




microbeMASST: a taxonomically informed mass spectrometry search tool for microbial metabolomics data

In the format provided by the authors and unedited

Supplementary Figures

 microbeMASST Dashboard - Version 1.1

Data Selection

GNPS Data Selection - Enter USI or Spectrum Peaks

Spectrum USI

Spectrum Peaks

Precursor m/z Charge

PM Tolerance (Da) Fragment Tolerance (Da) Cosine Threshold Minimum Matched Peaks

Analog Search Delta Mass Below (Da) Delta Mass Above (Da)

Supplementary Figure 1. microbeMASST web app

Users can access the microbeMASST web app at <https://masst.gnps2.org/microbemasst/>. They can search for MS/MS spectrum against the microbeMASST reference database by either providing a USI in `Spectrum USI` or by inputting fragment ions and their intensities and the precursor mass in `Spectrum Peak` and `Precursor m/z` respectively. Search parameters, such as parent ion and fragment ions tolerances, cosine threshold, and minimum matching peaks can be modified. Analog search can also be enabled. Finally, to submit a search query, users have just to click either on `Search microbeMASST by USI` or `Search microbeMASST by Spectrum Peak` based on the information that they have provided. Search jobs can be easily shared by clicking on `Copy Link`.

a Data Exploration

Library matches | Dataset matches | Taxa matches | Parameters

Copy | Excel | CSV | Show 4 | entries

Delta Mass	GNPSLibraryAccession	USI	Charge	Cosine	Matching Peaks	Adduct	CompoundName
0	CCMSLIB00005435750	mzspec:GNPS.GNPS-LIBRARY.accession.CCMSLIB00005435750	1	1	9	M+H	Yersiniabactin
0.01	CCMSLIB00005435756	mzspec:GNPS.GNPS-LIBRARY.accession.CCMSLIB00005435756	1	0.89	6	M+H	Yersiniabactin

Library Spectrum Information

GNPS Metabolomics USI

USI Data Selection

Data Visualization

Figure Size: 10 | 20 | X | 6 | 20

Mass Range: min | max | min | max | min | max

Maximum Intensity: 125 | %

Label Precision: 4 | decimal

Label Rotation: 90 | °

Code: Standard | +

Fragment Tolerance: 0.1 | m/z

Show Grid: Yes | -

UI Adjustment

Clipboard Size

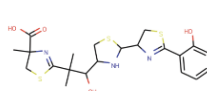
Top: mzspec:GNPS.LIBRARY.accession.CCMSLIB00005435750
Reference: m/z: 432 | 130 (Charge: 1)

Bottom: mzspec:GNPS.LIBRARY.accession.CCMSLIB00005435750
Reference: m/z: 432 | 130 (Charge: 1)

Y-axis: Abundance

X-axis: m/z

Yersiniabactin



b Data Exploration

Library matches | Dataset matches | Taxa matches | Parameters

Copy | Excel | CSV | Show 4 | entries

Name	NCBI	Rank	Cosine	Matching signals	USI	MassIVE	File
Escherichia_coli_Nissle_1917	316435	strain	0.96	8	mzspec:MSV000083383:stl_mic3_Fa_conc46_3_longscan:1892	MSV000083383	Open file
Escherichia_coli_MS_200-1	749550	strain	0.94	8	mzspec:MSV000083383:stl_mic3_Fa_conc46_1_longscan:1221	MSV000083383	Open file
Escherichia_coli_Nissle_1917	316435	strain	0.92	7	mzspec:MSV000083383:stl_mic3_Fa_conc46_2_longscan:1910	MSV000083383	Open file
Escherichia_coli_Nissle_1917	316435	strain	0.92	7	mzspec:MSV000083383:stl_mic3_Fa_conc46_1_longscan:1898	MSV000083383	Open file

Showing 1 to 4 of 129 entries

GNPS Metabolomics USI

USI Data Selection

Data Visualization

Top: mzspec:GNPS.LIBRARY.accession.CCMSLIB00005435750
Reference: m/z: 432 | 130 (Charge: 1)

Bottom: mzspec:MSV000083383:stl_mic3_Fa_conc46_3_longscan:1892
Reference: m/z: 432 | 130 (Charge: 1)

Y-axis: Abundance

X-axis: m/z

MassIVE MSV000083383

GNPS - E. Coli Nissle wild type versus knockout strains grown with altered zinc levels

Description

Metabolomics data from E. Coli Nissle wild type, WT1 and T.E. Coli Nissle knockout (KO) strains grown in 8.9 media and enriched in media. [url="https://doi.org/10.25541/MSV000083383"]Dataset license: CC-BY 4.0 International (CC BY)

Keywords: Ecol, E. Coli Nissle

Contact

Principal Investigators: Peter Domertsen, University of California San Diego, USA
[url="mailto:pd@ucsd.edu"]pd@ucsd.edu

Submitting User: altoparam

Number of Files: 1,082

Total Size: 19,30 GB

Spectra: 195,918

Substrates: 1

Owner Name(s):

Identification Results

Protein (Reported): N/A, N/A

Protein(s): N/A, N/A

Peptide(s): N/A, N/A

Peptide: N/A, N/A

PPM Download Link (URL to report): [url="http://massive.ucsd.edu/MSV000083383/"]http://massive.ucsd.edu/MSV000083383/

c Data Exploration

Library matches | Dataset matches | Taxa matches | Parameters

Copy | Excel | CSV | Show 4 | entries

Name	NCBI	Rank	Matches	Samples	Fraction
root	131567	cellular organisms	129	60306	0.0021
Prokaryote	2_placeholder	superkingdom_placeholder	110	44836	0.0025
Bacteria	2	superkingdom	110	44836	0.0025
Proteobacteria	1224	phylum	107	11567	0.0093

Showing 1 to 4 of 21 entries

Previous 1 | 2 | 3 | 4 | 5 | 6 | Next

Supplementary Figure 2. Complementary output of microbeMASST

- The queried MS/MS spectrum is searched against the GNPS libraries and, if matches are found, possible annotations are returned. Information on cosine similarity and number of matching peaks is provided and users can explore the associated GNPS Library Spectrum page and inspect mirror plots.
- Information of matching scans in the sample from the different taxa is provided. In addition to cosine similarity score, matching fragments, and the possibility to inspect mirror plots, users can retrieve the MassIVE ascension number of the project together with contact information on who deposited the data.
- Number of matches for each taxonomic level are returned. Users can observe how many samples for that specific taxon are part of microbeMASST and see how broadly the molecule is distributed within it (fraction).

Mevastatin



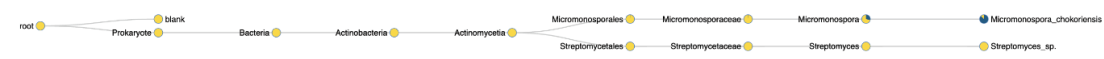
Arylomycin A4



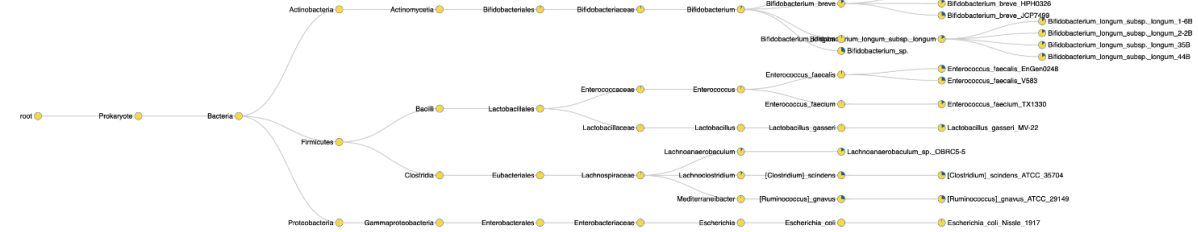
Yersiniabactin



Promicroferrioxamine



Glutamate-Cholic Acid

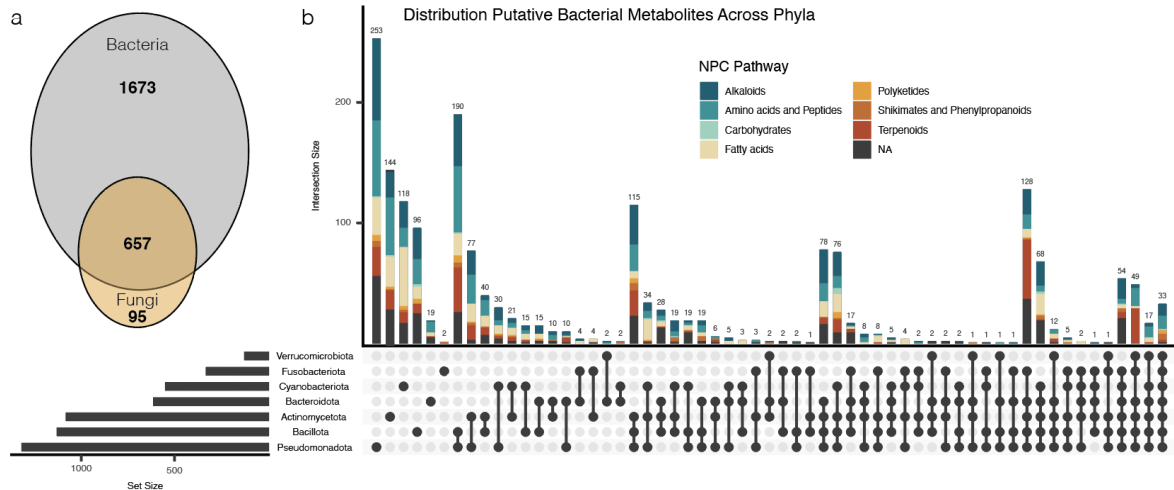


Glutamate-Deoxycholic Acid



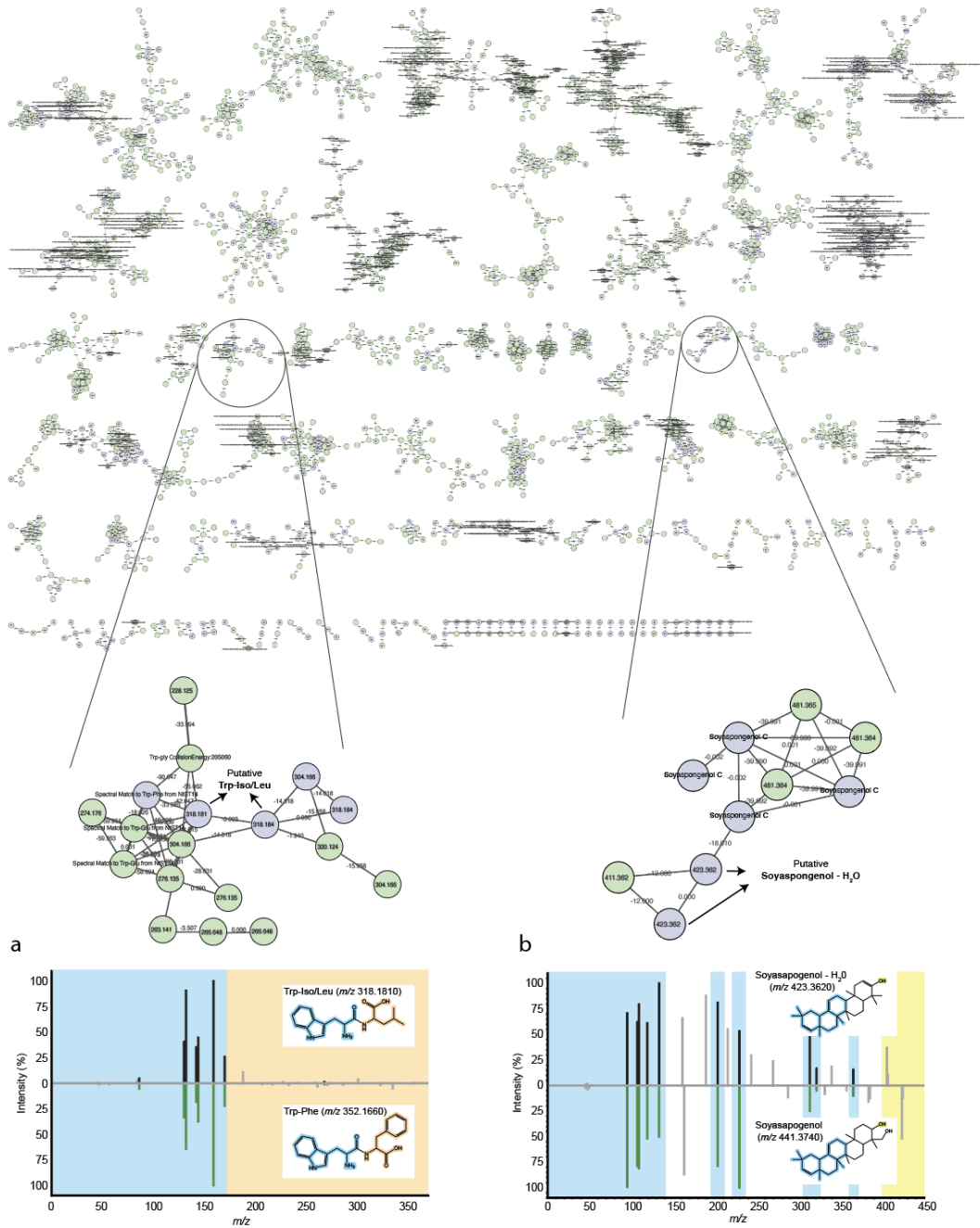
Supplementary Figure 3. Examples of microbeMASST outputs

Additional examples of results obtained from searching from [mevastatin](#), [arylomycin A4](#), [yersiniabactin](#), [promicroferrioxamine](#), [glutamate-cholic acid](#), and [glutamate deoxycholic acid](#). In all cases the molecules were found in the monocultures of known producers and not in human cell lines, confirming that microbeMASST can be used to search for microbial-derived molecules.



Supplementary Figure 4. Distribution putative microbial metabolites across phyla

a) Of the 2,425 MS/MS spectra that had a match exclusively to microbial monocultures, 1,673 were found only in bacteria, 95 in fungi, and 657 in both. b) Chemical classes were then predicted using SIRIUS and CANOPUS and their distribution across the different phyla was visualized using an Upset plot.



Supplementary Figure 5. Contextualization of the molecular network

The selected 512 MS/MS spectra were mapped back to the full molecular network generated from the data acquired from GF and SPF mice (MSV00079949). Only molecular families containing at least one of the MS/MS spectra of interest were retained and used to generate putative annotations of the unannotated spectra of interest. Each node represents a spectrum (ion), light blue indicates MS/MS of interest while green indicates spectra that were not retained in the performed downstream analysis. Within each node the m/z of the precursor ion is indicated, while on each edge the m/z difference between two connected nodes is reported. a) Ions with precursor mass 318.181 and directly connected to a spectrum annotated as the dipeptide Trp-Phe (m/z 352.144) can be tentatively annotated as Trp-Iso/Leu. b) Ions with precursor mass 423.362 connected to Soyasapogenol C, which can represent a soyasapogenol molecule with a loss of water (delta mass 18.01). Abbreviations: Trp, tryptophan; Phe, phenylalanine; Iso, isoleucine; Leu, leucine.