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IDBac Website

chasemc.github.io/IDBac

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

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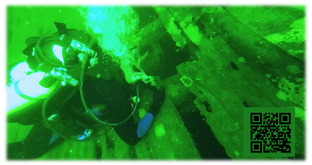
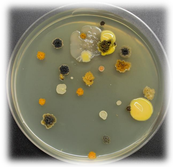
Freshwater Sponge Microbiomes are an Understudied Resource

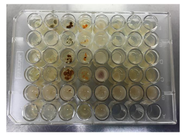
Since 2015 our lab and citizen scientist divers have collected 90 freshwater sponges across the Great Lakes. For this antibiotic-discovery study we chose two *Eunapius fragilis* var. *minuta* sponges collected one mile apart.



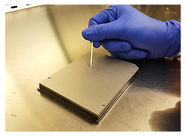
Every Distinguishable Colony of a Cultured Microbiome is Isolated



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



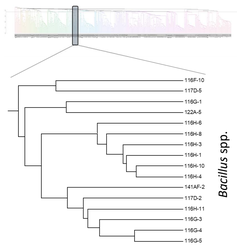
Isolated 900 bacteria and inoculated onto a single media type.

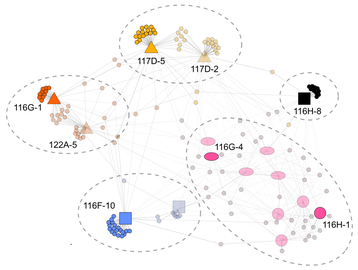


Sample preparation and data collection for ~125 isolates x 3 replicates can be acquired in 4 hours.

[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

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





Protein Data

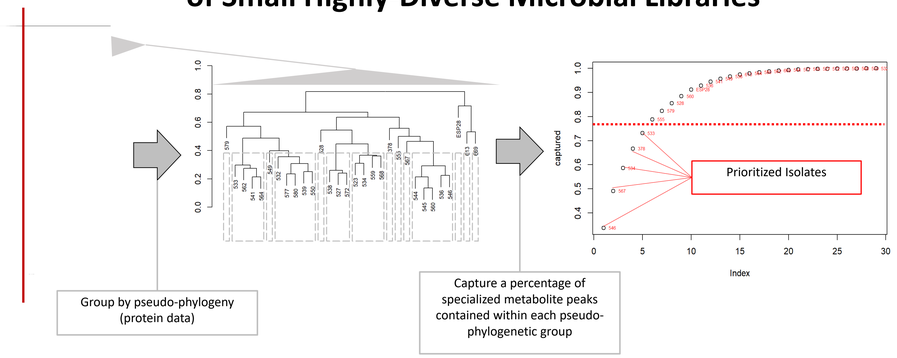
Allows IDBac to create pseudo-phylogenetic groups based on Matrix Assisted Laser Desorption Time of Flight Mass Spectrometry (MALDI-TOF MS) spectra.
These groupings are highly specific and correlate well to 16S rRNA gene groupings.

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

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

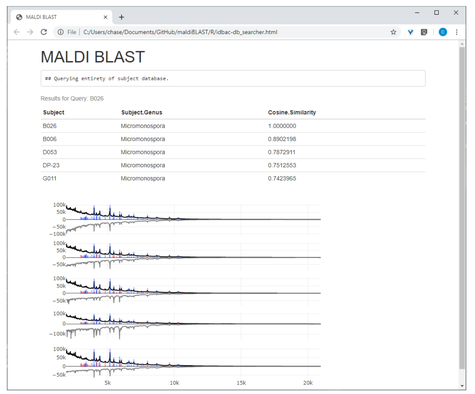


[3] Costa MS, Clark CM, Omarsdottir S, Sanchez LM, Murphy BT. Minimizing Taxonomic and Natural Product Redundancy in Microbial Libraries using MALDI-TOF MS and the bioinformatics pipeline IDBac. *J Nat Prod; In Press.*

[4] Automation: Unpublished- Chase M Clark

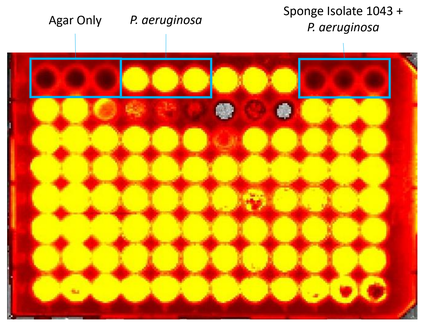
Tentatively Identify Prioritized Bacteria with IDBac Databases of Known Organisms

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).



[5] Unpublished- Chase M Clark;
Available: github.com/chasemc/maldiSearch

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.

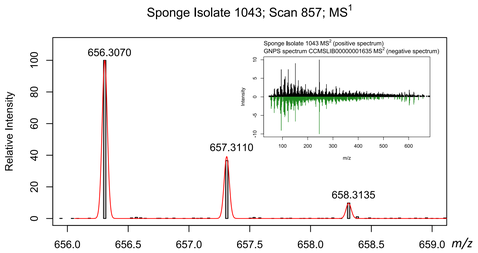
An agar-based OSMAC screen against the fluorescently-labelled pathogen P*seudomonas aeruginosa* 10145GFP (among other ESKAPE pathogens) was used to further prioritize isolates for antibiotic discovery.

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

Deprioritize Bioactive Isolates Producing Known Antibiotics



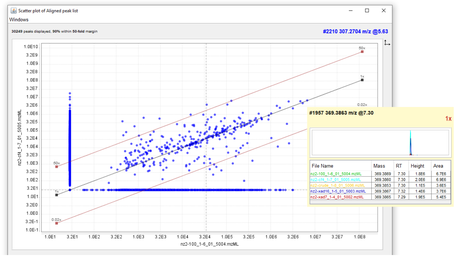
The red line represents the theoretical MS^1 spectrum of Rifamycin W. Grey bars represent experimental data. LC-MS/MS data acquired using UHPLC(C18)-QTOF.

Before scaling cultures, we screened bioactive colonies from agar for commonly-isolated antibiotics using UHPLC-QTOF and GNPS[7]. 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.

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.



Above: A GNPS Feature-Based Molecular Network of isolate fractions and controls is used to quickly compare and prioritize LC-MS/MS features for further isolation and elucidation.[8]

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

[8] Nothias L-F, Nothias-Esposito 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, Oresič 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

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:

chasemc.github.io/IDBac
chasemc.github.io/mzEasy
github.com/chasemc/mzPlotter
github.com/chasemc/electricShine

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