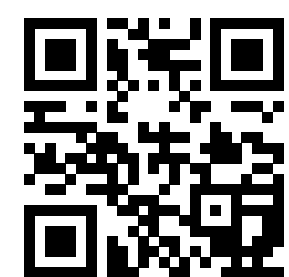
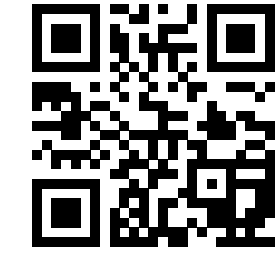


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IDBac Website



[chasecm.github.io/IDBac](https://chasecm.github.io/IDBac)

# Using IDBac to Investigate the Microbial and Natural Product Potential of Freshwater Sponges

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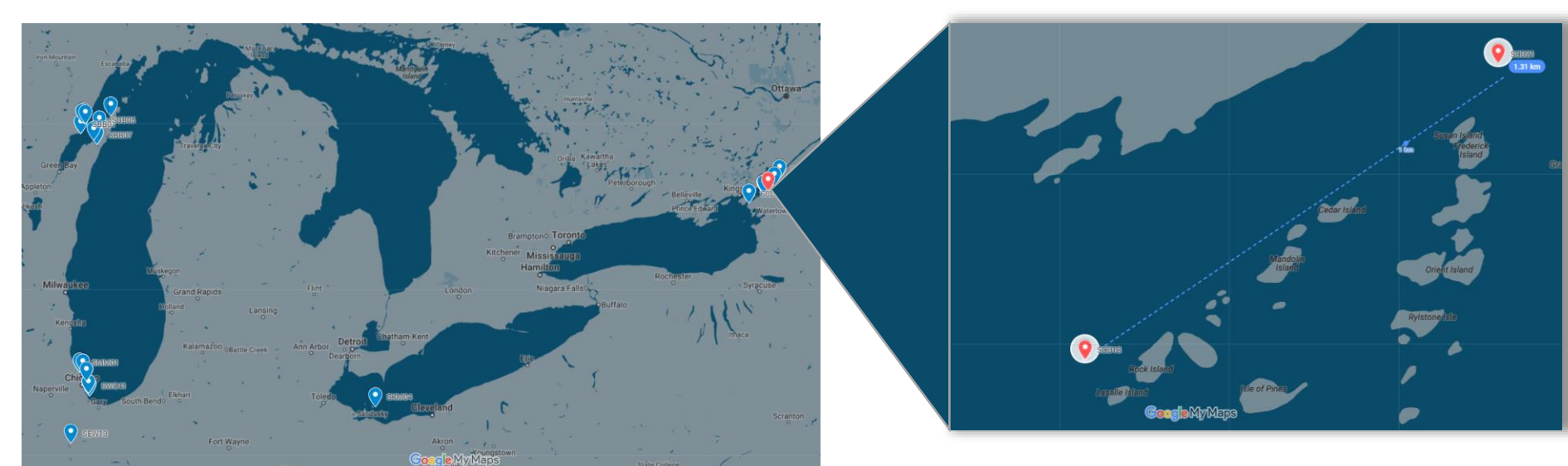
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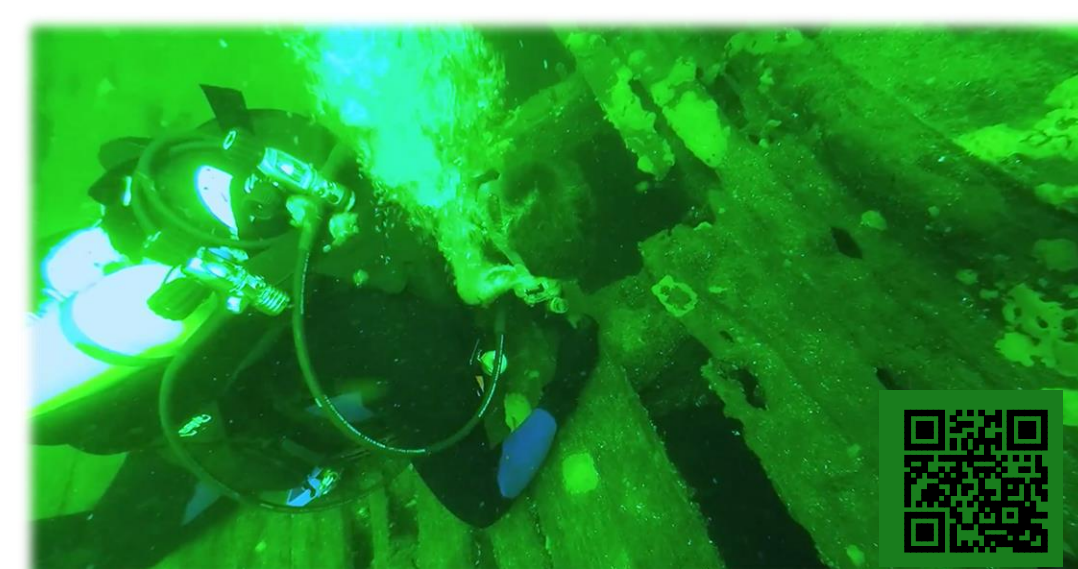


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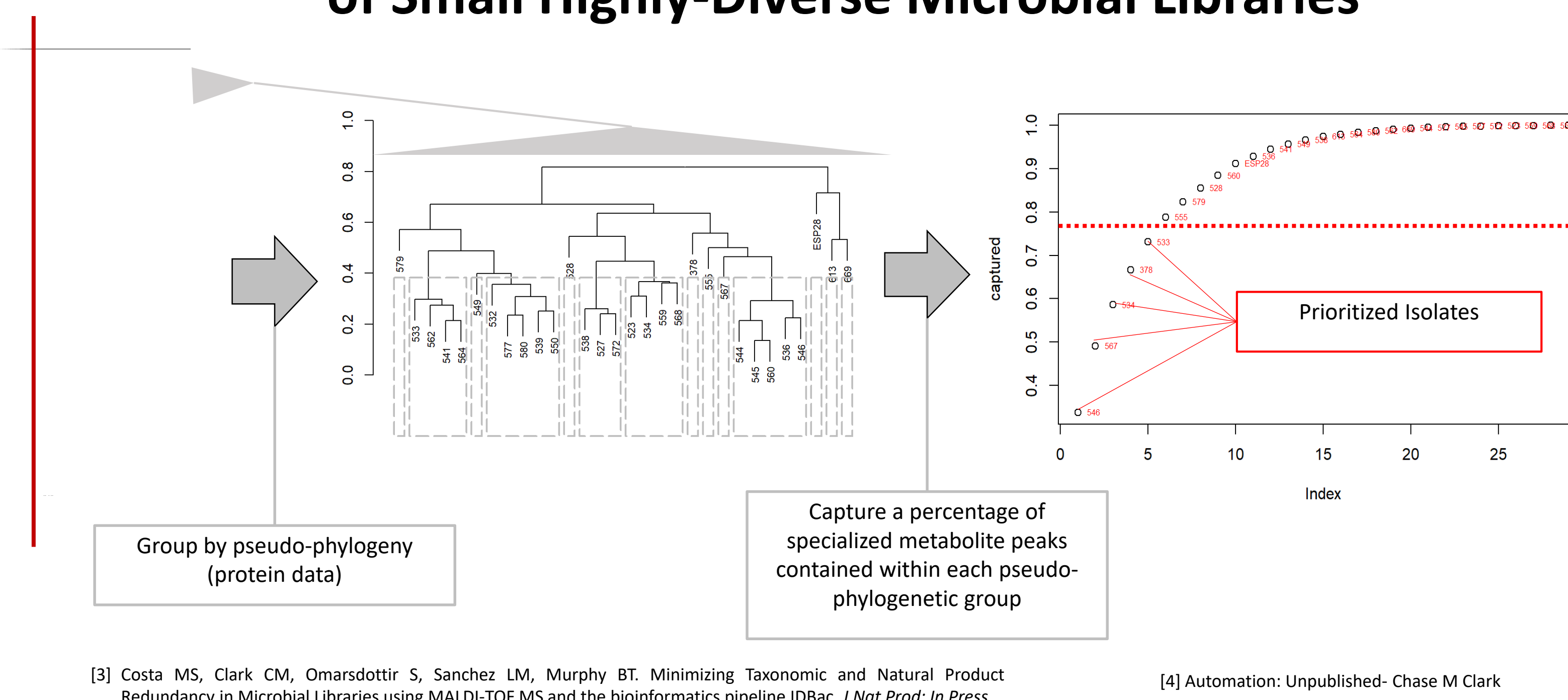
## Freshwater Sponge Microbiomes are an Understudied Resource



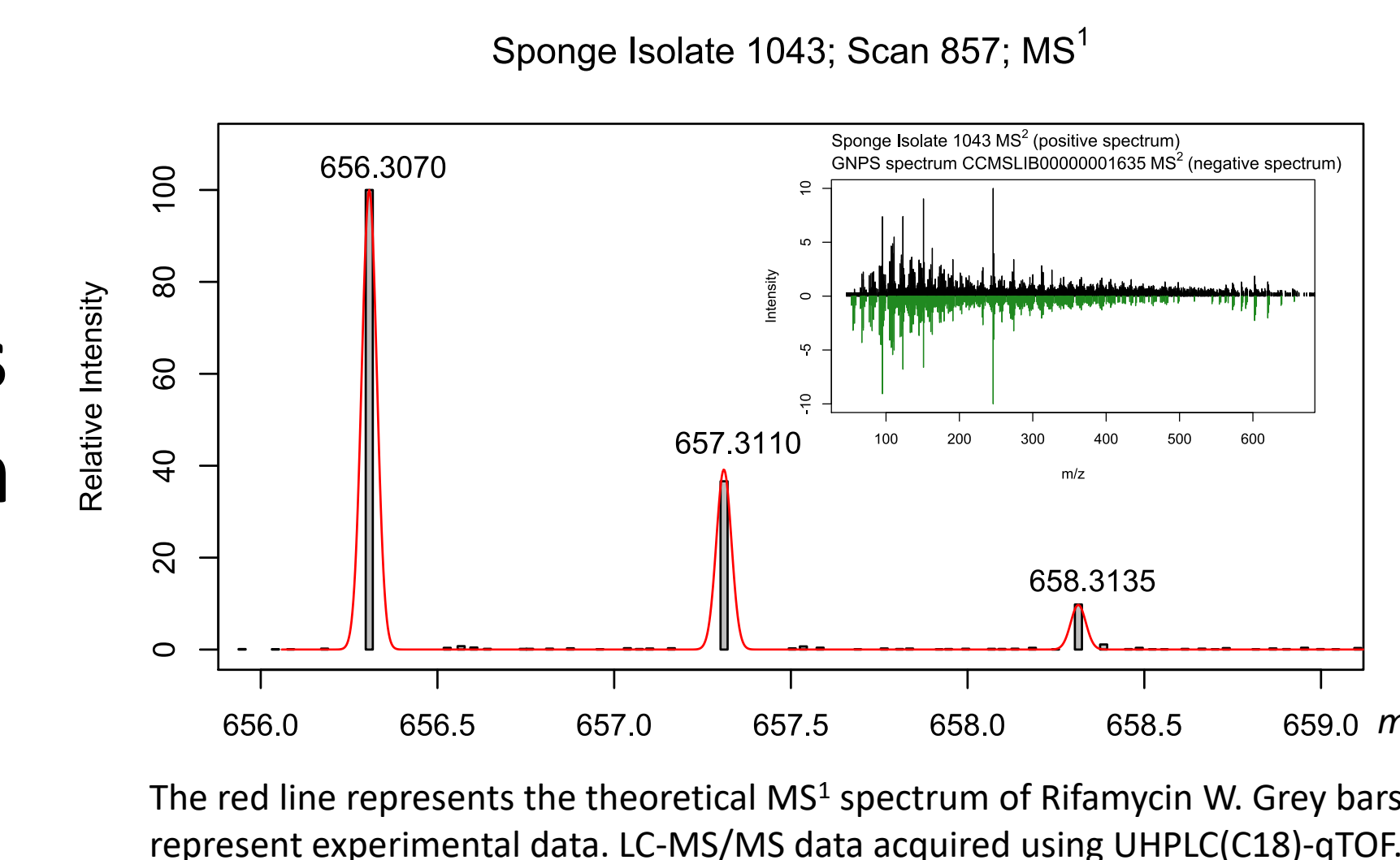
Since 2015 our lab and citizen scientist divers have collected 90 freshwater sponges across the Great Lakes. For this antibiotic-discovery study we chose two *Eunapius fragilis* var. *minuta* sponges collected one mile apart.



## New IDBac Algorithms Automate the Creation of Small Highly-Diverse Microbial Libraries



## Deprioritize Bioactive Isolates Producing Known Antibiotics



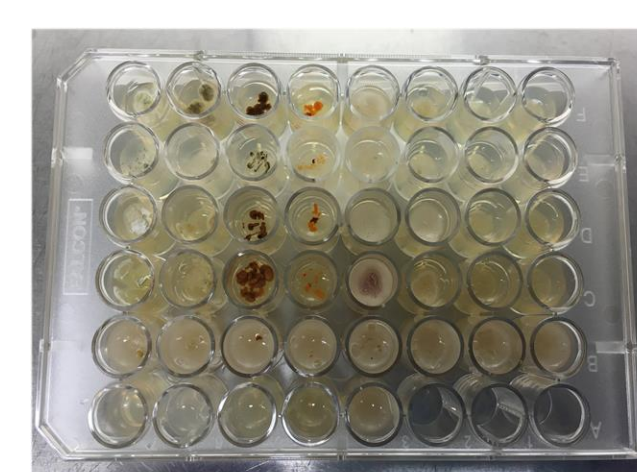
Before scaling cultures, we screened bioactive colonies from agar for commonly-isolated antibiotics using UHPLC-qTOF and GNPS<sup>[7]</sup>. In this case isolate 1043, while active, also produced a well-known antibiotic, rifamycin W, and was de-prioritized.

[7] Wang, Mingxun, et al. "Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking." *Nature Biotechnology* 34.8 (2016): 828-837.

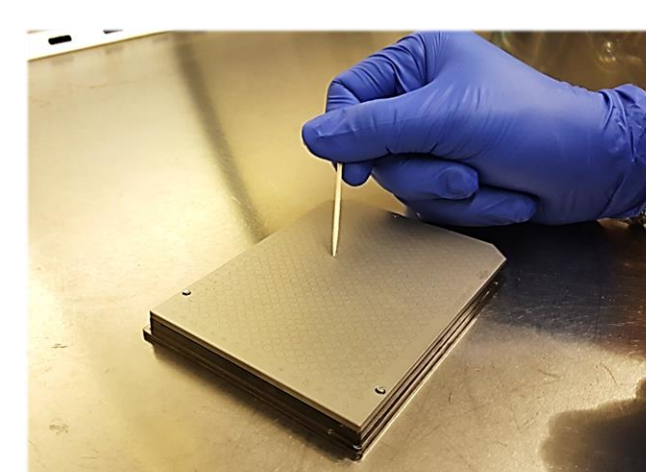
## Every Distinguishable Colony of a Cultured Microbiome is Isolated



Sponge-associated bacteria are cultivated using diverse sample treatments and media.



Isolated 900 bacteria and inoculated onto a single media type.



Sample preparation and data collection for ~125 isolates x 3 replicates can be acquired in 4 hours.

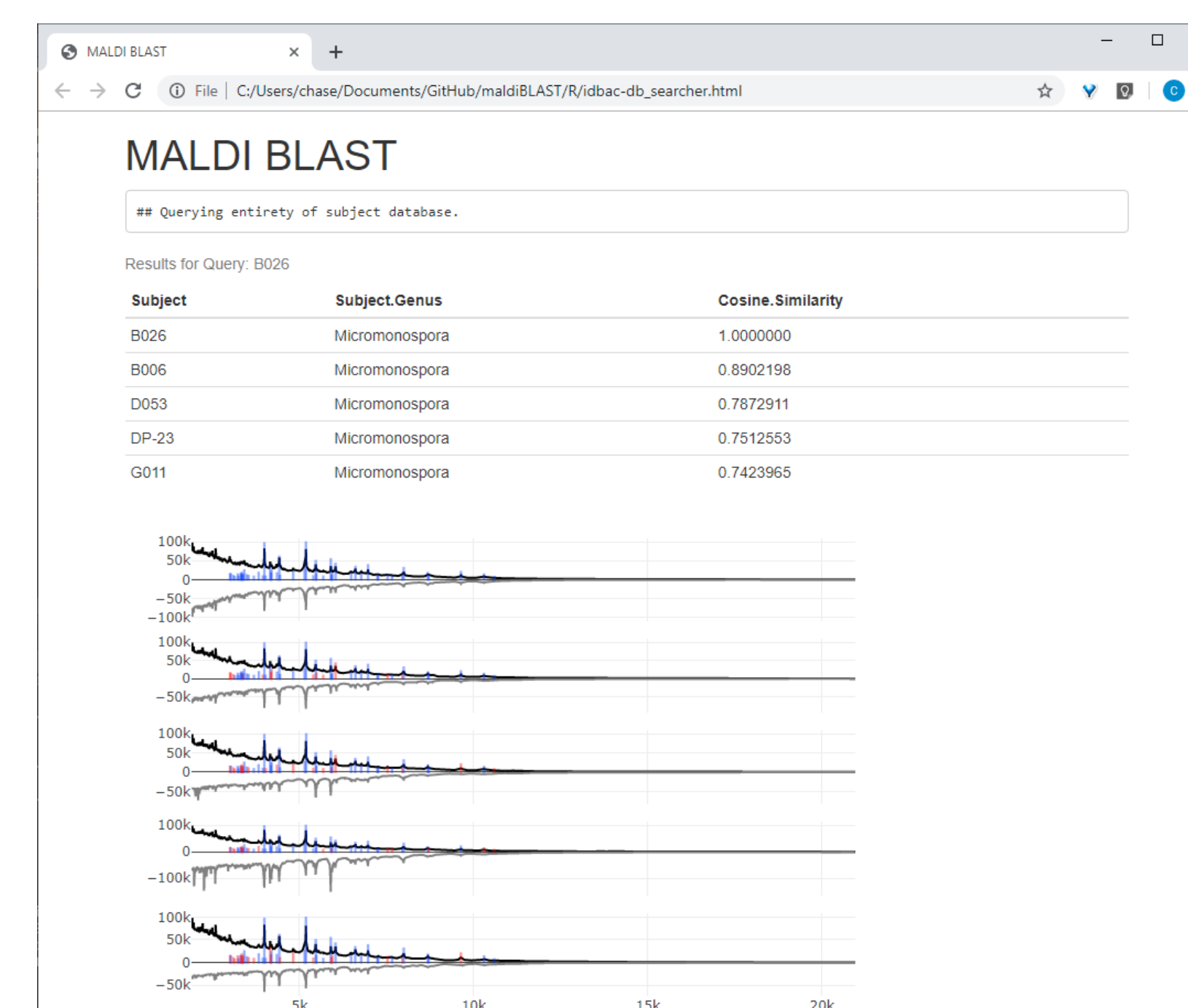
[1] Clark CM, Costa MS, Conley E, Li E, Sanchez LM, Murphy BT. Using the Open-Source MALDI-TOF-MS IDBac Pipeline for Analysis of Microbial Protein and Specialized Metabolite Data. *J Vis Exp*. 2019;(147):e59219. doi:10.3791/59219



## Tentatively Identify Prioritized Bacteria with IDBac Databases of Known Organisms

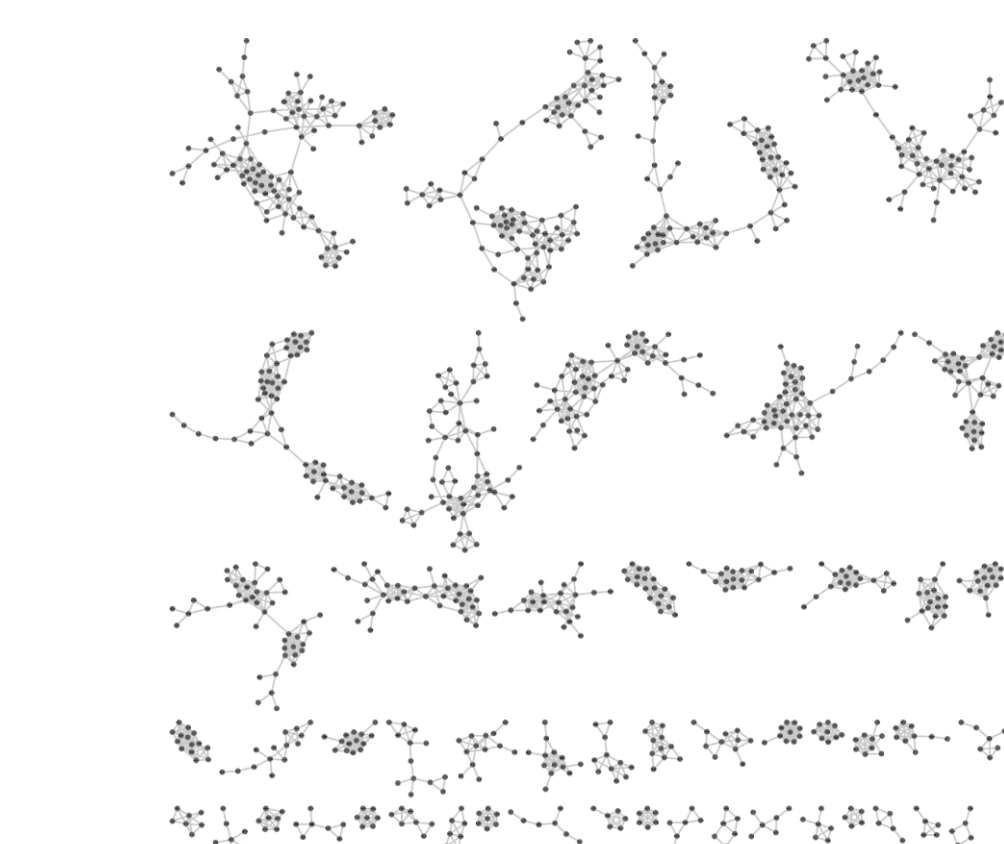
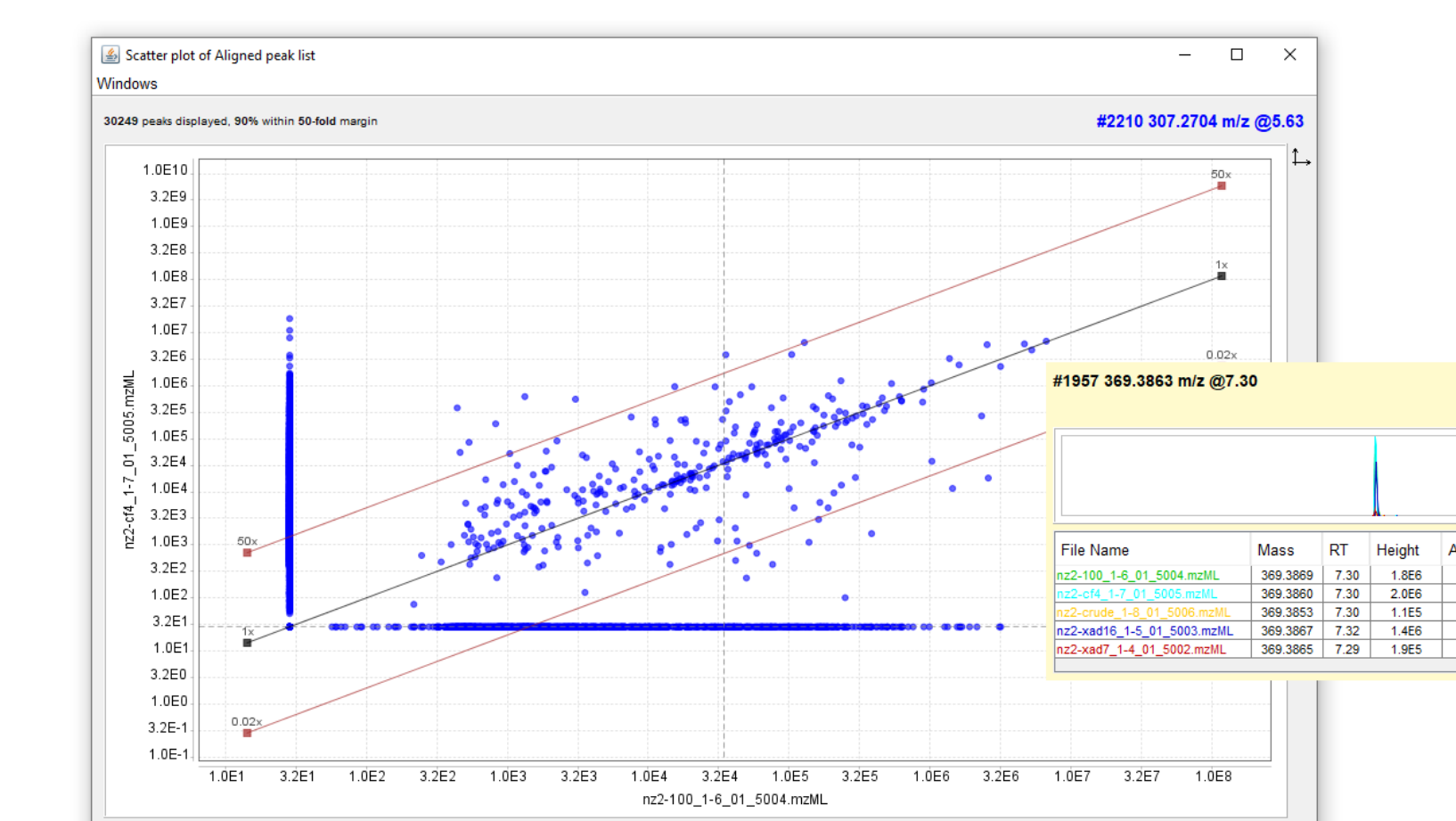
IDBac v1.0 or greater allows users to create shareable reference databases of known bacteria. Upcoming releases will include the ability to rapidly query spectra of unknowns against these databases.

We are working to make our database of 110 genera available soon (few replicates) and have begun digitizing a portion of the NRRL microbial library (high-quality, eight biological replicates).



## Remaining Prioritized Isolates Undergo Isolation and Elucidation

Another freshwater isolate, 112, exhibited bioactivity but dereplication has afforded no known antibiotics. Isolation and elucidation efforts are underway.

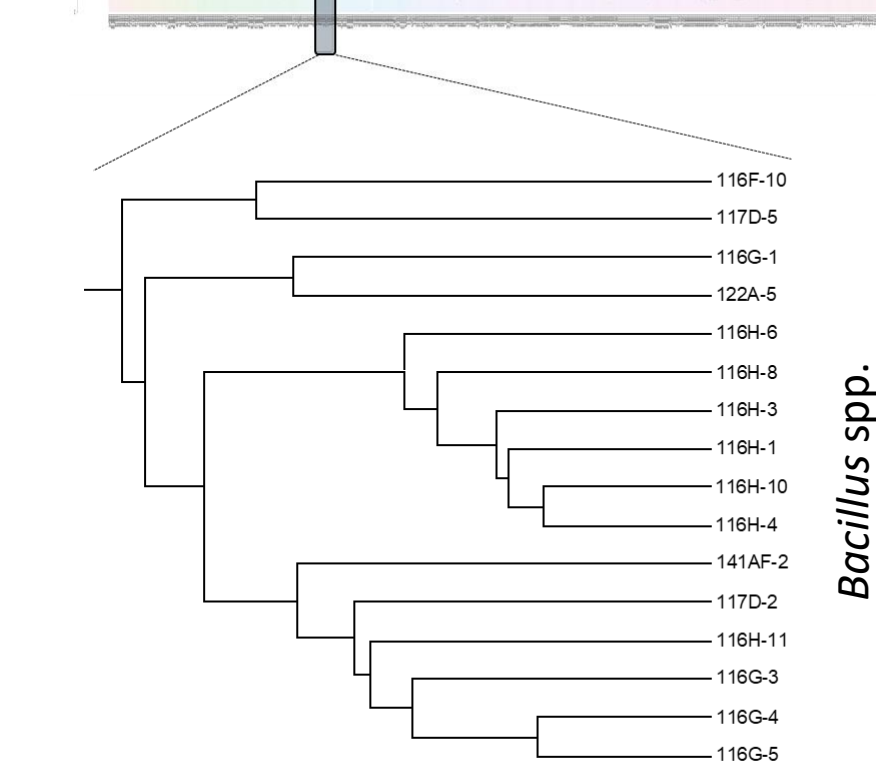
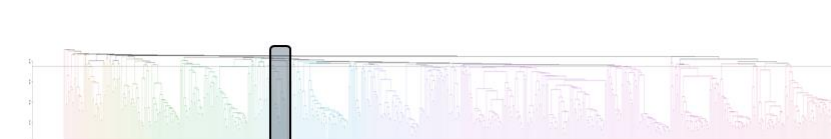


Left: Active and non-active C18-SPE fractions from isolate 112 are compared for differential occurrence/abundance of LC-MS/MS features.<sup>[9]</sup>

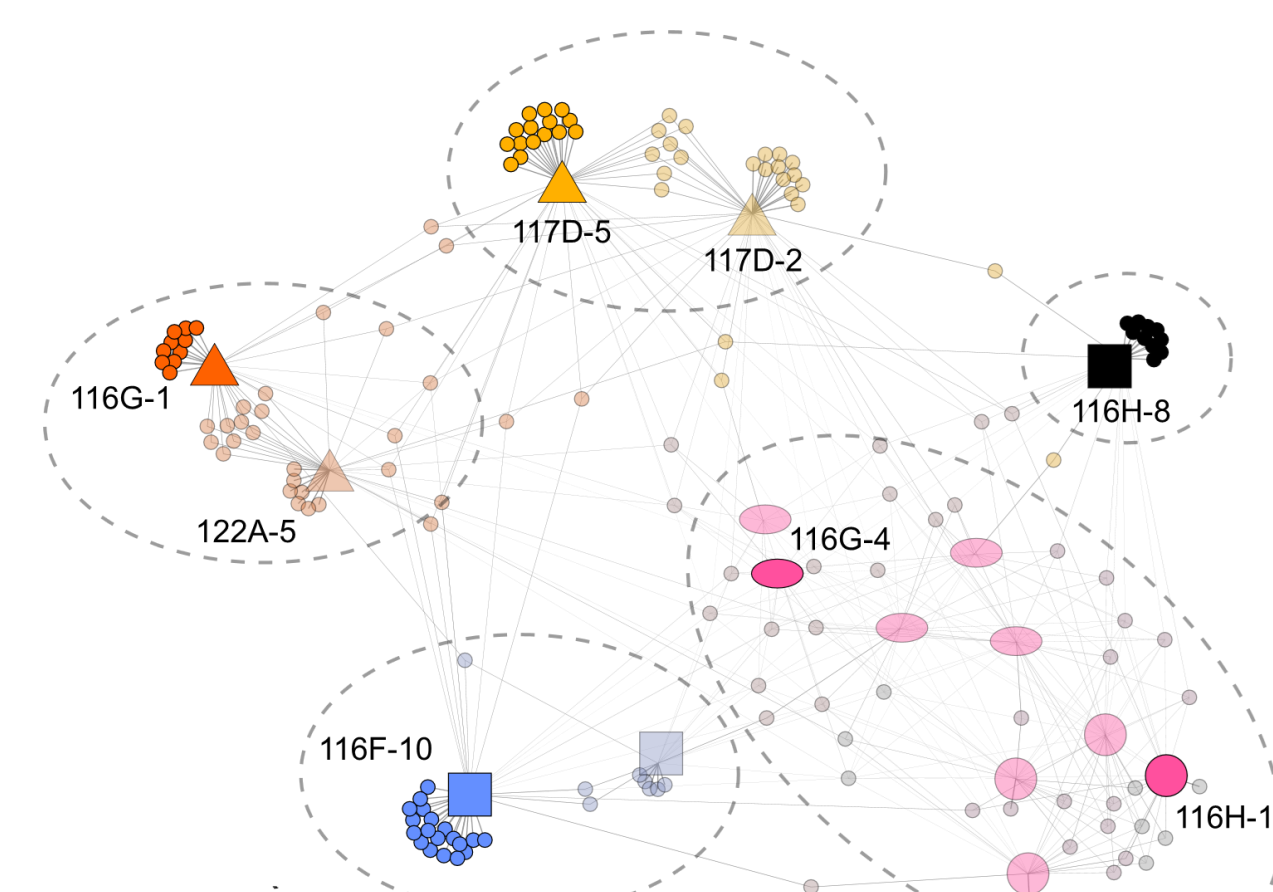
[8] Nothias L-F, Nothias-Esposto M, da Silva R, et al. Bioactivity-Based Molecular Networking for the Discovery of Drug Leads in Natural Product Bioassay-Guided Fractionation. *J Nat Prod*. 2018;81(4):758-767. doi:10.1021/acs.jnatprod.7b00737

[9] Pluskal T, Castillo S, Villar-Briones A, Orešič M. MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. *BMC Bioinformatics*. 2010;11(1):395. doi:10.1186/1471-2105-11-395

## MALDI-TOF MS Data is Collected for Protein and Specialized Metabolite Mass Regions



Protein Data  
Allows IDBac to create pseudo-phylogenetic groups based on Matrix Assisted Laser Desorption Time of Flight Mass Spectrometry (MALDI-TOF MS) spectra. These groupings are highly specific and correlate well to 16S rRNA gene groupings.

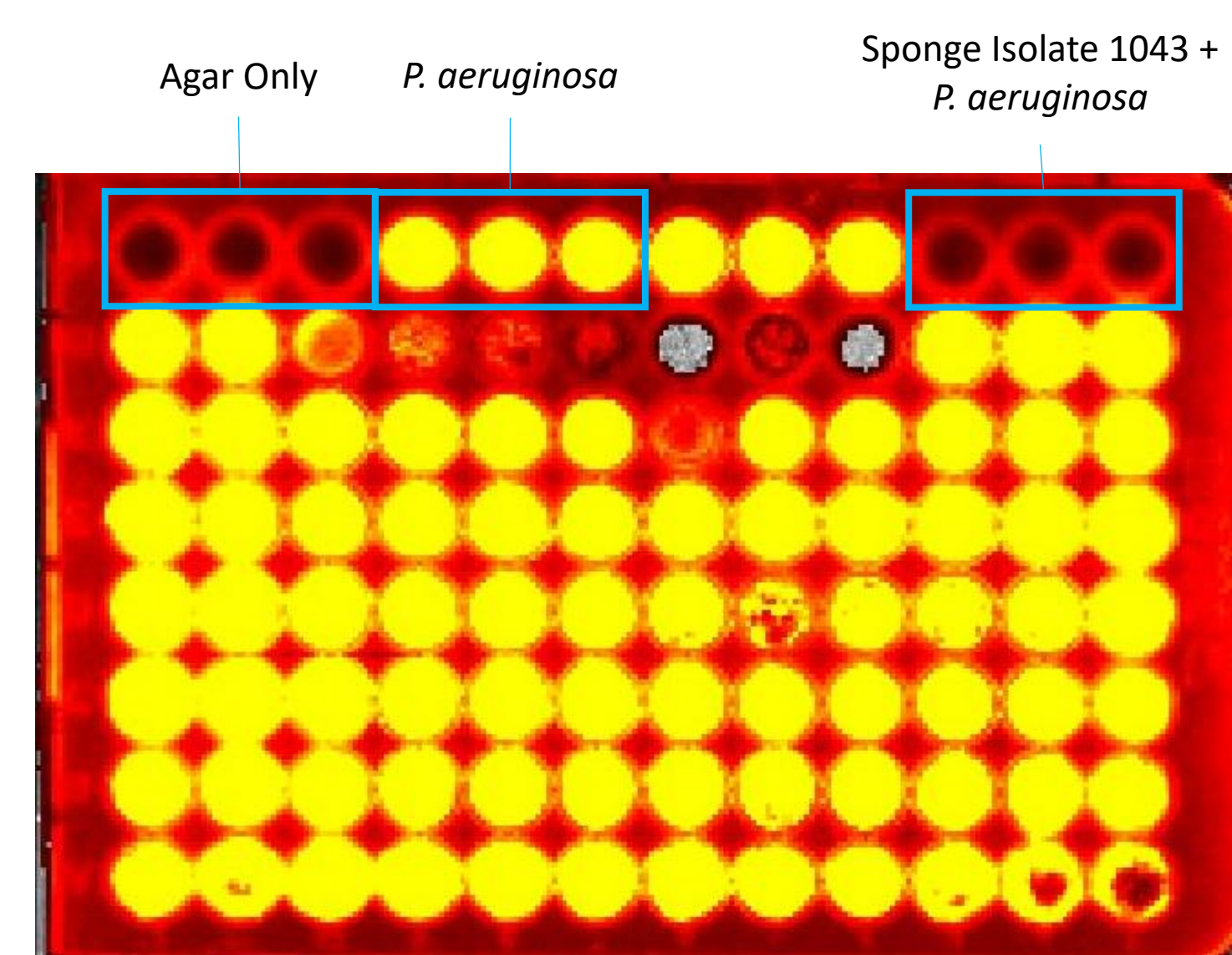


Specialized Metabolite Data  
Once isolates are grouped using protein data, IDBac is used to create sub-groups of isolates with similar specialized metabolite MALDI-TOF MS profiles.

[2] 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*. 2018;115(19):4981-4986. doi:10.1073/pnas.1801247115



## Further Prioritize the Highly-Diverse Microbial Library Using a Custom Agar-Based OSMAC Screen



Results of a screen of isolate 1043, which was found to inhibit *P. aeruginosa*. For more information on this assay, see poster P-016.

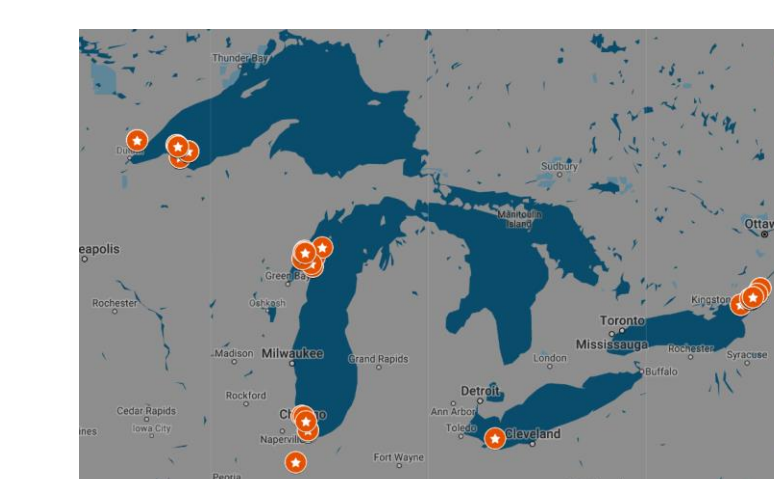
An agar-based OSMAC screen against the fluorescently-labelled pathogen *Pseudomonas aeruginosa* 10145GFP (among other ESKAPE pathogens) was used to further prioritize isolates for antibiotic discovery.

Some benefits:

- Screen doesn't require liquid culture
- GFP result confirmed with a secondary MTT assay
- Differential activity in different growth conditions is easily exposed
- 3D-printed 96-well plates are autoclavable and reusable

[6] Unpublished

## Future Directions



### Antibiotic Discovery:

In addition to elucidating the bioactive compound(s) from isolate 112, we are preparing to screen all 120 prioritized sponge isolates against a panel of ESKAPE pathogens using our newly-developed agar-based OSMAC assay.

### Freshwater Sponge Biogeography:

Utilizing existing, and not-yet-released IDBac analyses, we are working to understand the biogeographic distribution of Great Lakes freshwater sponges (map above), their associated bacteria, and bacterial specialized metabolites.

Software Links:

[chasecm.github.io/IDBac](https://chasecm.github.io/IDBac)  
[chasecm.github.io/mzEasy](https://chasecm.github.io/mzEasy)  
[github.com/chasecm/mzPlotter](https://github.com/chasecm/mzPlotter)  
[github.com/chasecm/electricShine](https://github.com/chasecm/electricShine)

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