



Bioremediation – 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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<https://superfund.arizona.edu/>

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



Iron King Mine and Humboldt Smelter Superfund site

Phytoremediation reduces movement of contaminants off-site through:

- Root sequestration of arsenic
- Reduction in dust generation

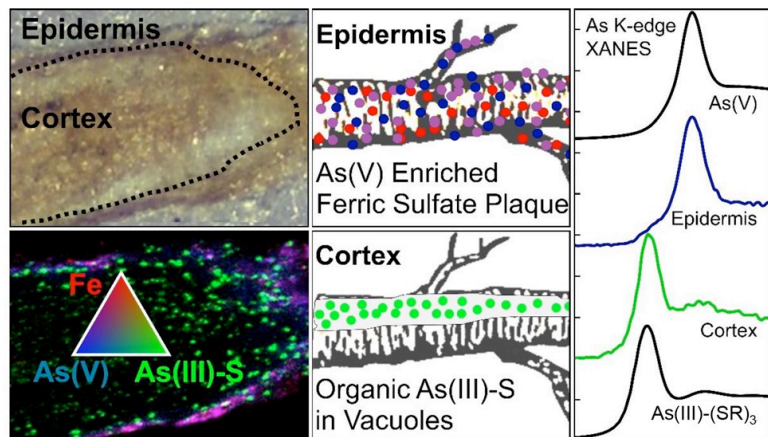
The challenge is to cost-effectively get plants to establish and survive on these types of sites. To do this efficiently, we need to understand plant-microbe-metal interactions.



Wind Erosion



Exposure



➔ Reduced As bioavailability

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.



Dr. Priyanka Kushwaha

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)



Dr. Julian Schroeder

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

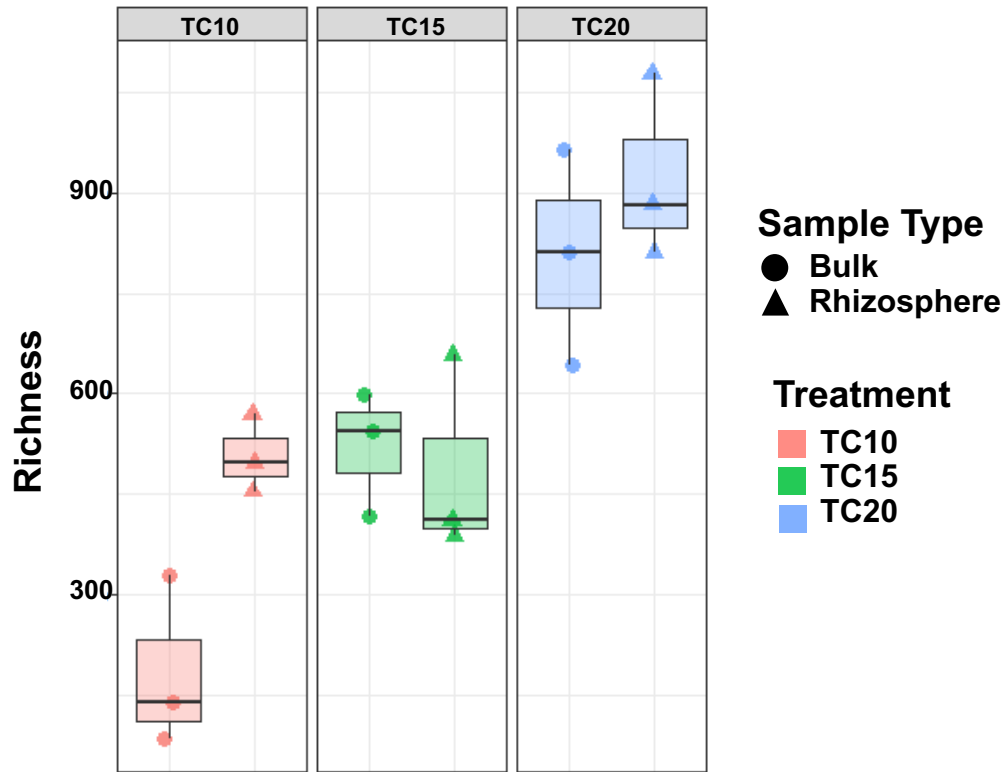


Quailbush
(*Atriplex lentiformis*)

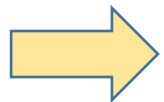
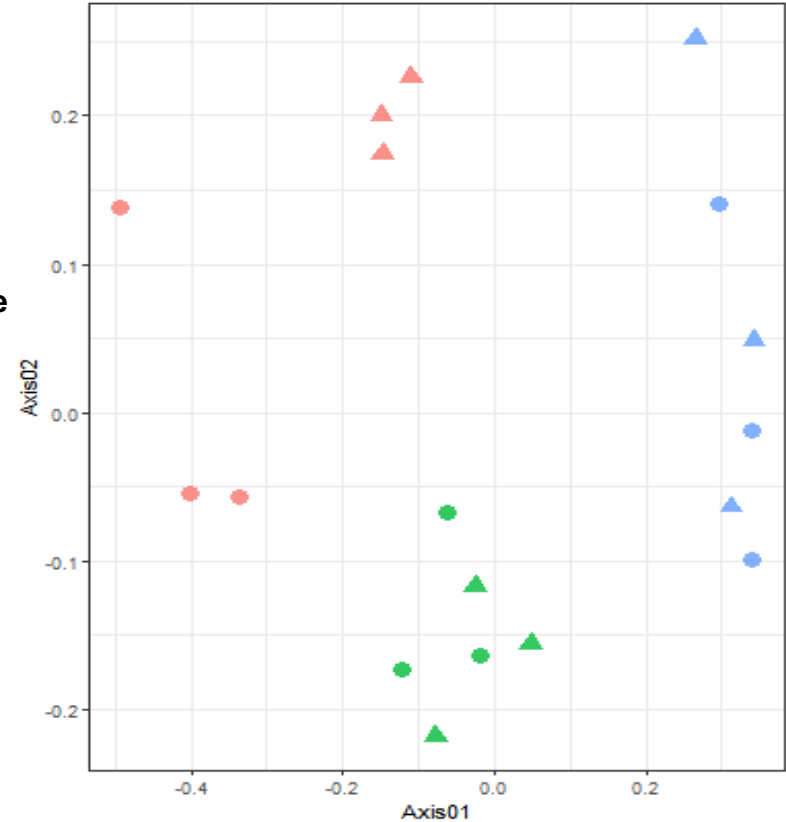
- native plant
- drought tolerant
- salt tolerant
- successful in field trials

16S Bacterial/Archaeal rDNA Richness and Beta Diversity

Richness

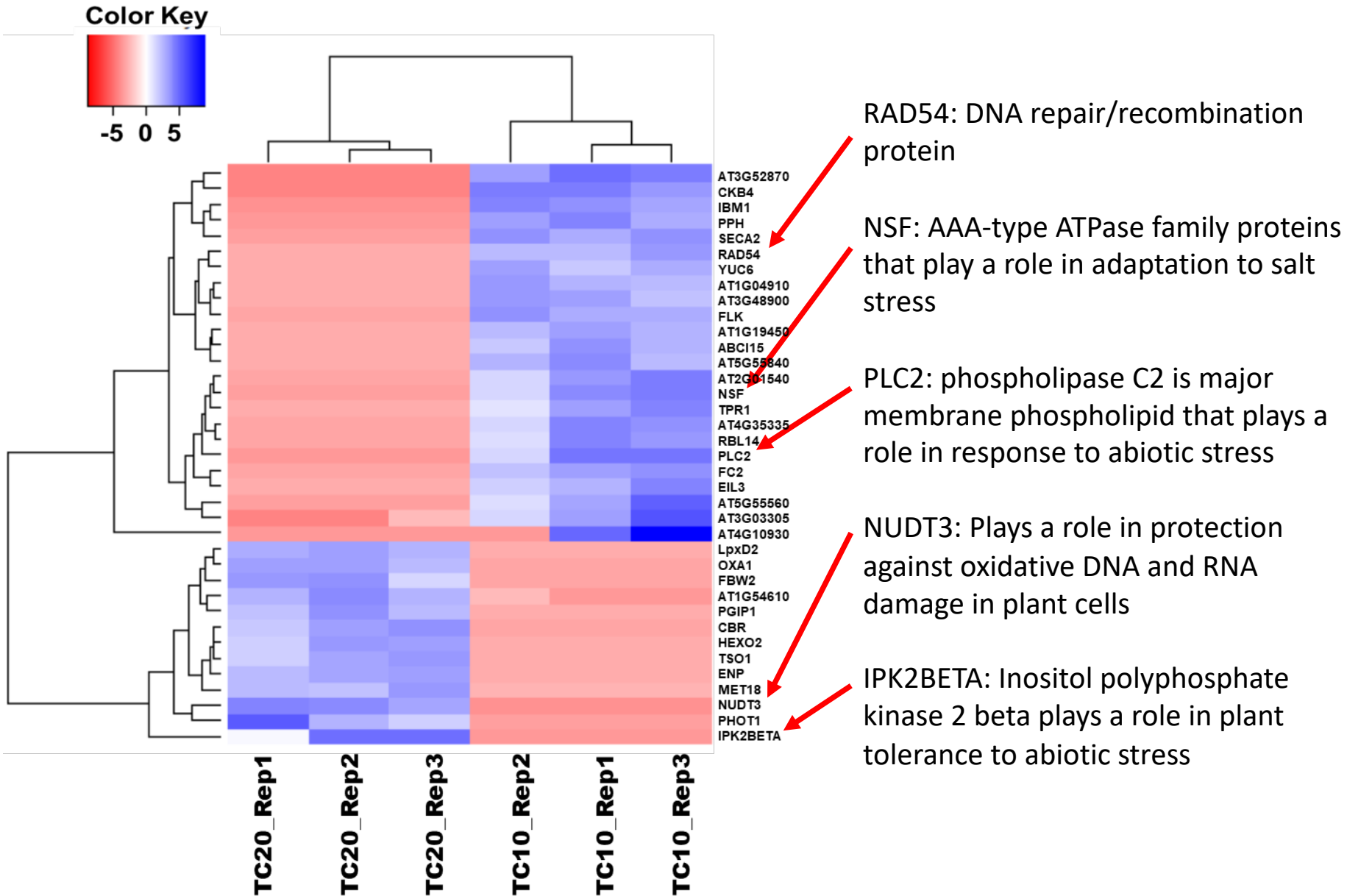


Beta Diversity



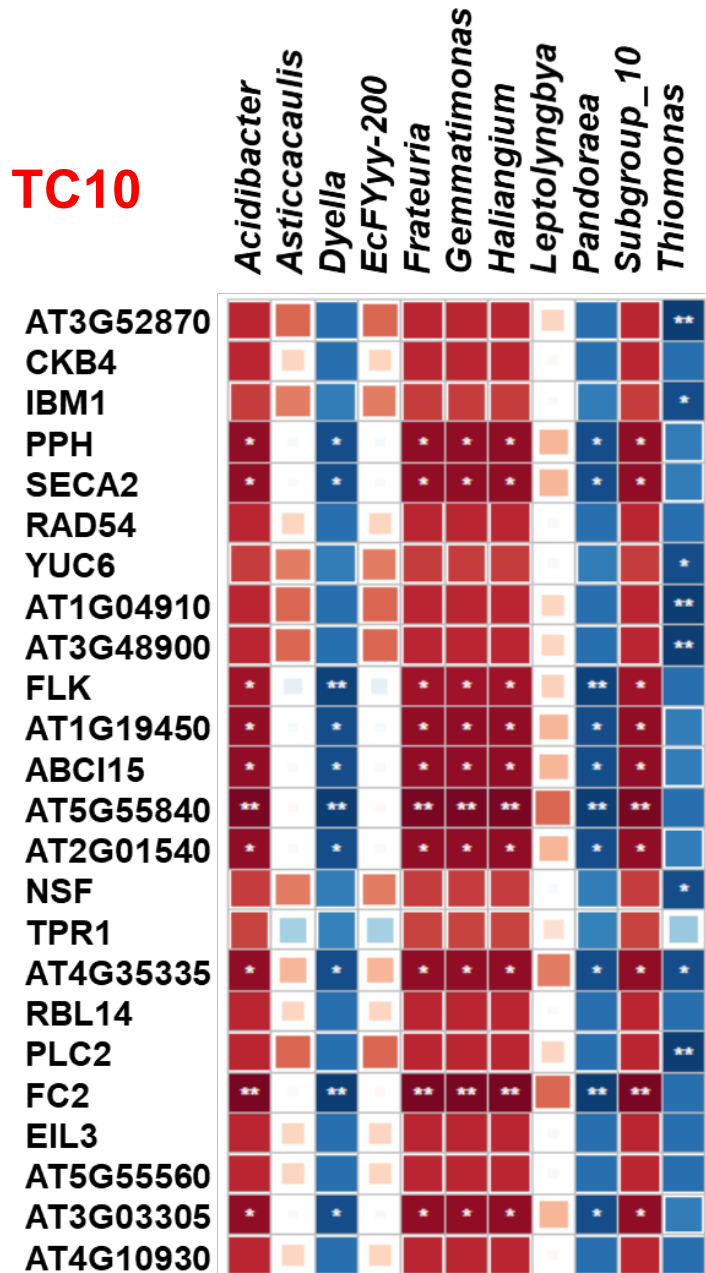
Rate of compost amendment (nutrients) positively impacts richness

Differentially expressed quailbush genes in the root samples comparing two treatments: 10 vs. 20% compost amendment



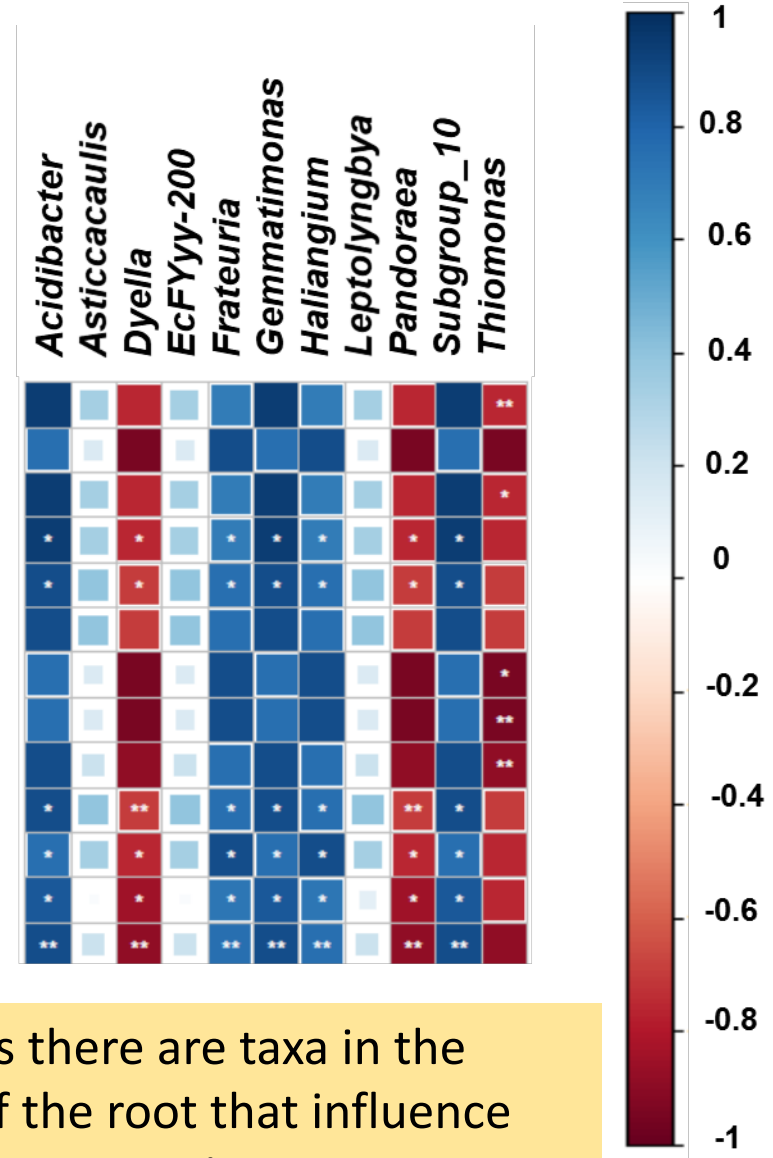
Plant gene-microbe interactions: root sample and rhizosphere influenced soil

TC10



TC20

- LpxD2
- OXA1
- FBW2
- AT1G54610
- PGIP1
- CBR
- HEXO2
- TSO1
- ENP
- MET18
- NUDT3
- PHOT1
- IPK2BETA



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:

Young, Ellen, et al. "Microbiome and ecotypic adaption of *Holcus lanatus* (L.) to extremes of its soil pH range, investigated through transcriptome sequencing." *Microbiome* 6.1 (2018): 48.

Yu, Peng, et al. "Root type and soil phosphate determine the taxonomic landscape of colonizing fungi and the transcriptome of field-grown maize roots." *new phytologist* 217.3 (2018): 1240-1253.

Dayama, Gargi, et al. "Interactions between the gut microbiome and host gene regulation in cystic fibrosis." *BioRxiv* (2019): 596312.



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

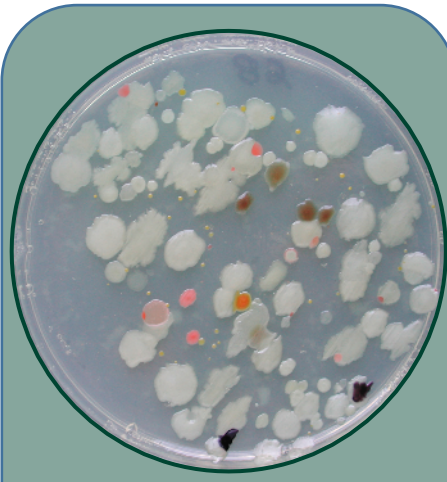


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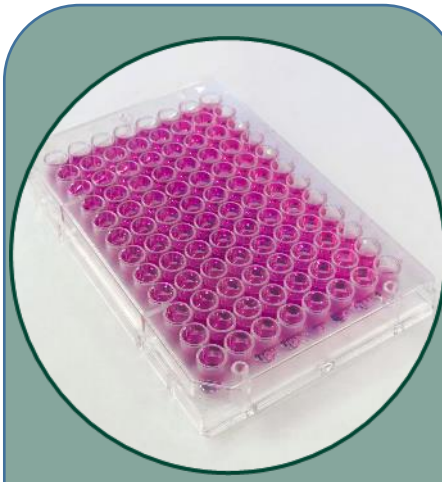
CariniLab.com



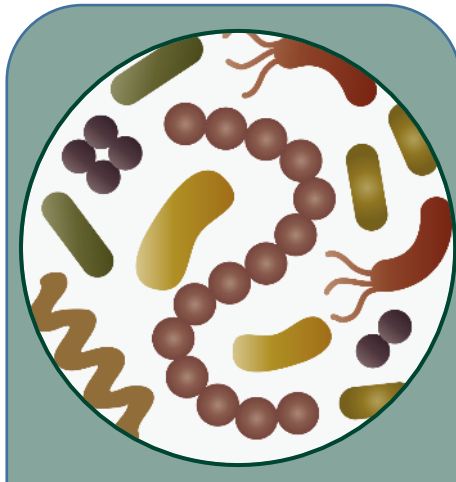
Microbial
cultivation: the
new-old
approach to
microbiology



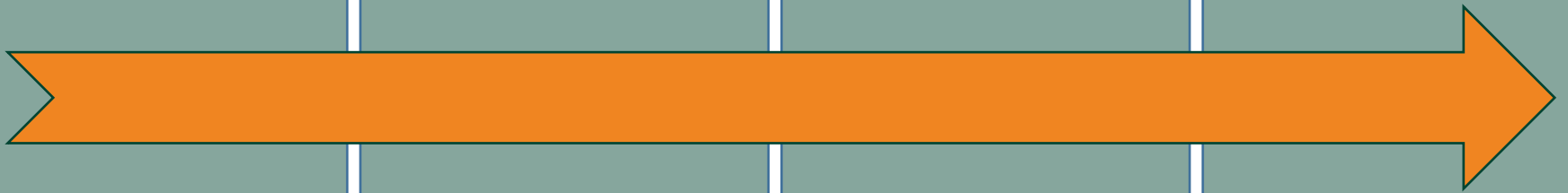
High -
throughput
microbial
cultivation
approaches

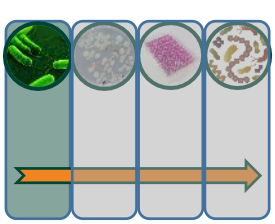


Screening
cultures for
microbes or
activities of
interest

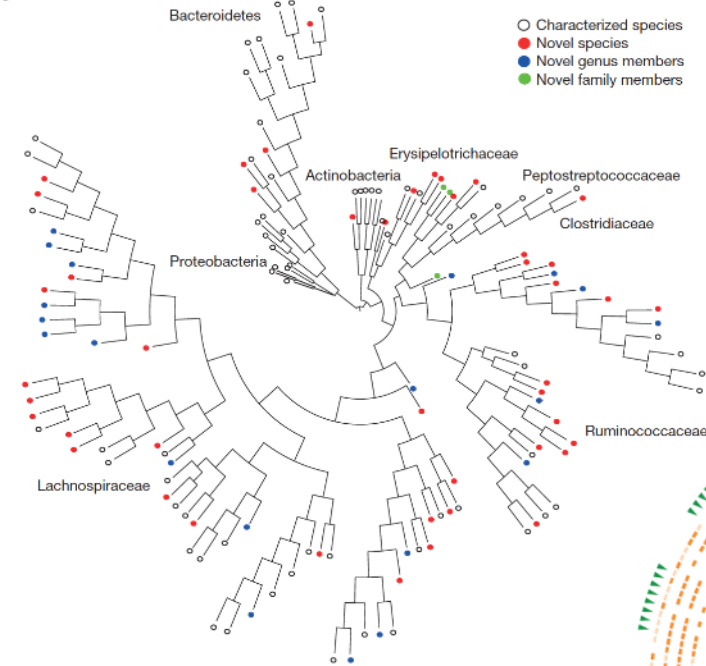


Synthetic
microbial
communities for
cost-effective &
efficient
remediation



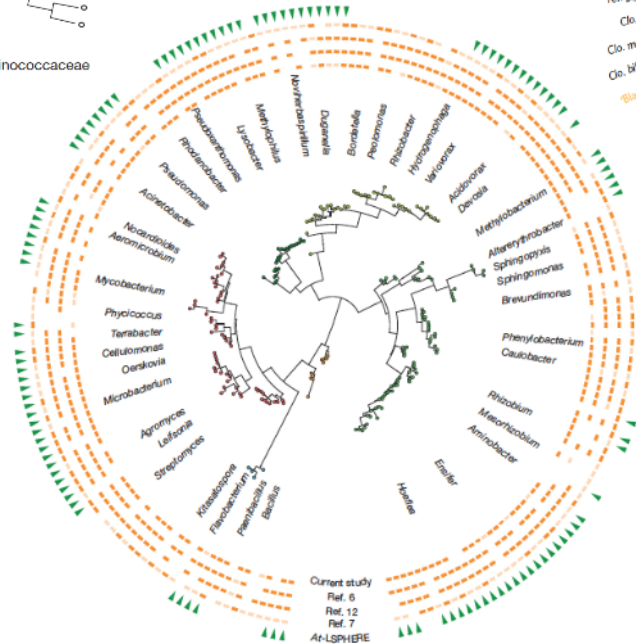


A 'cultural' renaissance



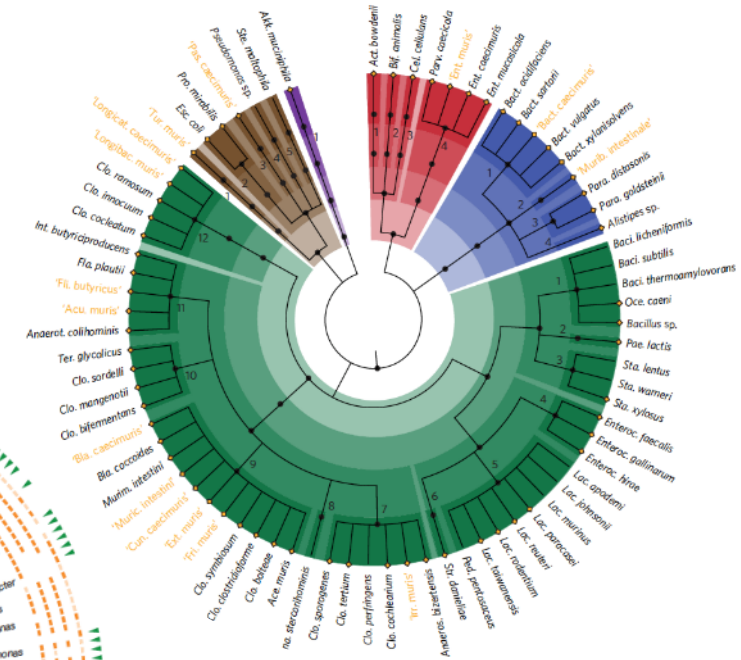
Human gut
culture collection

73% of human gut microbiome cultured



Arabidopsis rhizosphere
culture collection

Up to 65% of the abundant phylotypes



Mouse gut

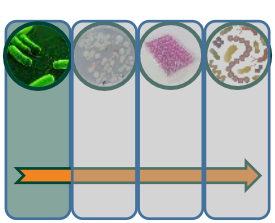
culture collection

Up to 75% of functional potential

Browne, et al., 2016

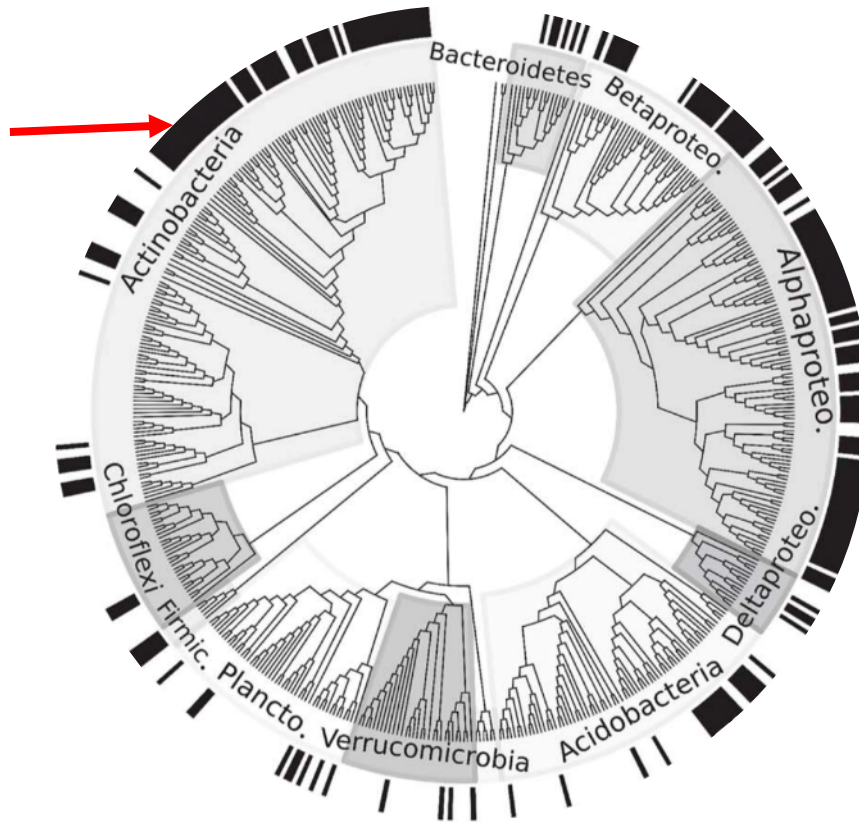
Lagkouvardos, et al., 2016

Bai, et al., 2015

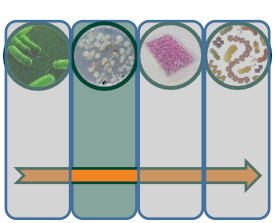


Culturing abundant soil microbes is still a challenge

Cultured lineages



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

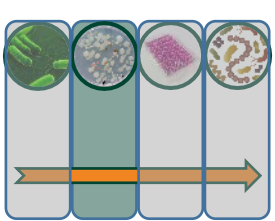
Solid media

2

Dilute liquid media
"oligotrophs"

3

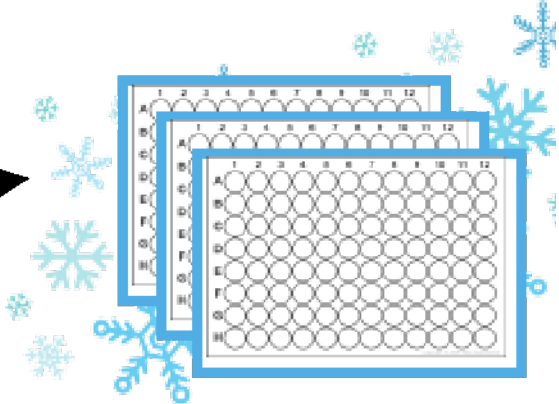
Rich liquid media; ultra high
throughput



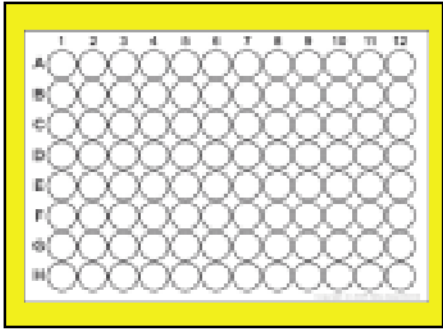
1

Solid media

cryo-preserve
cultures at -80°C

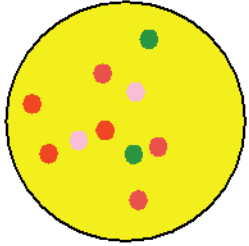
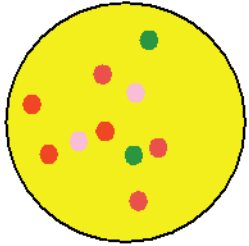


Pick colonies to
deep 96-well plates
containing liquid
growth medium

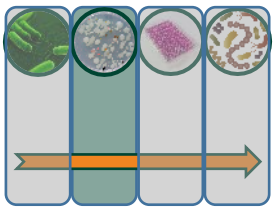


incubate

screen
cultures



solid
medium



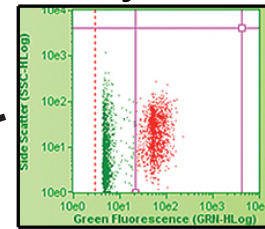
2

Dilute liquid media
"oligotrophs"



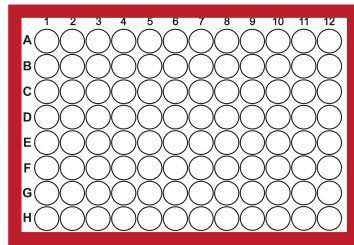
separate
cells

count cells with
flow cytometry

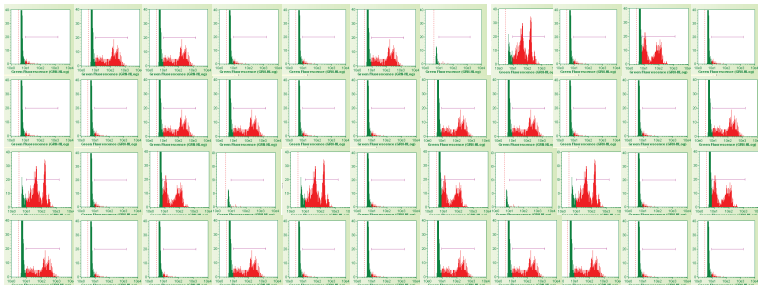
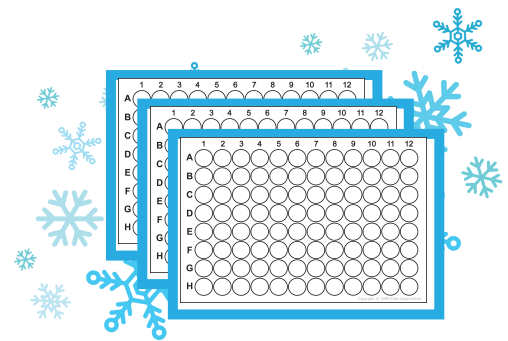


Dilute cells into
96-well plates
containing dilute
medium to ~1-5
cells well⁻¹

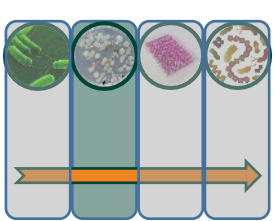
Screen for growth
with
high-throughput
flow cytometry



array growing
wells only &
cryo-preserve
culture aliquots



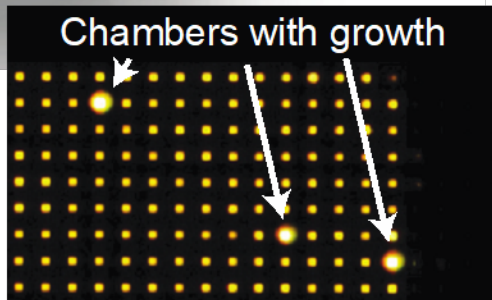
Screen
growing
cultures



3

Rich liquid media; ultra high throughput

Screen arrays for growth with GALT Prospector

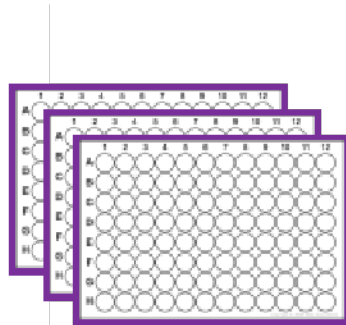


75 mm × 25 mm
~6,000 3.4 nl wells

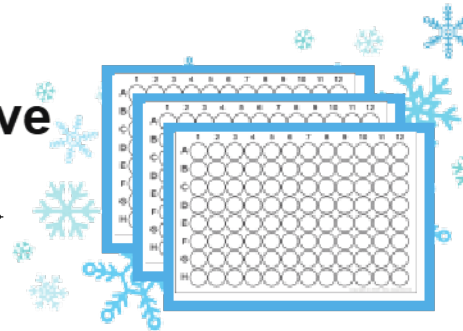


separate cells

Dilute cells into growth medium that contains a fluorometric growth indicator

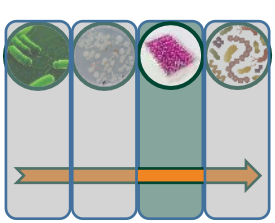


Cryo-preserve cultures

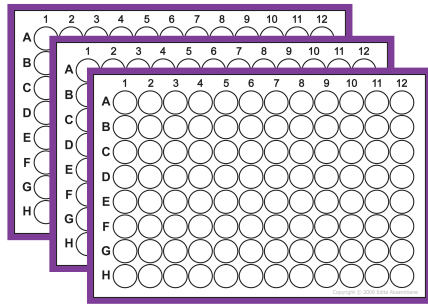


Array positive chambers into microtiter plates containing fresh medium & incubate

Screen cultures

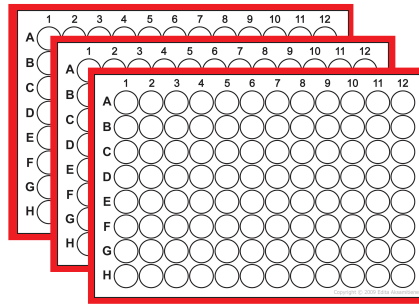
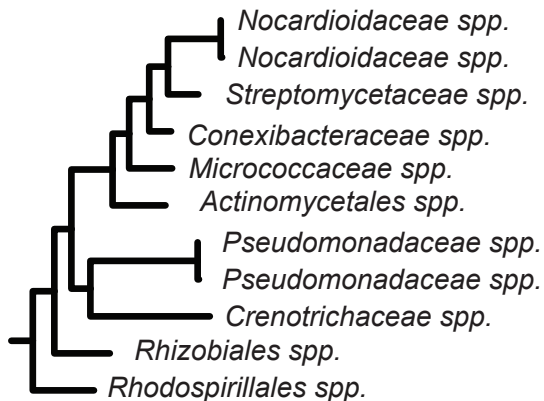


Screening culture libraries



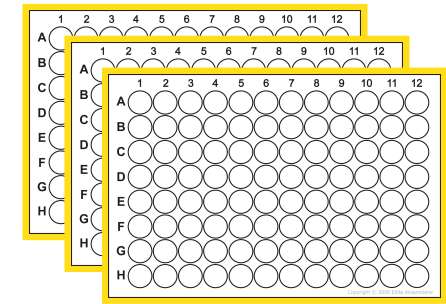
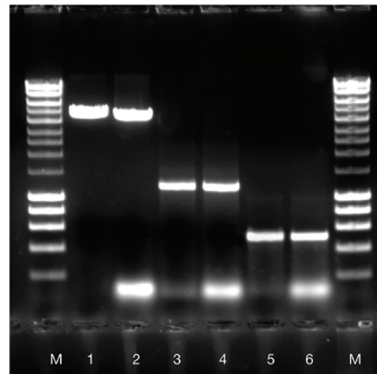
High-throughput classification

Illumina 16S rRNA gene sequencing



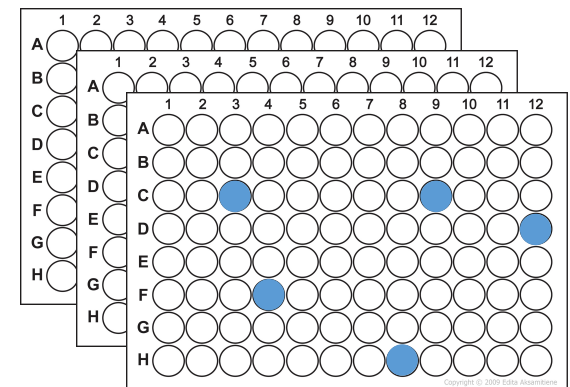
Functional gene screens

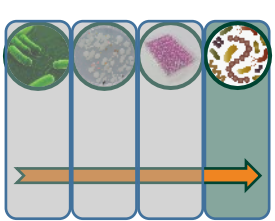
Presence-absence or coupled to sequencing



Colorimetric or fluorometric assays

Screen with plate reader

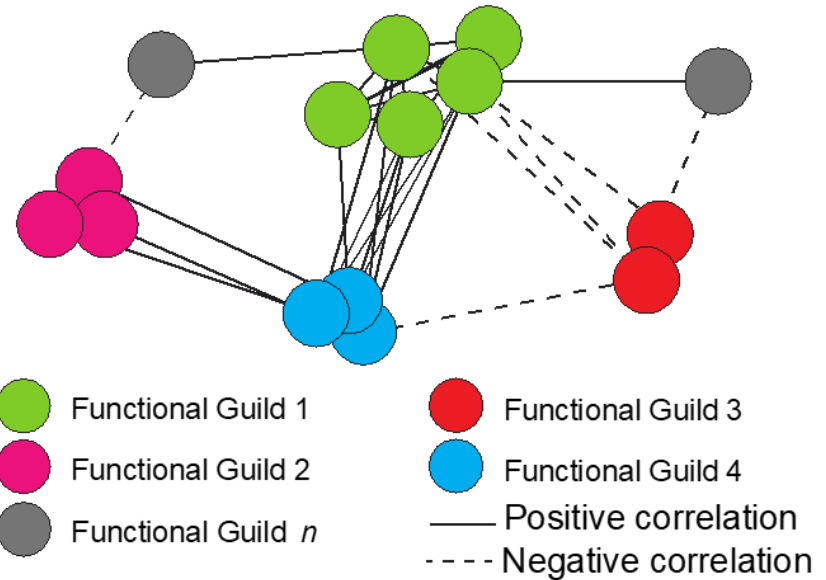




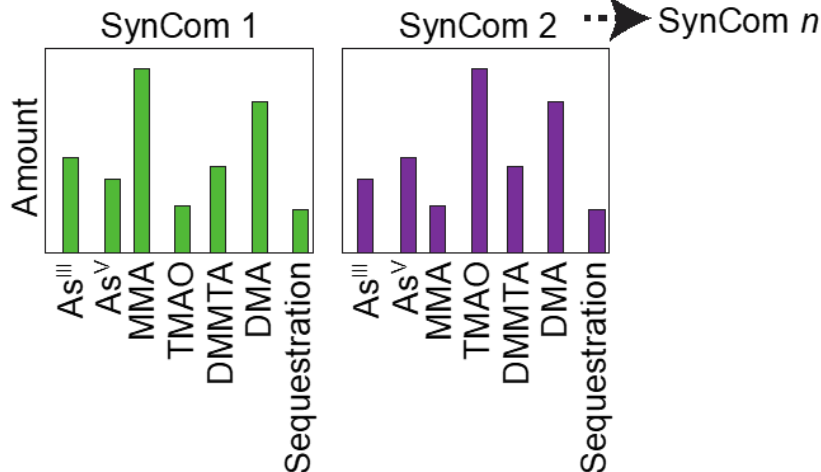
SynComs to deduce microbial arsenic transformations in gut



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



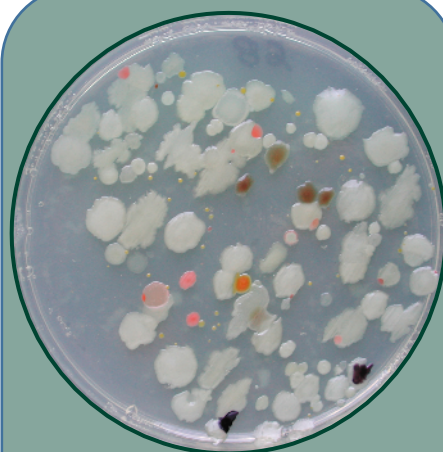
Quantify As biotransformation & sequestration capacity of each SynCom



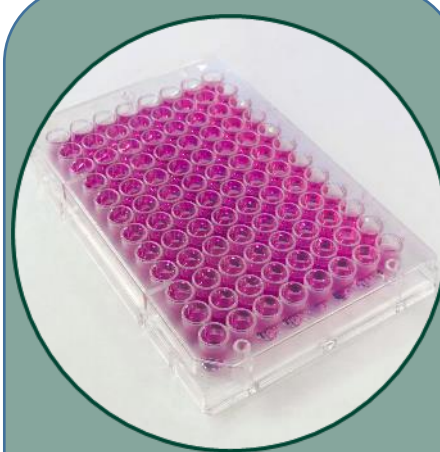
Identify As biotransformation-sequestration functional guilds & test *in vivo* or *in situ*



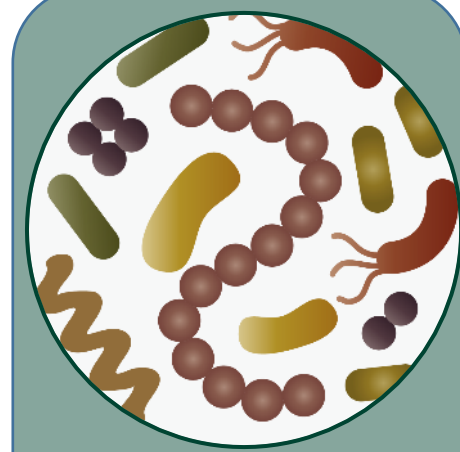
Microbial
cultivation: the
new-old
approach to
microbiology



High -
throughput
microbial
cultivation
approaches



Screening
cultures for
microbes or
activities of
interest



Synthetic
microbial
communities for
cost-effective &
efficient
remediation

