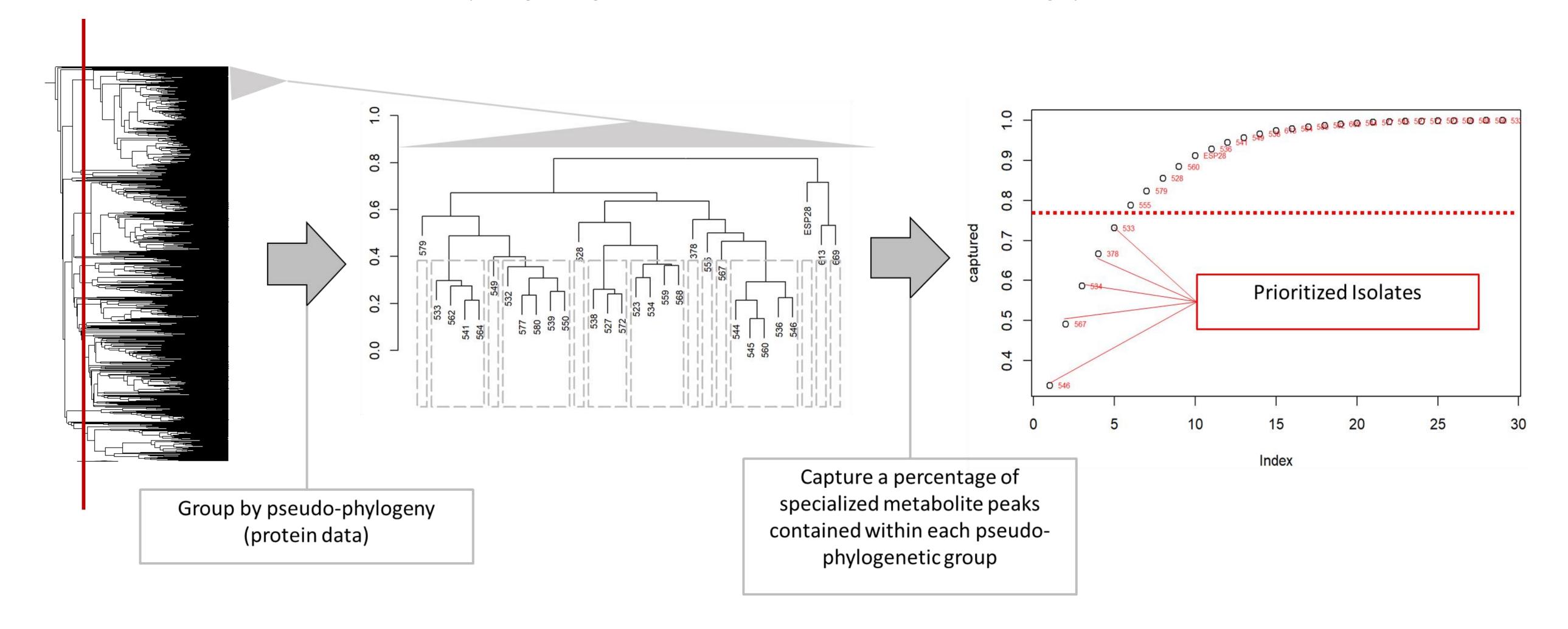
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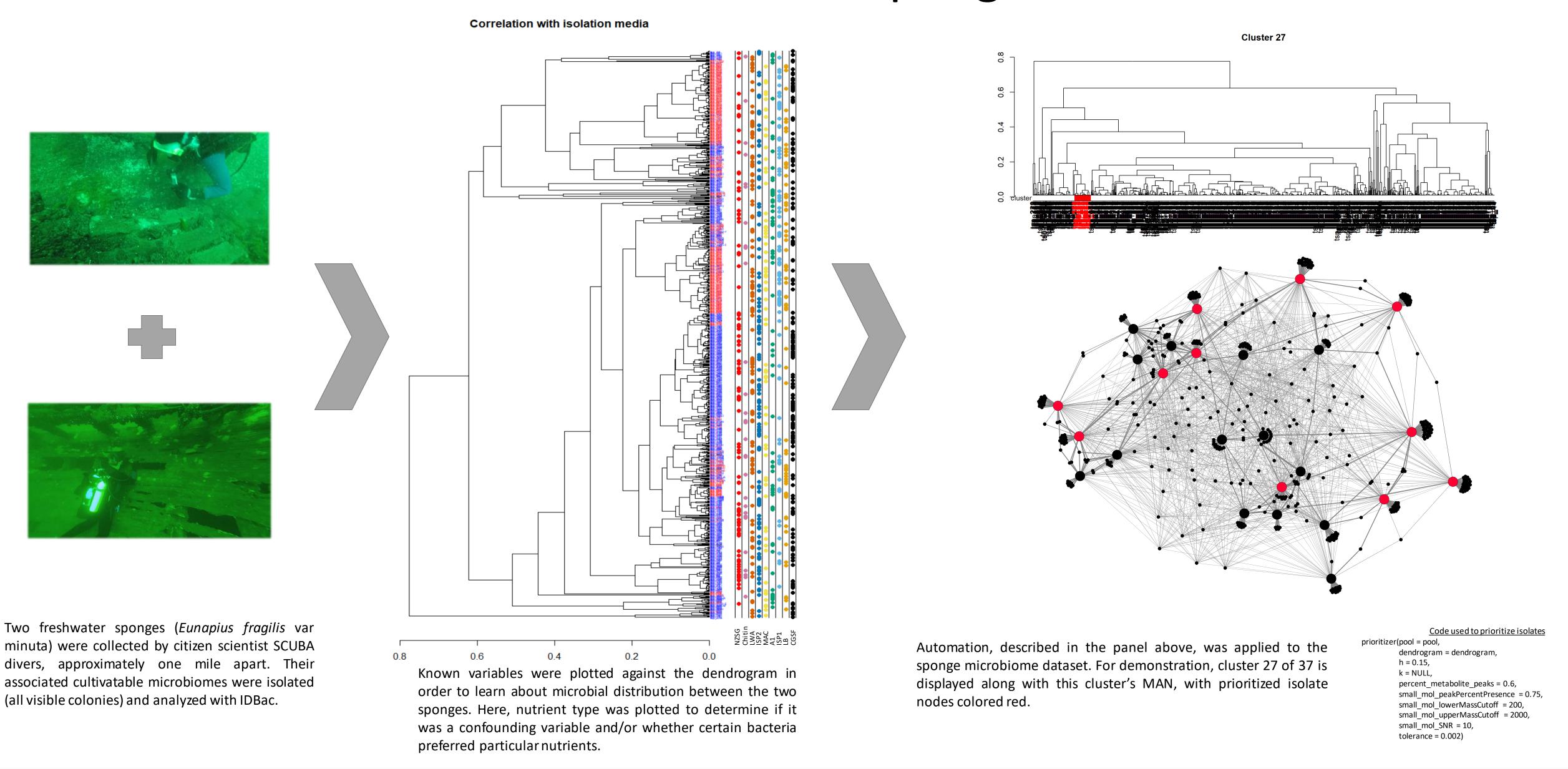
Open-Source Mass Spectrometry-Based Methods for Microbial

Automating Microbial Library Prioritization

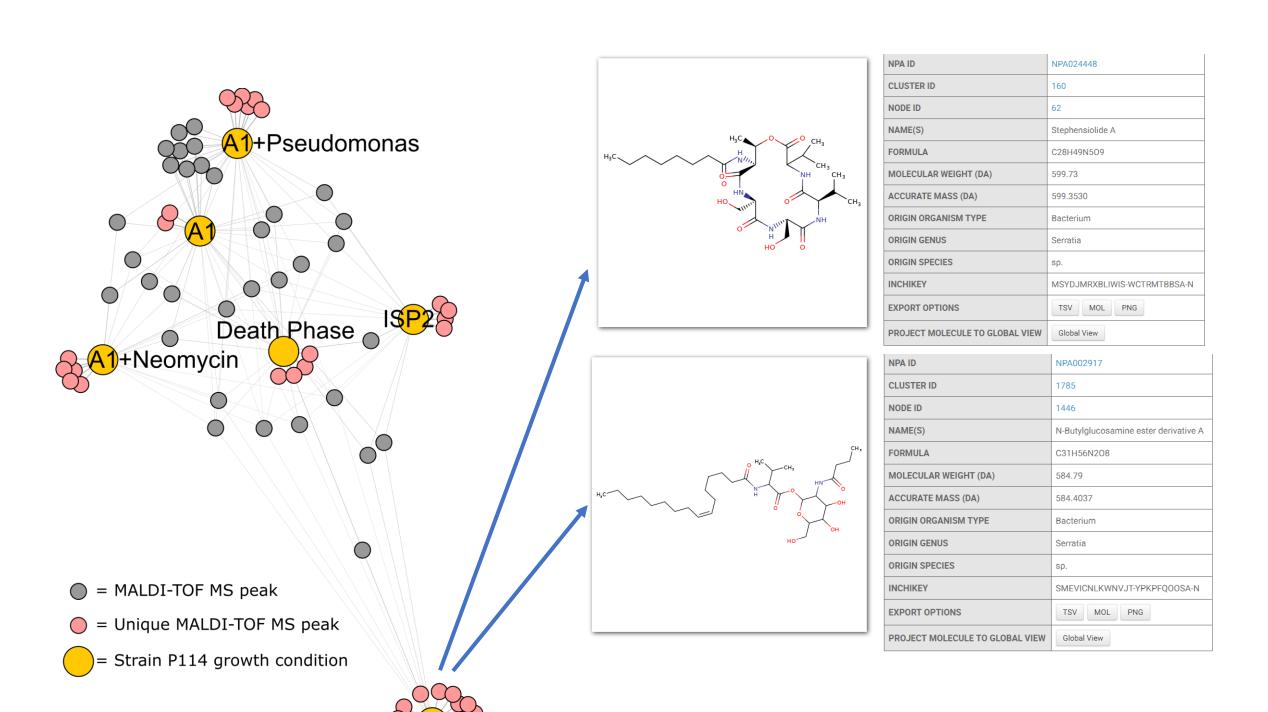
The next version of IDBac will contain automated functions to assist users in quickly parsing through 100's to 1000's of strains to create small but highly-diverse libraries.



Using IDBac to Understand Microbial Natural Product Distribution in Freshwater Sponge Microbiomes



Analyzing the Effect of Culture Conditions on the Production of Antibiotics



differences in MALDI-TOF MS small molecule

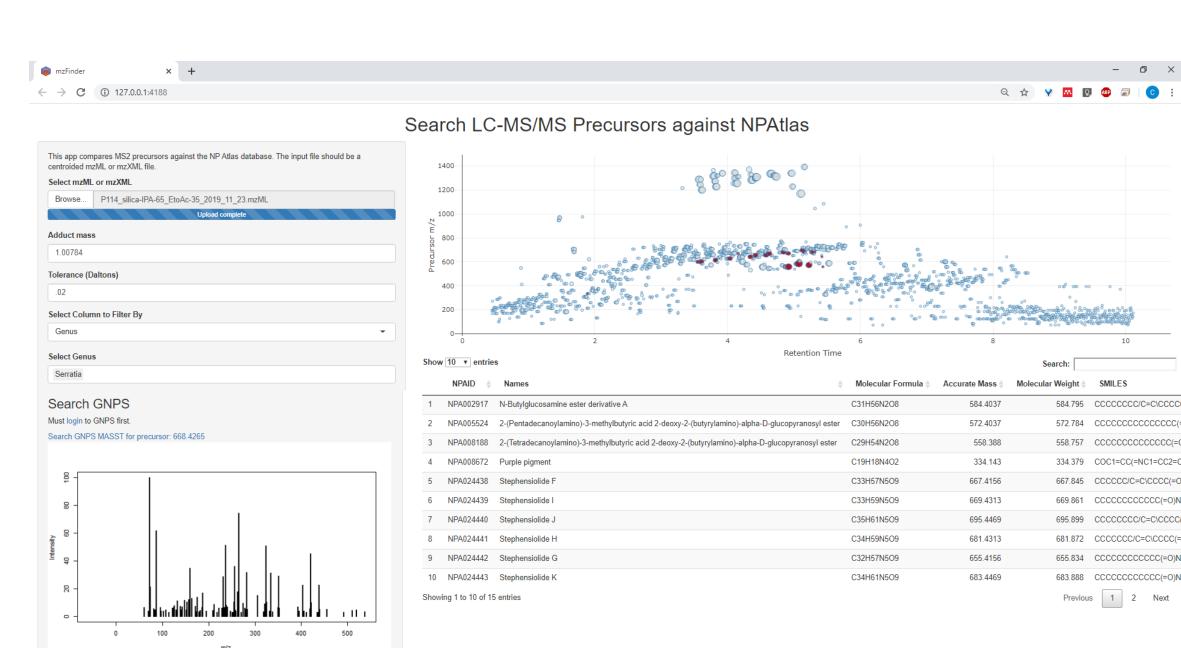
peaks when grown in CGS media compared

to other conditions and media blanks.

The CGS-associated small molecule peaks were then dereplicated using LC-MS/MS and NMR, to be stephensiolide and Nbutylglucosamine ester analogues. Sponge Isolate "P114" showed significant

Due to the N-butylglucosamine ester derivatives' previously hypothesized activity against *Mycobacterium tuberculosis*¹ we have grown "P114" at 30 L scale and currently have fractions undergoing testing with collaborators at UIC's Institute for Tuberculosis Research

[1] Dwivedi D, Jansen R, Molinari G, Nimtz M, Johri BN, Wray V. Antimycobacterial Serratamolides and Diacyl Peptoglucosamine Derivatives from Serratia sp. J Nat Prod.





HR-ESI-UPLC-MS/MS afforded no matches from GNPS¹ (other than from culture media). An R package {mzfinder} was written to search all precursor matches against the Natural Products Atlas². This aided dereplication of the Nbutylglucosamine ester derivatives.

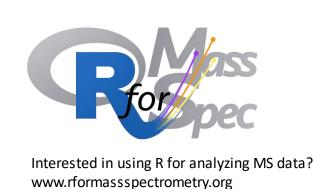
[1] Wang M, Carver JJ, Phelan V, et al. Sharing and community curation of mass spectrometry data with Global Natural

Products Social Molecular Networking. Nat Biotechnol. 2016;34(8):828-837. doi:10.1038/nbt.3597 van Santen JA, Jacob G, Singh AL, et al. The Natural

Funding + Extra Resources

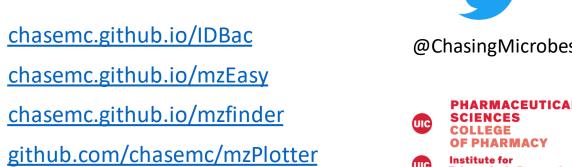
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(Note: Authors not affiliated)





github.com/chasemc/electricShine

PHARMACEUTICA SCIENCES **OF PHARMACY**

Redundancy in Microbial Libraries Using MALDI-TOF MS and the Bioinformatics Pipeline IDBac. J Nat Prod American Chemical Society; 2019 Aug 23;82(8):2167–2173. https://doi.org/10.1021/acs.jnatprod.9b00168

2008;71(4):637-641. doi:10.1021/np7007126

Products Atlas: An Open Access Knowledge Base for Microbial Natural Products Discovery. ACS Cent Sci. 2019;5(11):1824-1833. doi:10.1021/acscentsci.9b00806