JGI JOINT GENOME INSTITUTE
UNITED STATES DEPARTMENT OF ENERGY

Linking Sequence to Metabolic Function

Case Studies

March 22, 2016
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Case Studies

1. Secondary Metabolism

Integration of genome sequencing, analysis of biosynthetic clusters, and secondary metabolite data

- Biochemical evidence for aminobacteriohopanetriol biosynthesis in proteobacteria

2. Primary Metabolism

Identification of primary metabolites required for Chloroflexi growth in the Great Boiling Spring, Nevada

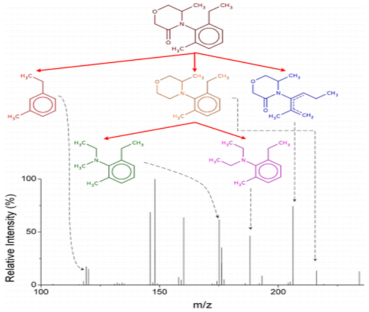
- Growth of *T. hugenholtzii* in the lab

Primary and Secondary Metabolism

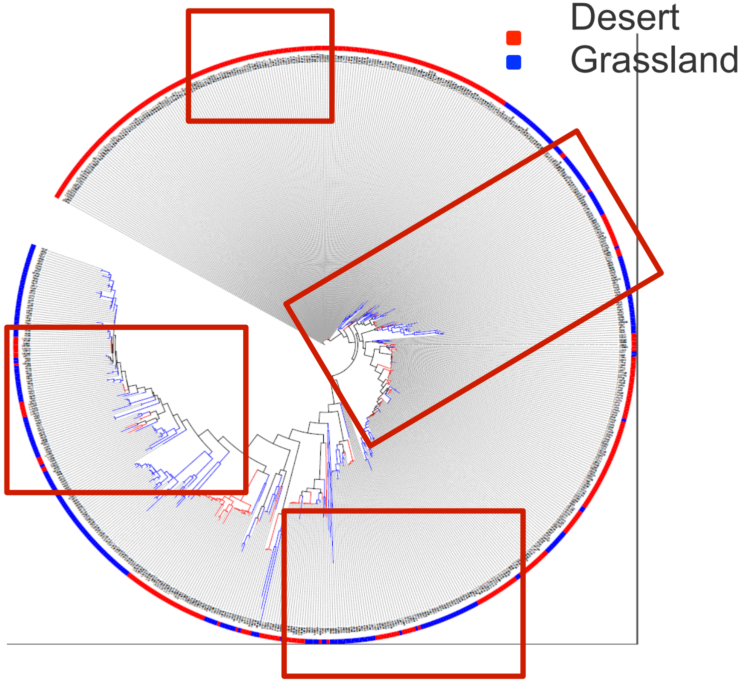
- **Primary Metabolism**
  - Involved in normal growth, development and reproduction
- **Secondary Metabolism**
  - Not directly involved in those processes, but has an important ecological function
  - Typically present in a taxonomically restricted set of organisms

Secondary Metabolite Identification from Diverse Soil Proteobacteria

MIDAS/PACTOLUS

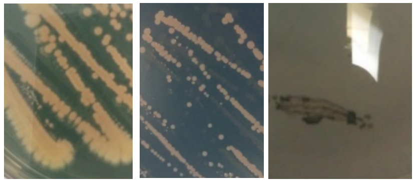


- Putative identification of metabolites missing from our standard libraries
- Link these putative IDs to their respective biosynthetic clusters

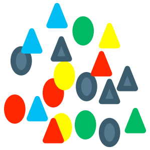


- Leveraged an existing isolate collection Eoin Brodie (UCB/LBNL)

Secondary Metabolite Identification Protocol



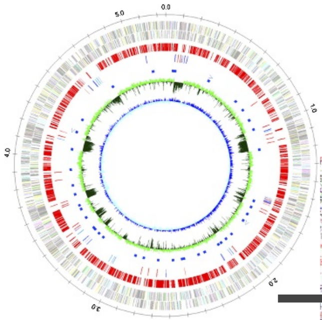
Proteobacteria isolates cultured in 1/10 R2A media

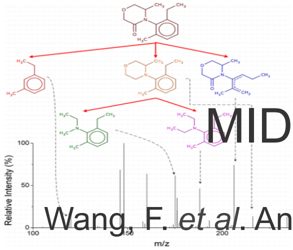


Exometabolites extracted from the media of each sample (quadruplicate)

Reconstitute in MeOH + internal standards (D_4-Lysine, ABMBA, D_5-Benzoic Acid)

Sequence





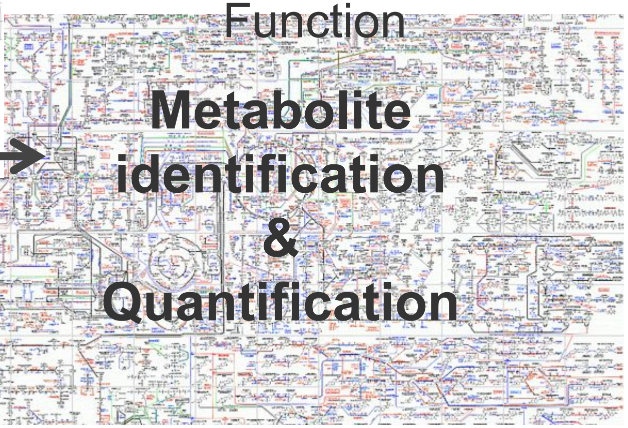
MIDAS

Wang, F. *et al*. Anal. Chem., 2014

Secondary Metabolites

What are the microbes releasing?

50 mm x 2.1mm, 1.8µm Zorbax RRHD C18 (Agilent Technologies)



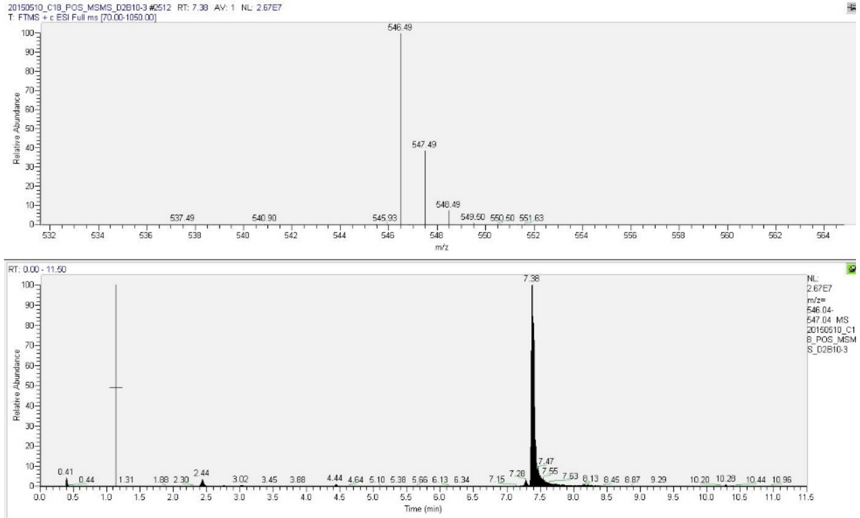
Function

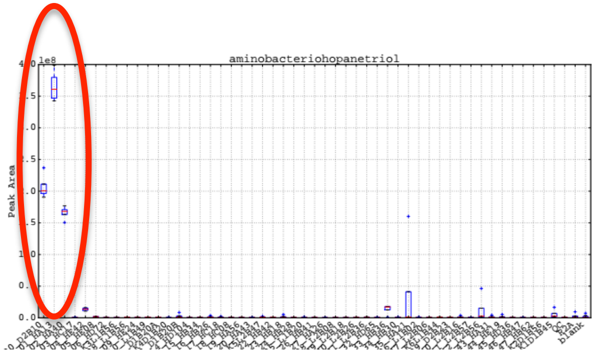
Metabolite identification & Quantification

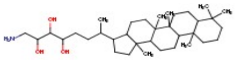
3/23/16

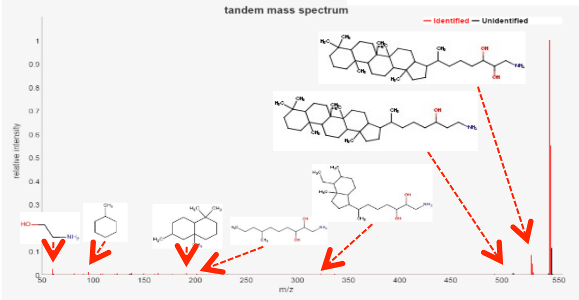
Identification of Aminobacteriohopanetriol

- **Pactolus putatively identified 100’s of compounds per sample.**
- **One compound of interest was only found to be released by 3 strains**





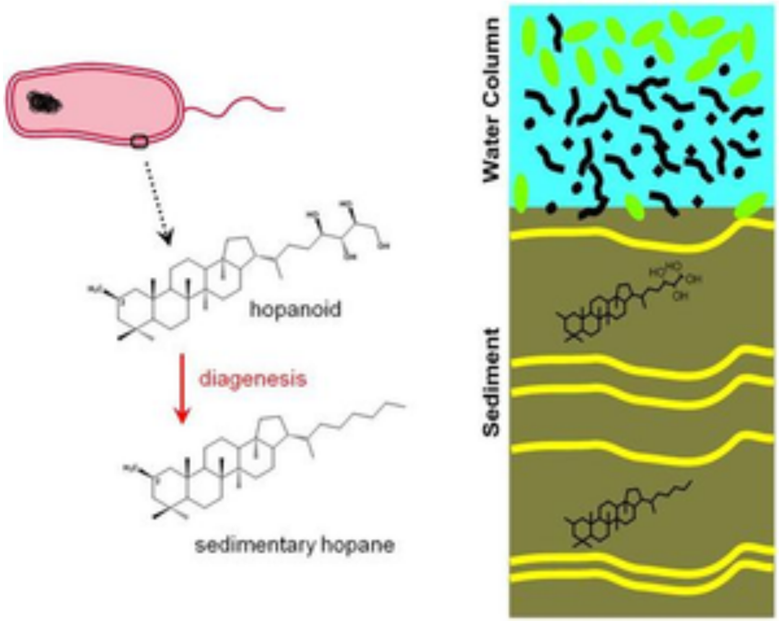




Secondary Metabolite Identification

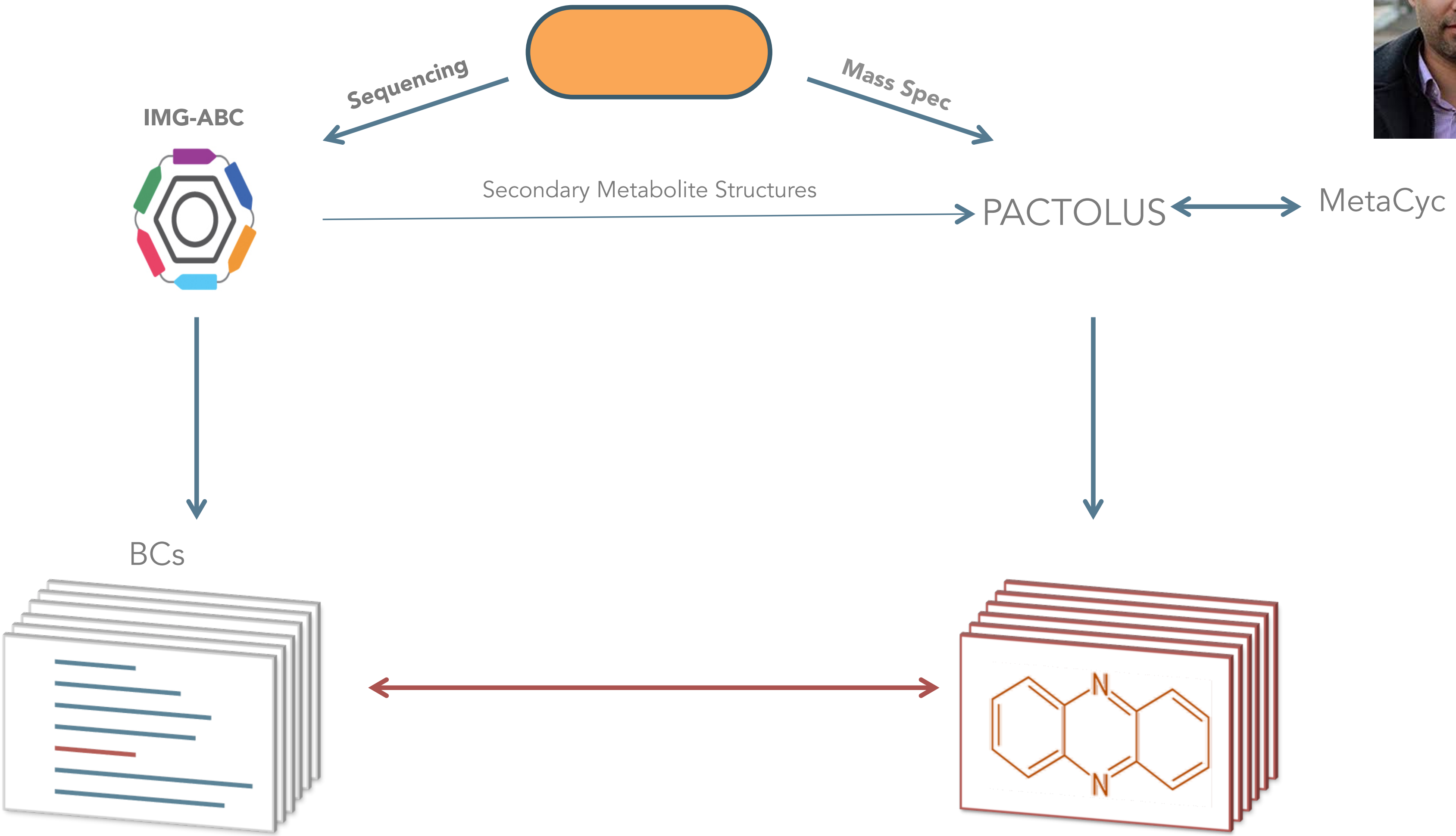
Aminobacteriohopanetriol

- **Hopanoids** – useful molecular fossil biomarkers in reconstruction of early evolution and geology
- They insert themselves in lipid bilayers, and their hydrocarbon derivatives are abundant in organic-rich sediments as old as 2500 Myr



Summons *et al*. Nature, 1999.

IMG/ABC: Bridging genomics and metabolomics



Sequencing

Mass Spec

IMG-ABC

Secondary Metabolite Structures

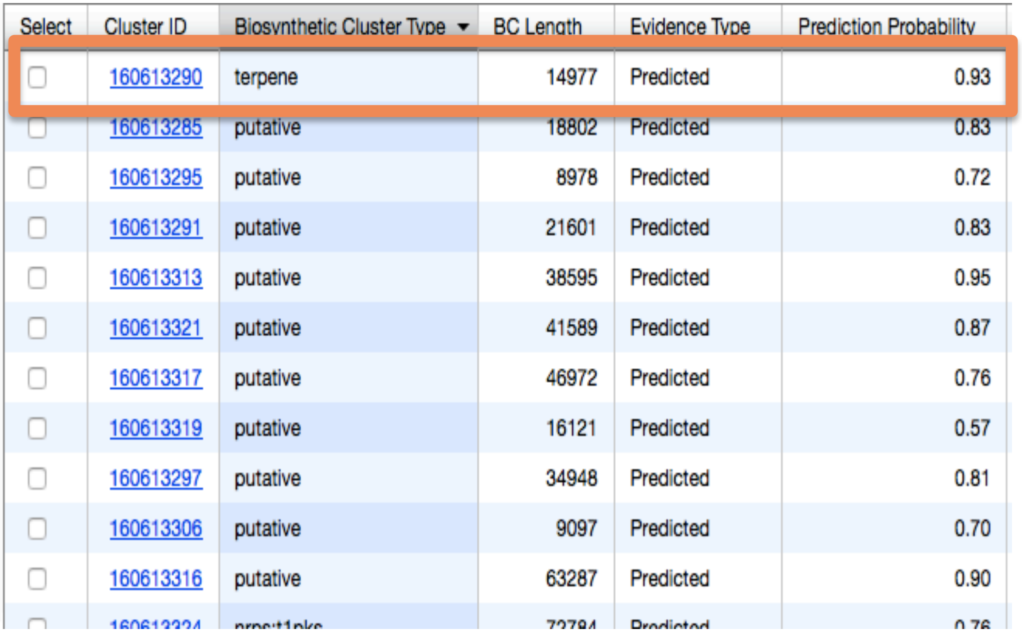
PACTOLUS

MetaCyc

BCs

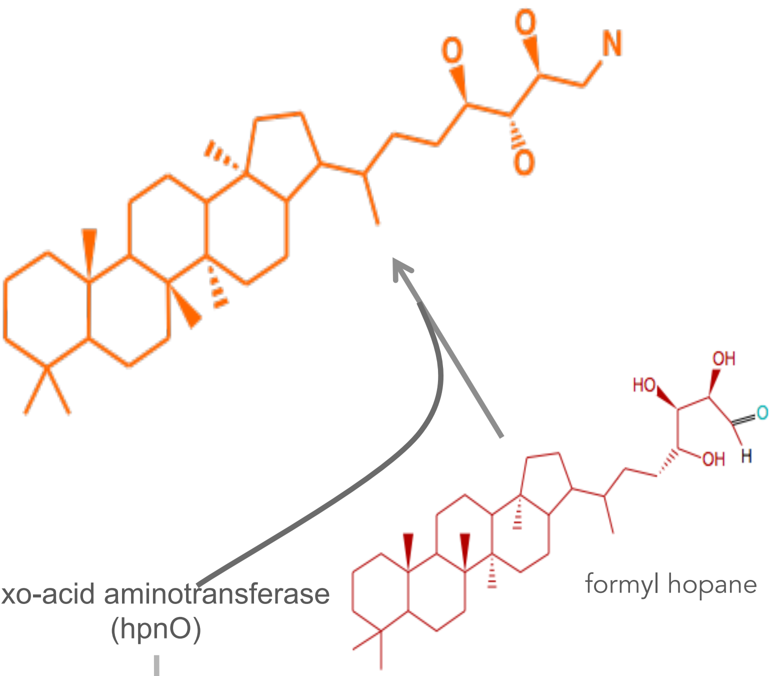
Linking secondary metabolites to biosynthetic clusters

Genomics



Select	Cluster ID	Biosynthetic Cluster Type	BC Length	Evidence Type	Prediction Probability
☐	160613290	terpene	14977	Predicted	0.93
☐	160613285	putative	18802	Predicted	0.83
☐	160613295	putative	8978	Predicted	0.72
☐	160613291	putative	21601	Predicted	0.83
☐	160613313	putative	38595	Predicted	0.95
☐	160613321	putative	41589	Predicted	0.87
☐	160613317	putative	46972	Predicted	0.76
☐	160613319	putative	16121	Predicted	0.57
☐	160613297	putative	34948	Predicted	0.81
☐	160613306	putative	9097	Predicted	0.70
☐	160613316	putative	63287	Predicted	0.90
☐	160613324	nrps;t1pks	72784	Predicted	0.76

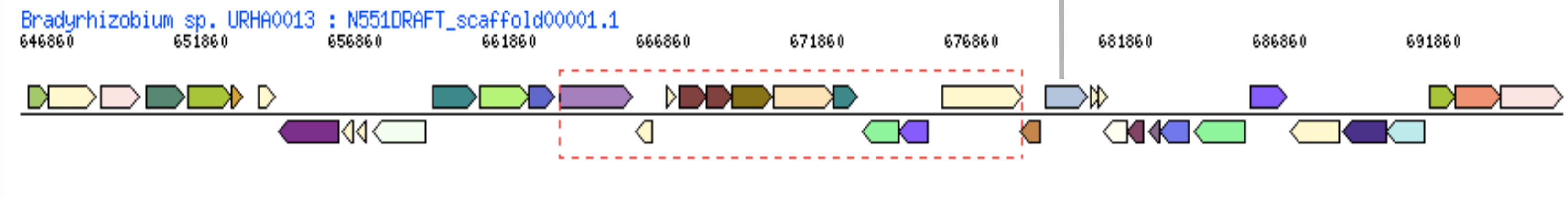
Metabolomics



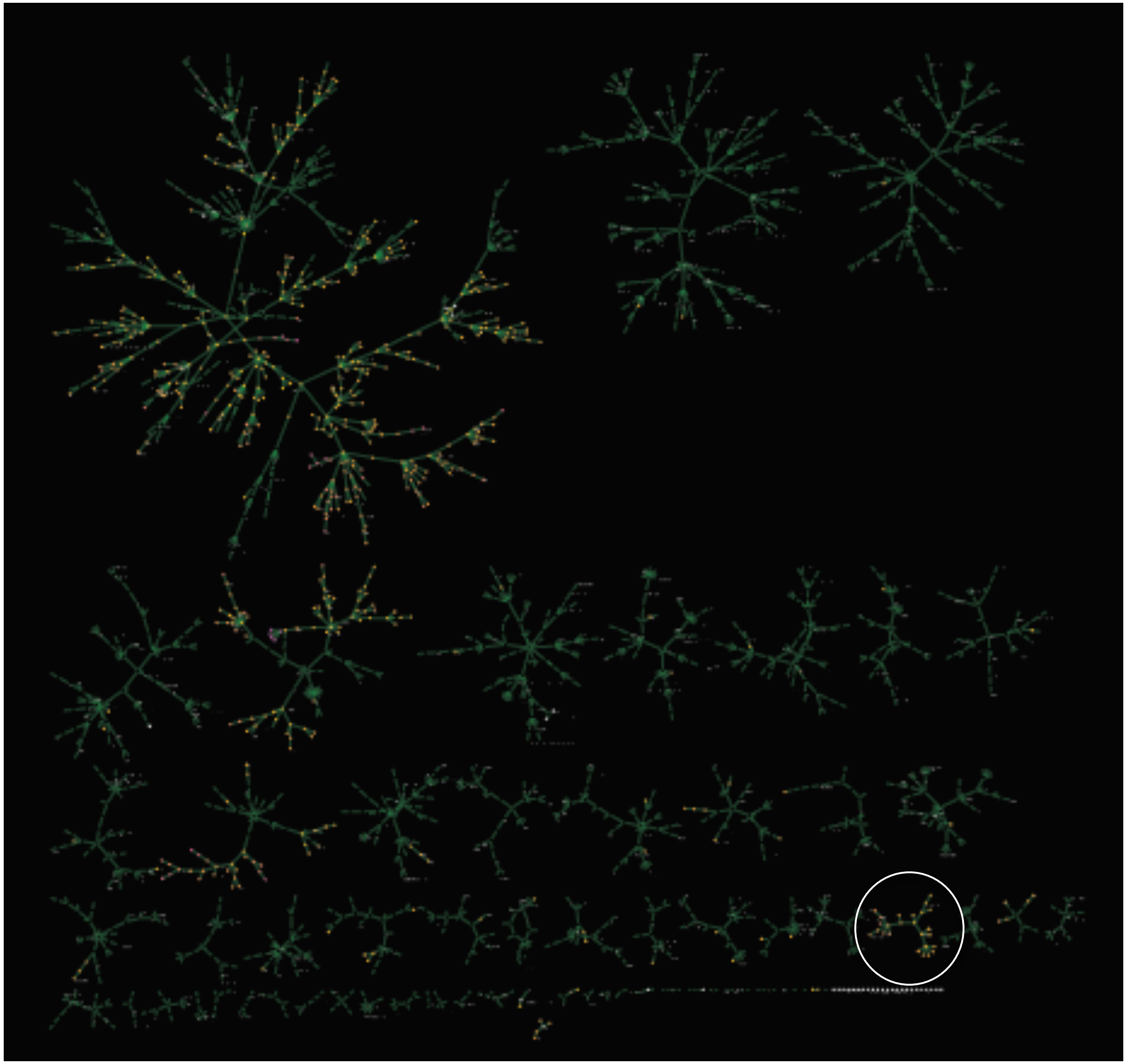
Aminobacteriohopanetriol
(hopanoid, terpene)

formyl hopane

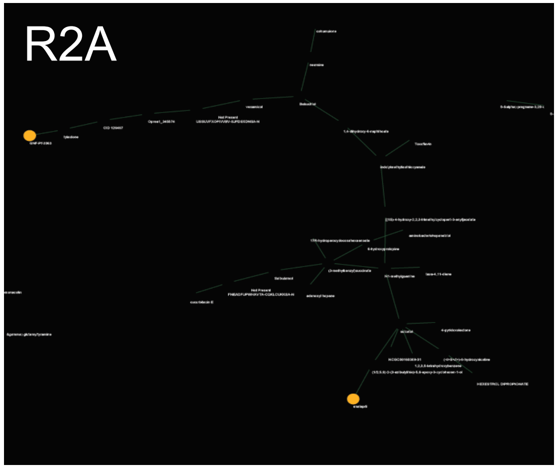
oxo-acid aminotransferase
(hpnO)

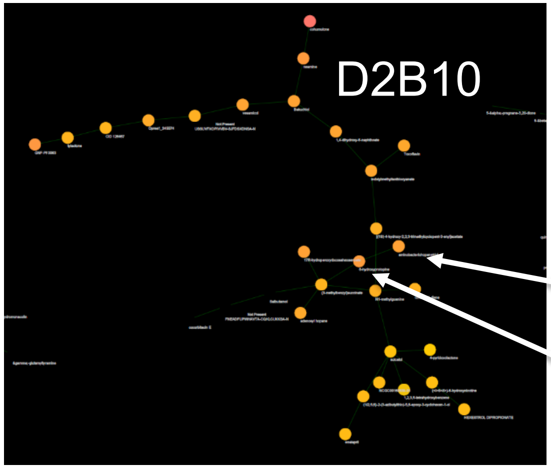


The use of chemical networks to identify closely-related secondary metabolites



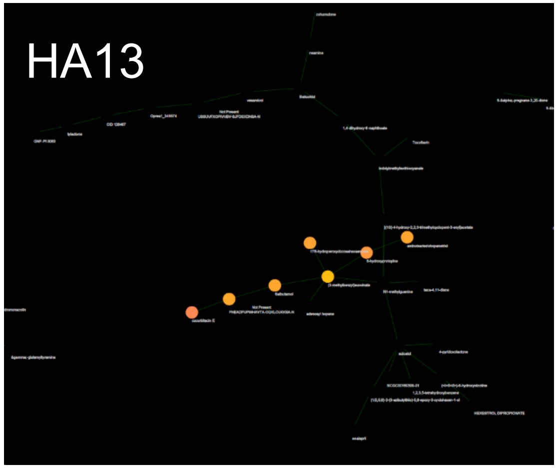
Secondary metabolites that co-cur with aminobacteriahopanetriol

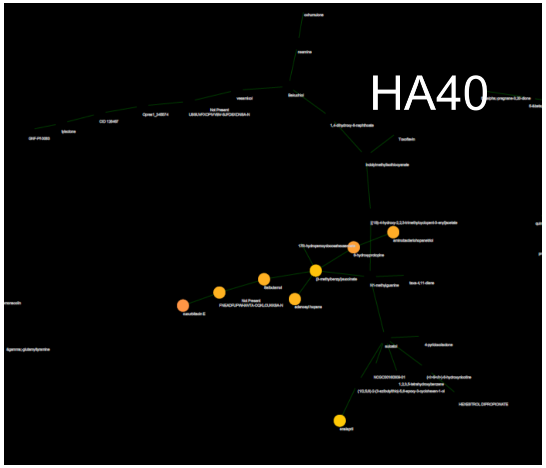




Aminobacteriohopanetriol

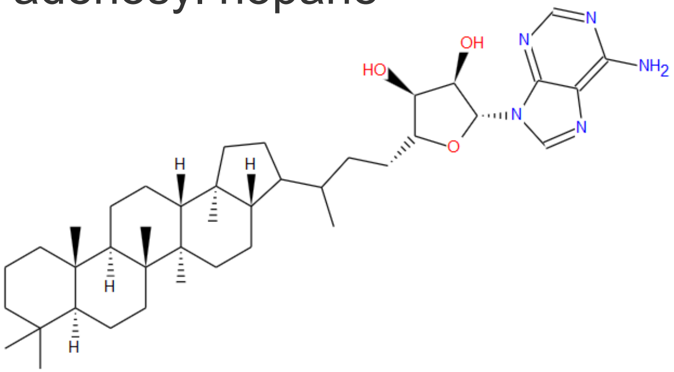
Adenosyl hopane



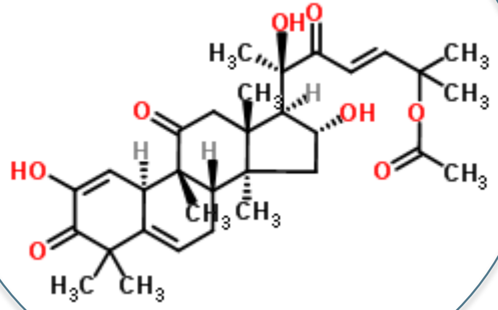


How accurate are these chemical network predictions?

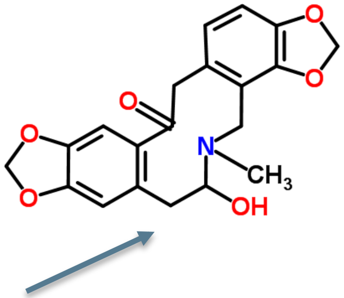
adenosyl hopane



Cucurbitacin E

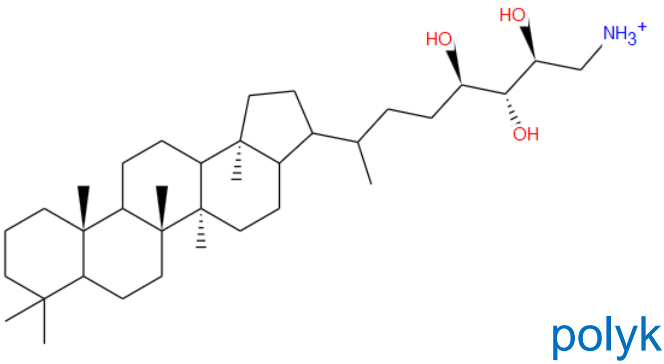


6-hydroxyprotopine

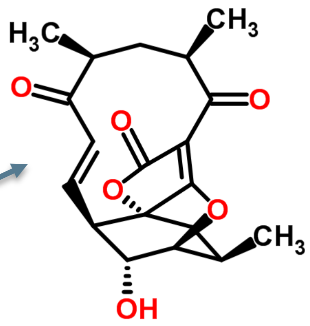


alkaloid

aminobacteriohopanetriol

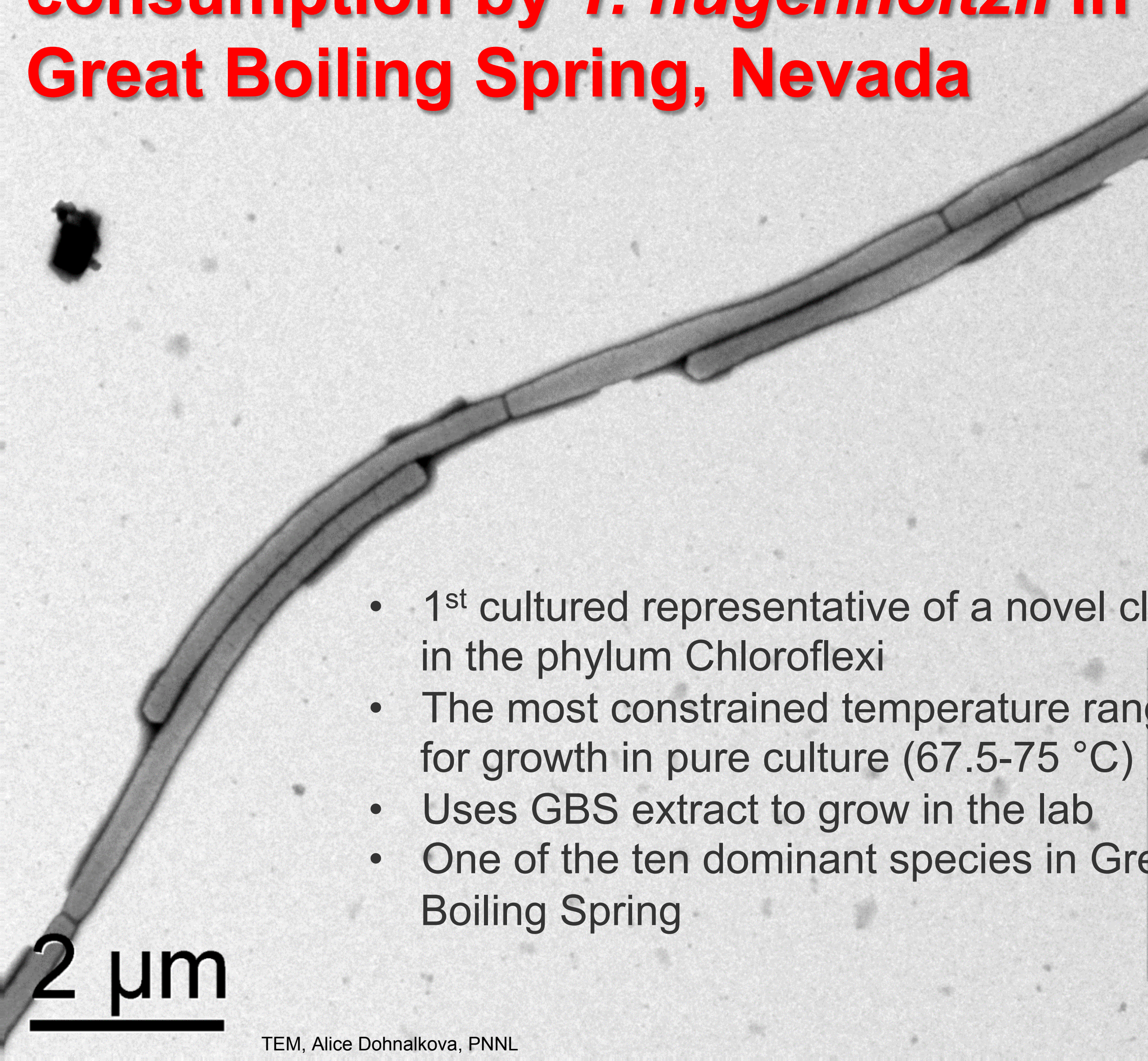


Atrop-abyssomicin C



polyketide

Understanding metabolic consumption by *T. hugenholtzii* in Great Boiling Spring, Nevada



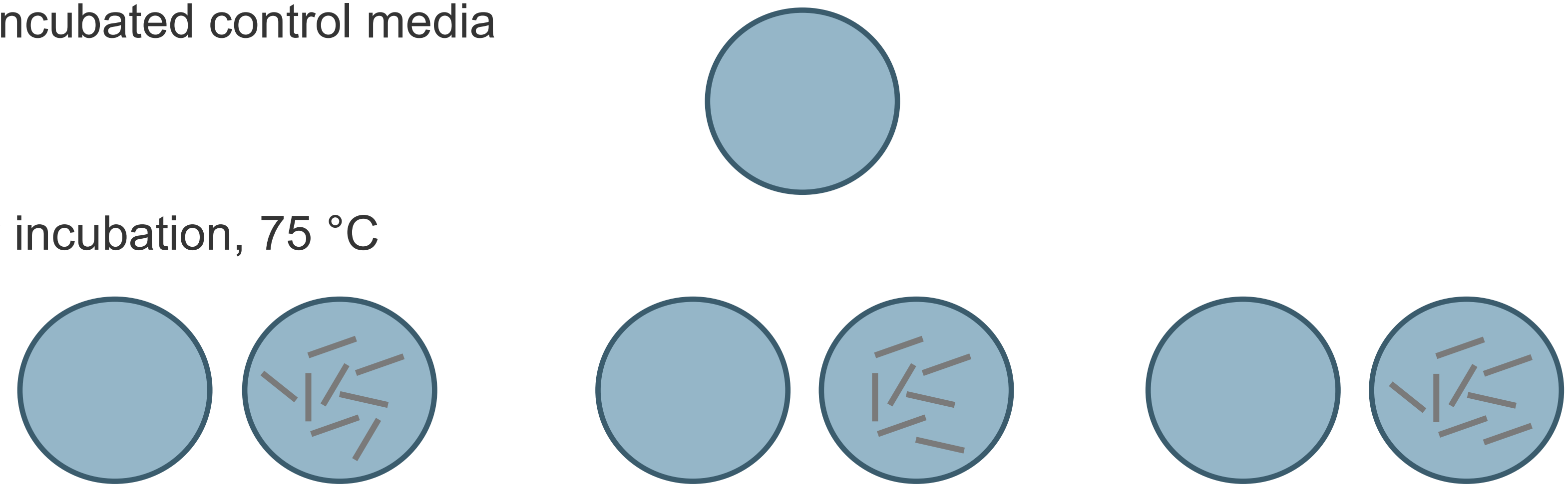
TEM, Alice Dohnalkova, PNNL

- 1st cultured representative of a novel class in the phylum Chloroflexi
- The most constrained temperature range for growth in pure culture (67.5-75 °C)
- Uses GBS extract to grow in the lab
- One of the ten dominant species in Great Boiling Spring

Great Boiling Spring, Gerlach, Nevada

Phase Contrast, Jeremy Dodsworth

What small molecules are in GBS extract that allow *T. hugenholtzii* to grow?



Non-incubated control media

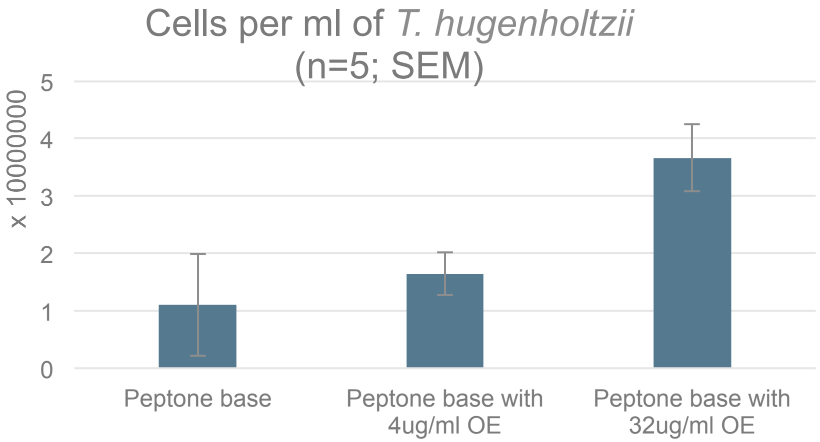
7 day incubation, 75 °C

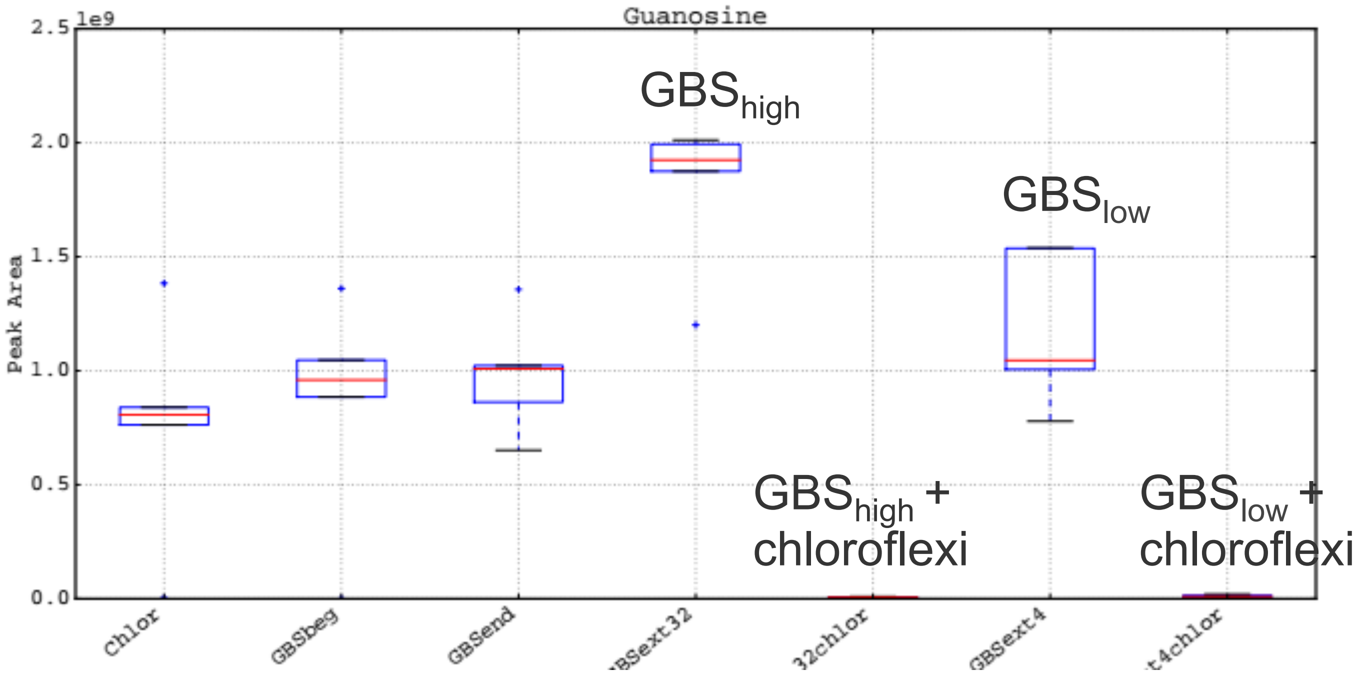
Culture medium

+ high dose GBS extract

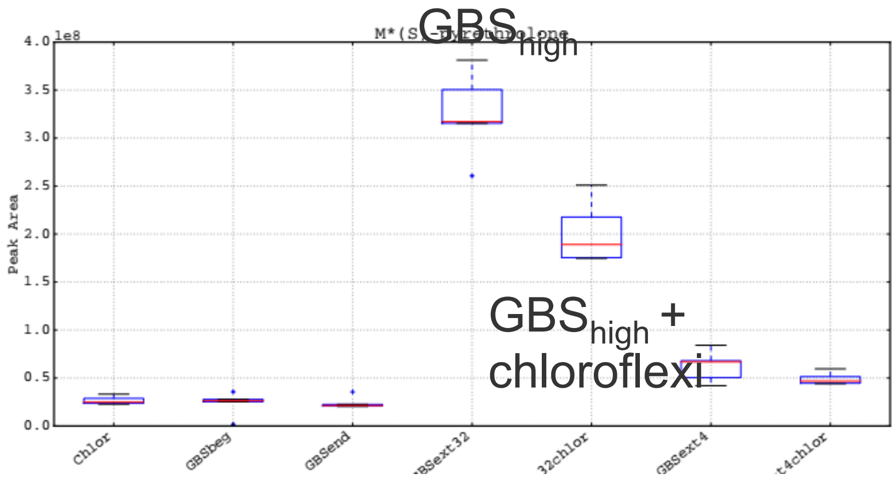
+ low dose GBS extract

T. hugenholtzii grew best with GBS extract



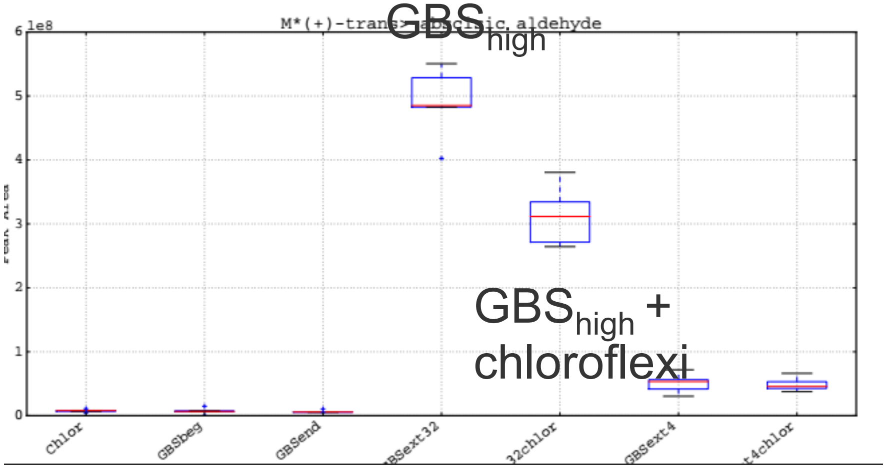


Molecules present only in GBS extract



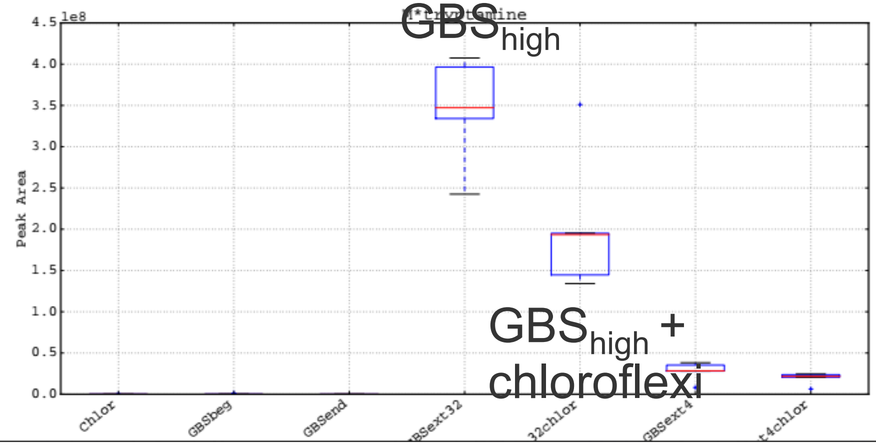
GBS_{high}

GBS_{high} + chloroflexi



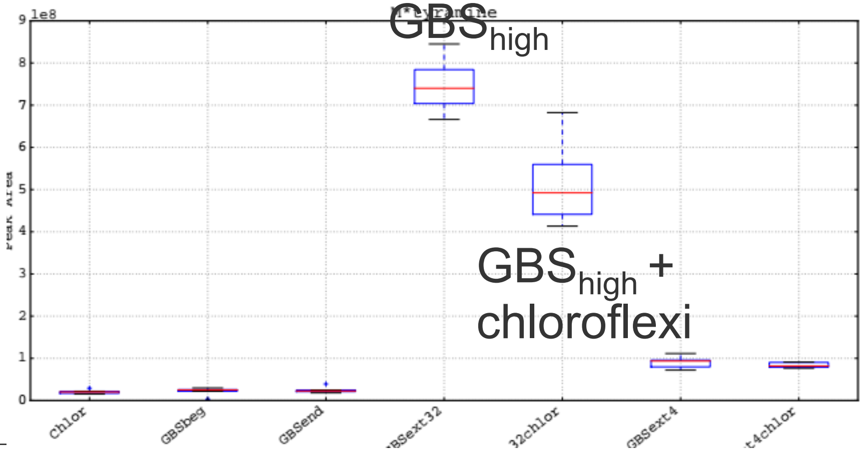
GBS_{high}

GBS_{high} + chloroflexi



GBS_{high}

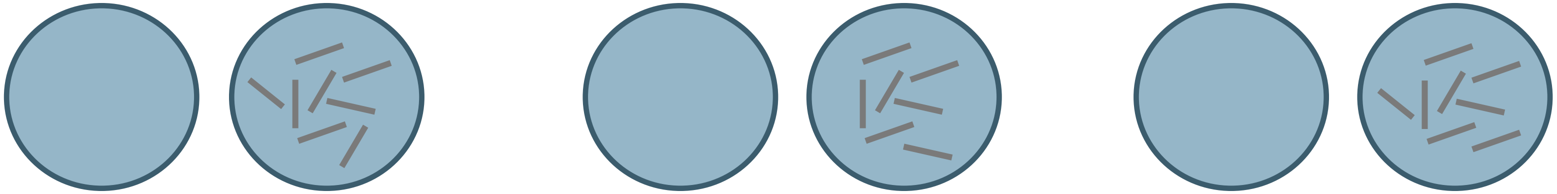
GBS_{high} + chloroflexi



GBS_{high}

GBS_{high} + chloroflexi

Preliminary Finding



Peptone medium

+ high dose GBS extract

+ identified molecules

Supplementing media with these molecules leads to *T. hugenholtzii* growth, comparable to GBS extract

Identified a way to grow *T. hugenholtzii* comparably in the lab without GBS extract

Acknowledgements

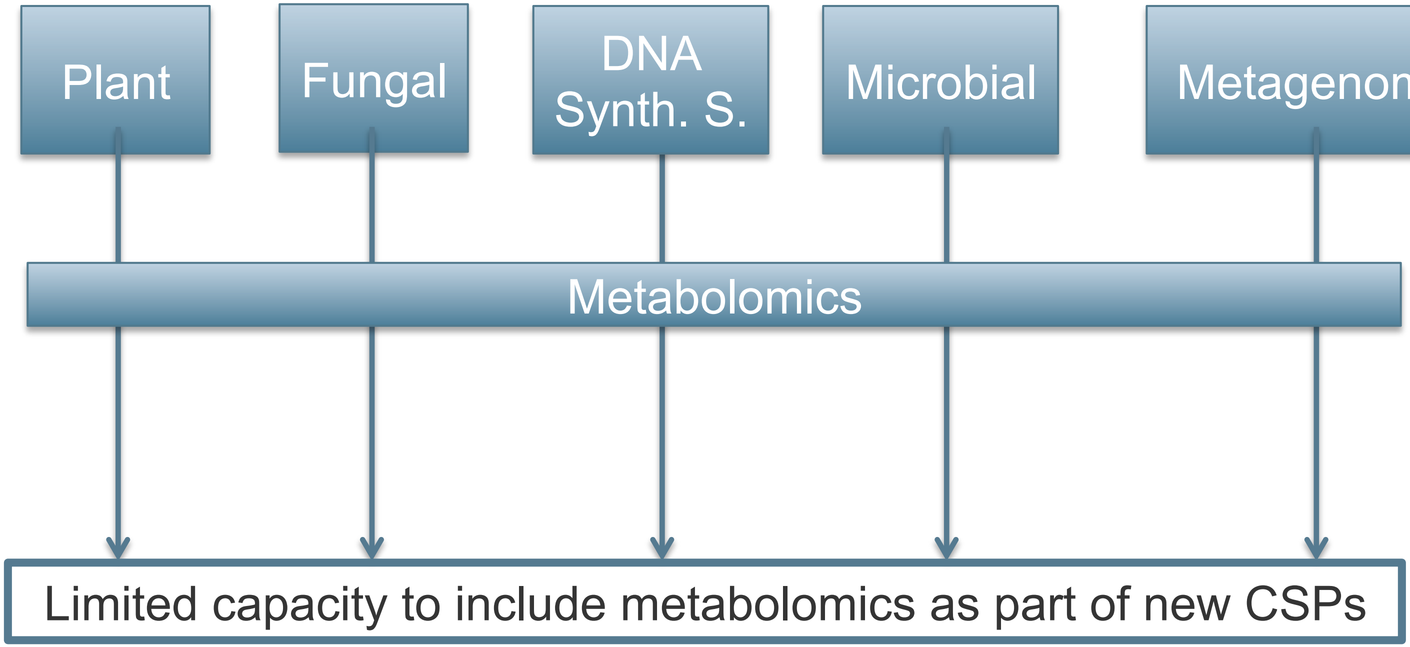
JGI
Trent Northen
Ben Bowen
Katherine Louie
Rebecca Lau
Michalis Hadjithomas
Natalia Ivanova
Nikos Kyrpides

UNLV
Brian Hedlund
Scott Thomas
Enmin Zhou

Now accepting applications for metabolomics with your CSP!

LOI due April 7th, 2016

Consider including metabolomics as part of new CSPs



Plant

Fungal

DNA Synth. S.

Microbial

Metagenome

Metabolomics

Limited capacity to include metabolomics as part of new CSPs

- ***Metabolomics should be in all cases tightly linked with sequencing and/or DNA synthesis***
- Typical metabolomics experiments are around 50-200 samples for polar metabolite analysis and 50-500 samples for secondary metabolite analysis.
- Larger requests will be considered on a case by case basis.

For questions about the appropriateness of projects, program specifics or application process, please contact Susannah Tringe: SGTringe@lbl.gov

http://bit.ly/CSP-2017