Bioresmediation – Expanding the Toolbox:
Session I – The Microbiome

Understanding Plant-Microbe-Metal Interactions in Metal-Contaminated Soils
(with a focus on arsenic)

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As, Pb, and Zn up to 4000 mg/kg

The challenge is to cost-effectively get plants to establish and survive on these types of sites. To do this efficiently, we need to understand plant-microbe-metal interactions.

Phytoremediation reduces movement of contaminants off-site through:
- Root sequestration of arsenic
- Reduction in dust generation

Gil-Loaiza et al., 2018, ES&T 15:5851
Hammond et al., 2018, ES&T 3:1156-1164
Gil-Loaiza et al., 2016, STOTEN 565:451-461
We have used traditional microbiome analysis combined with biogeochemical analyses to examine microbial diversity as a function of treatment and time for phytoremediation of mine tailings that are highly acid and contain high levels of arsenic. We have learned that:

- key microbial populations associated with pH transitions are bioindicators for either sustained future plant growth or for acid generation conditions that inhibit further plant growth
- these key indicators can be used to manage field sites and the need for re-application of compost or an alternate buffering material in regions susceptible to re-acidification to maintain beneficial bacterial communities for long-term plant establishment.

Hottenstein et al., DOI: 10.3389/fmicb.2019.01211
Honeker et al., DOI: 10.3389/fmicb.2019.01209

What I would like to focus on today is a tool that combines microbiome and biogeochemical analysis with plant metatranscriptomics. This work has been done in collaboration with the University of California-San Diego Superfund Research Program through a KC Donnelly exchange.

The data I will show have just been generated and are not yet published but show the potential for this tool to provide insights to the phytoremediation process and the way that both microbes and plants deal with arsenic.

Priyanka Kushwaha and Raina Maier (UA)
Qi Yu, Alexandria Tan and Julian Schroeder (UCSD)
Experimental set-up:

- Greenhouse study in Iron King tailings (low pH, high arsenic content)
- Treatments: 10, 15, 20% compost amendment and a potting soil control
- Soil samples were taken for amplicon sequencing and biogeochemical analyses
- Plant root and shoot tissues were taken for metatranscriptomic analysis

Quailbush
(Atriplex lentiformis)

- native plant
- drought tolerant
- salt tolerant
- successful in field trials
16S Bacterial/Archaeal rDNA Richness and Beta Diversity

Rate of compost amendment (nutrients) positively impacts richness
Differentially expressed quailbush genes in the root samples comparing two treatments: 10 vs. 20% compost amendment

RAD54: DNA repair/recombination protein

NSF: AAA-type ATPase family proteins that play a role in adaptation to salt stress

PLC2: phospholipase C2 is major membrane phospholipid that plays a role in response to abiotic stress

NUDT3: Plays a role in protection against oxidative DNA and RNA damage in plant cells

IPK2BETA: Inositol polyphosphate kinase 2 beta plays a role in plant tolerance to abiotic stress
Plant gene-microbe interactions: root sample and bulk soil

### TC10

<table>
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<tr>
<th>AT3G52870</th>
<th>CKB4</th>
<th>IBM1</th>
<th>PPH</th>
<th>SECA2</th>
<th>RAD54</th>
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<th>AT3G03305</th>
<th>AT4G10930</th>
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### TC20

| LpxD2 | OXA1 | FBW2 | AT1G54610 | PGIP1 | CBR | HEXO2 | TSO1 | ENP | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 | MET18 |
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Color scale: -1 to 1, indicating the strength and direction of gene-microbe interactions.
It appears there are taxa in the vicinity of the root that influence plant gene expression.
Take-Home Messages:

• Combining microbiome and plant transcriptome analyses is a new tool that shows great promise to identify key microbes important for plant establishment and survival.

• Importance of investment in long-term studies and developing collaboration.

• The next step is to go beyond identification and actually culture these microbes to allow study of mechanisms of plant-microbe-metal interactions.

• My colleague Paul Carini will take over and discuss high-throughput cultivation for just this purpose.

References:


High-throughput cultivation to link key taxa to specific contaminant biotransformations

paulcarini@email.arizona.edu
CariniLab.com
Synthetic microbial communities for cost-effective & efficient remediation

Screening cultures for microbes or activities of interest

High-throughput microbial cultivation approaches

Microbial cultivation: the new-old approach to microbiology
A ‘cultural’ renaissance

Human gut culture collection
73% of human gut microbiome cultured

Mouse gut culture collection
Up to 75% of functional potential

Arabidopsis rhizosphere culture collection
Up to 65% of the abundant phylotypes

Browne, et al., 2016
Lagkouvardos, et al., 2016
Bai, et al., 2015
Culturing abundant soil microbes is still a challenge

How do we access uncultured biodiversity to identify taxa that play key roles in bioremediation?
High-throughput cultivation approaches

How do we construct culture libraries that can be screened for properties of interest with high-throughput?

Can we grow ‘uncultured’ lineages using new approaches?

1. Solid media
2. Dilute liquid media “oligotrophs”
3. Rich liquid media; ultra high throughput
1. Solid media

- Pick colonies to deep 96-well plates containing liquid growth medium

- Incubate

- Cryo-preserve cultures at -80°C

- Screen cultures
2

Dilute liquid media “oligotrophs”

count cells with flow cytometry

separate cells

count cells with flow cytometry

Screen for growth with high-throughput flow cytometry

Dilute cells into 96-well plates containing dilute medium to ~1-5 cells well⁻¹

array growing wells only & cryo-preserve culture aliquots

Screen growing cultures
3. Rich liquid media; ultra high throughput

Screen arrays for growth with GALT Prospector

75 mm × 25 mm 
~6,000 3.4 nl wells

Inoculate GALT Prospector array chip

Dilute cells into growth medium that contains a fluorometric growth indicator

Cryo-preserve cultures

Array positive chambers into microtiter plates containing fresh medium & incubate

Screen cultures
Screening culture libraries

High-throughput classification
Illumina 16S rRNA gene sequencing

Functional gene screens
Presence-absence or coupled to sequencing

Colori- or fluorometric assays
Screen with plate reader

Nocardioidaceae spp.
Nocardioidaceae spp.
Streptomycetaceae spp.
Conexibacteraceae spp.
Micrococcaceae spp.
Actinomycetales spp.
Pseudomonadaceae spp.
Pseudomonadaceae spp.
Crenotrichaceae spp.
Rhizobiales spp.
Rhodospirillales spp.
SynComs to deduce microbial arsenic transformations in gut

Build correlation network from SynComs with significantly and strongly correlated As transformation-sequestration profiles

Quantify As biotransformation & sequestration capacity of each SynCom

Identify As biotransformation-sequestration functional guilds & test in vivo or in situ
Synthetic microbial communities for cost-effective & efficient remediation

Screening cultures for microbes or activities of interest

High-throughput microbial cultivation approaches

Microbial cultivation: the new-old approach to microbiology