

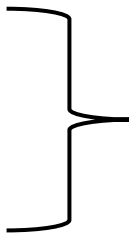
Creating a bioremediation model system—
choosing and modifying a chassis

Module Outline

- M2D1: Environmental heavy metal contamination
- M2D2: Model system – target selection and engineering approach
- **M2D3: Model system – choosing a chassis host**
- M2D4: Screening a system—high throughput vs functional screens
- M2D5: Analysis of elemental metals – laboratory and field approaches
- M2D6: Applying remediation strategies—advantages and pitfalls
- M2D7: Engineering a problem-specific bioremediation solution
- M2D8: Comm Lab




Intro



Design



Test



Apply



Review

Lecture overview

- Engineered bioremediation system
 - Modified transporter
 - Host to express transporter
- Chassis is the host cell used to house and support engineered biological material
 - replication machinery
 - metabolic resources
- Common chassis/hosts for bioremediation
 - Cyanobacteria
 - Microalgae
 - Yeast



What do you want from a chassis?

What do you want from a chassis?

Biological relevance

- Has relevant endogenous machinery
 - To support the biologically engineered material
- Able to grow in desired environment

Genetically tractable

- Straightforward protocols for genetic manipulation
- Laboratory strains of bacteria/cells/animals = highly genetically tractable
 - Environmental strains = less so, but possible

Economically viable

- Scalable
 - Relatively rapid growth
 - Resource usage
- Able to thrive without extensive assistance

Additional useful host features

- Robust growth
 - Support production of non-native proteins
 - Increased survival rates following manipulation
- Able to operate under non-ideal conditions
 - temperature range
 - pH range
 - low resource availability
- Well characterized
 - genome
 - growing conditions
 - pathogenicity
 - DNA delivery tools



Single-cell organisms for bioremediation

Natural defense mechanisms against heavy metal toxicity that can be utilized for bioremediation

- Defenses most useful to modulate for remediation
 - Metal transformation
 - Metal sequestration
- Good chassis hosts for remediation
 - Bacteria
 - Microalgae
 - Yeast

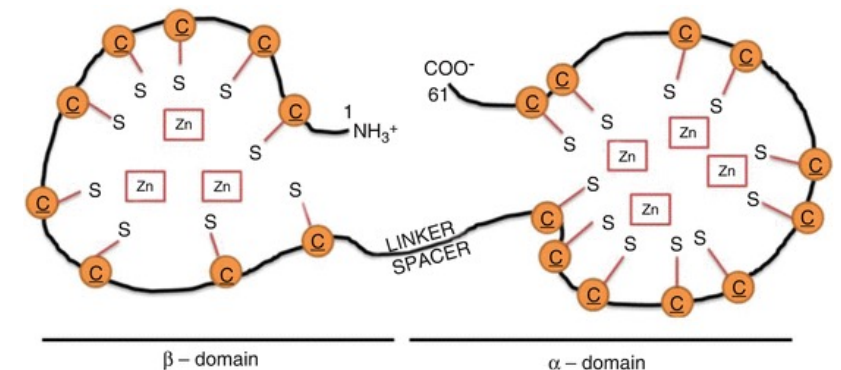
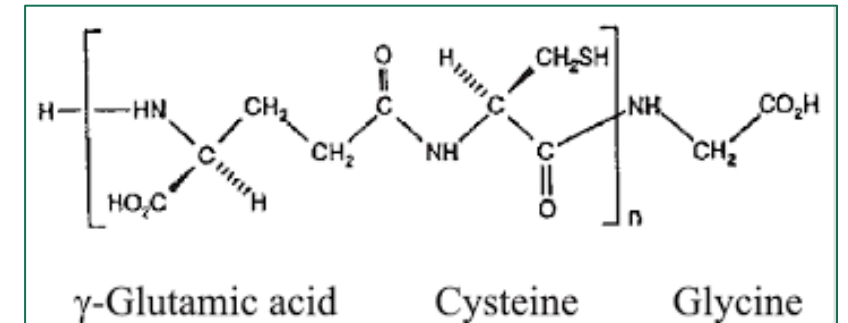
Metal transformation by bioremediation hosts

Oxidation, reduction, methylation of metal to less toxic form

- Example: Cr(VI) and Cr(III) are the most stable oxidation states of chromium
 - Cr(VI) is most toxic but is reduced to Cr(III) by chromium reductase enzyme or interaction with intracellular reducing agents

Chelation by cysteine-rich polypeptides

- Phytochelatins
 - Enzymatically synthesized from glutathione
- Metallothioneins
 - Gene-encoded
 - Contain two cysteine-rich metal binding domains

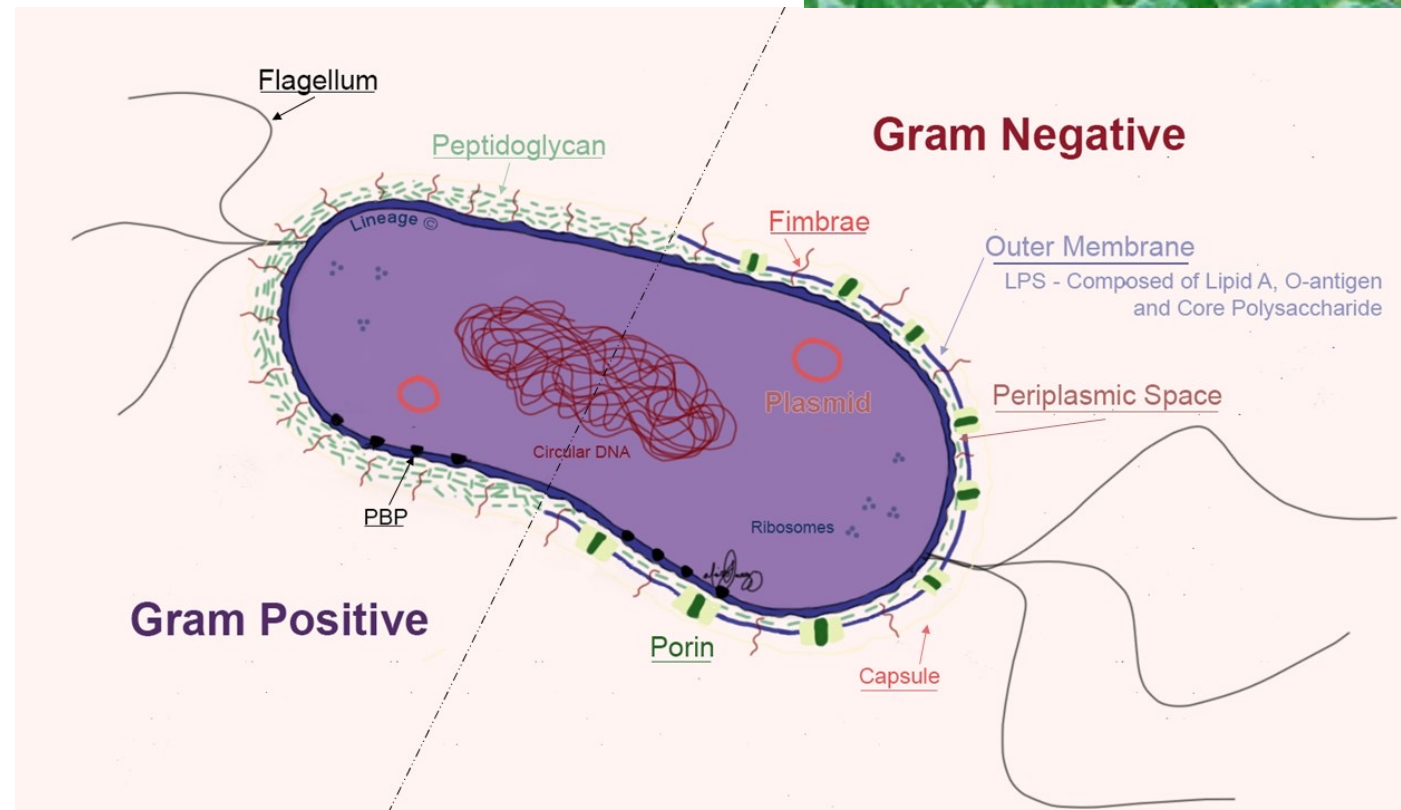


Metal sequestration

- **Adsorption** to the cell surface can be mediated by
 - Binding of heavy metal cations with negatively charged groups
 - Interactions with polysaccharides
 - Interactions with proteins containing carboxyl, hydroxyl, carbonyl, amide functional groups
- **Accumulation** in an intracellular vesicle
 - GSH-conjugated metals can be transported into cell vacuoles by ATP-Binding Cassette (ABC) transporters for sequestration

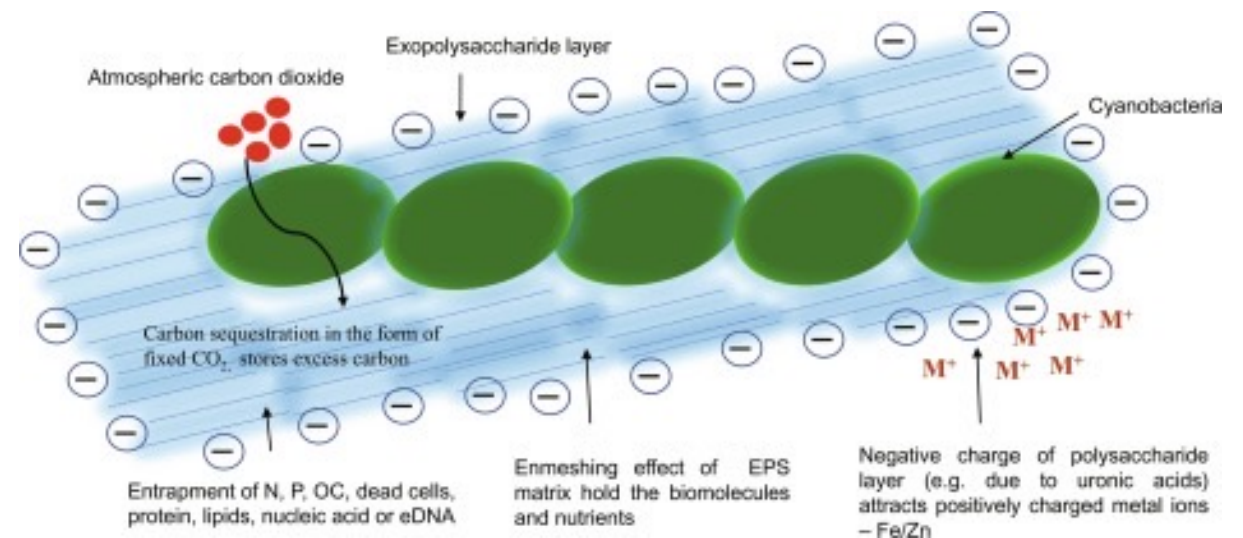
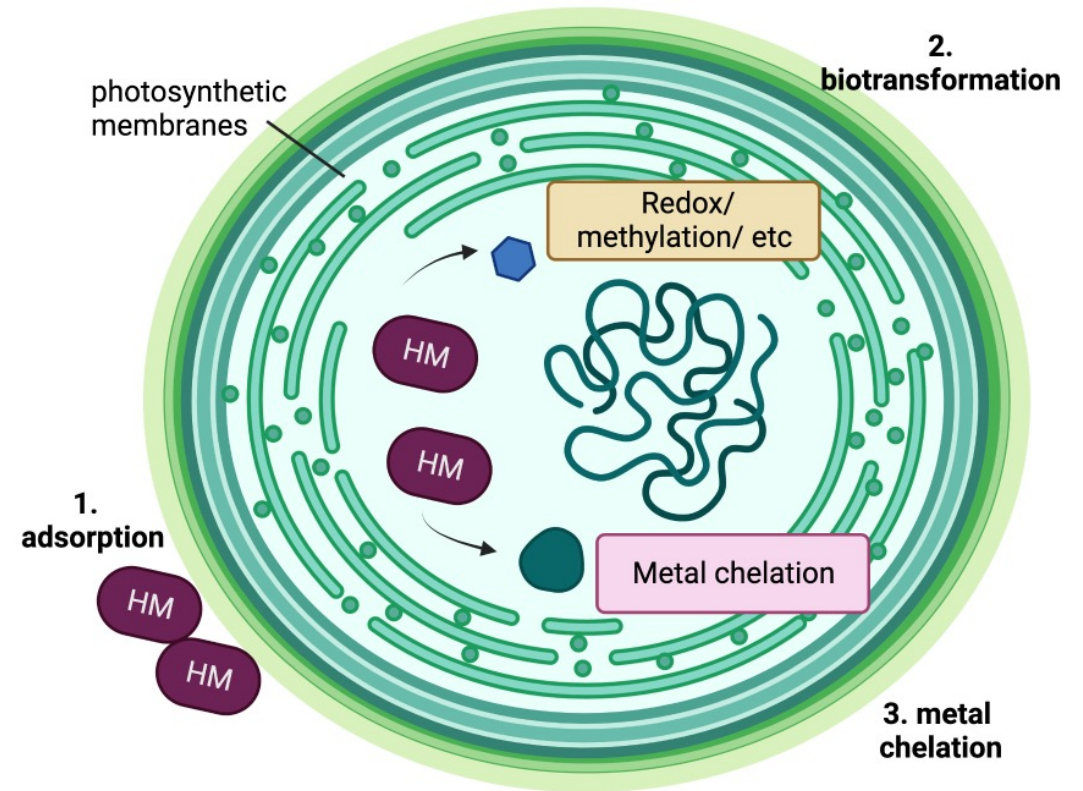
Cyanobacteria

- Gram negative bacteria
 - prokaryotes
- Undergoes photosynthesis
- Grows in soil and water
 - Can produce biofilm matrix to group bacterial cells together or to a surface
- Has metal transporters, but lacks the specificity of other host organisms



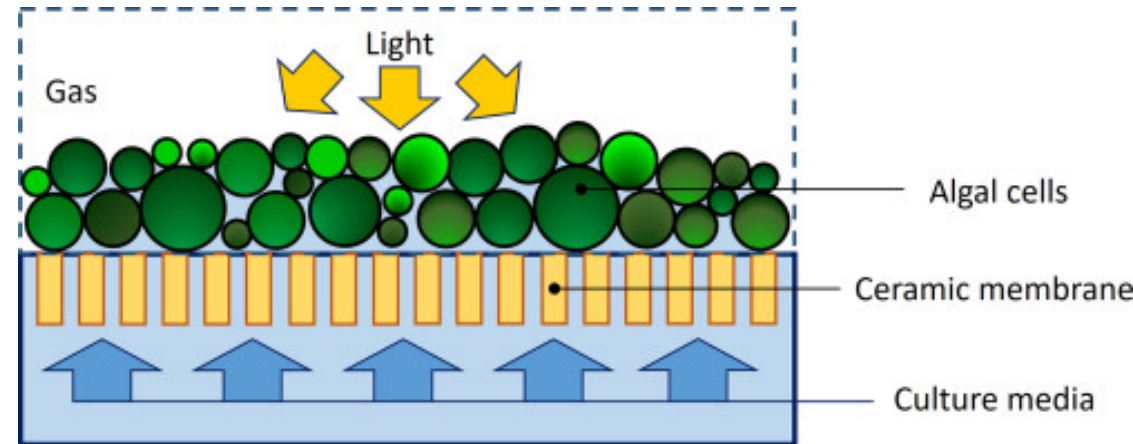
Cyanobacteria in bioremediation

- EPS-mediation adsorption
 - Extracellular Polymeric Substances
 - Can be live or dead for adsorption
- MT and PC
 - metal chelation
- Biotransformation machinery



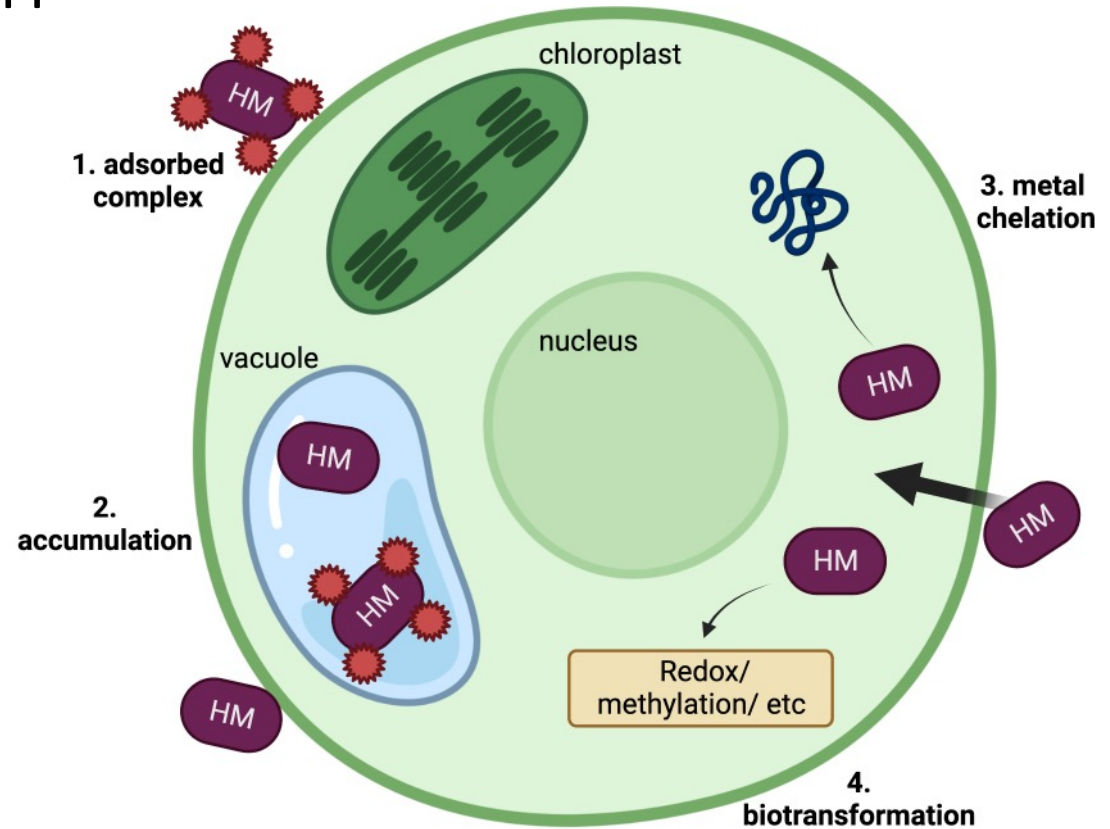
Microalgae

- Single cell eukaryote
- Undergoes photosynthesis
- Can form biofilm to attach to a surface
 - reduces resources requirements for growth
- Some membrane transporters for metals, but not the complexity/diversity of yeast



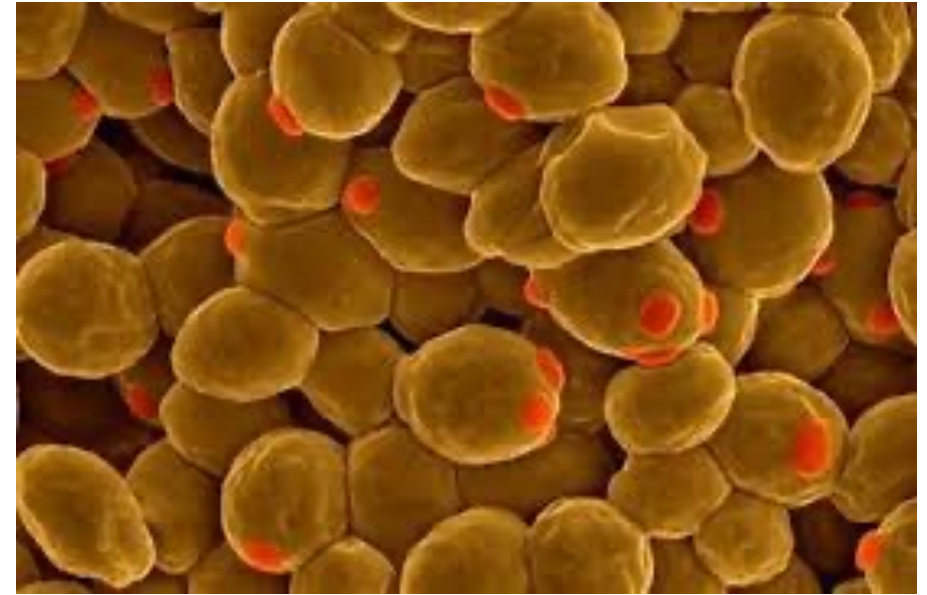
Microalgae in bioremediation

- MT and PC
 - metal chelation
 - Vacuole
 - bioaccumulation
 - Adsorption
 - Biotransformation machinery
- Heavy metals can interact with surface groups to form complexes which can be transported internally where they are accumulated or transformed



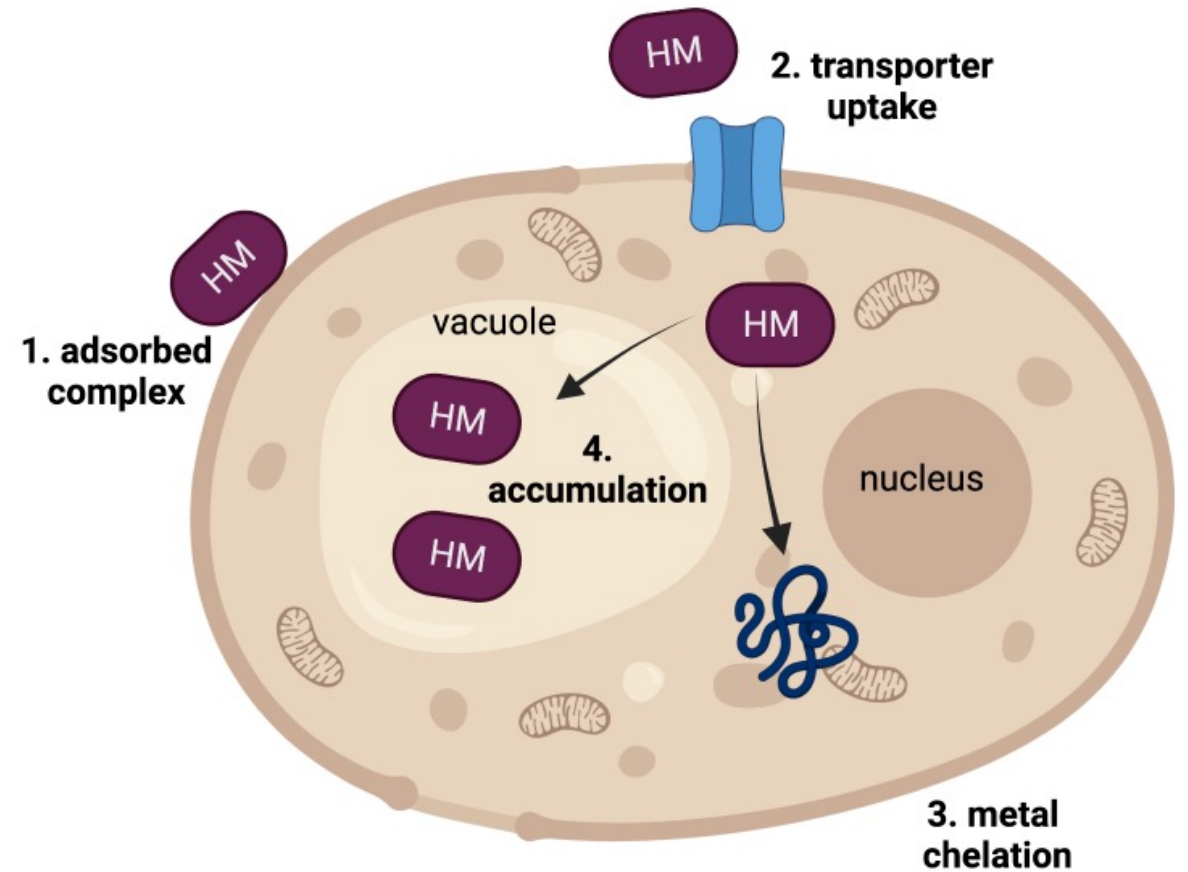
Yeast

- Single-cell eukaryote
 - fungi
 - *Saccharomyces cerevisiae*
- Widely studied and genetically tractable
- Resilient growth under non-ideal pH and osmolarity conditions
- *S. cerevisiae* is generally considered non-pathogenic and safe
- Slower growth rate than bacteria or algae



Yeast in bioremediation

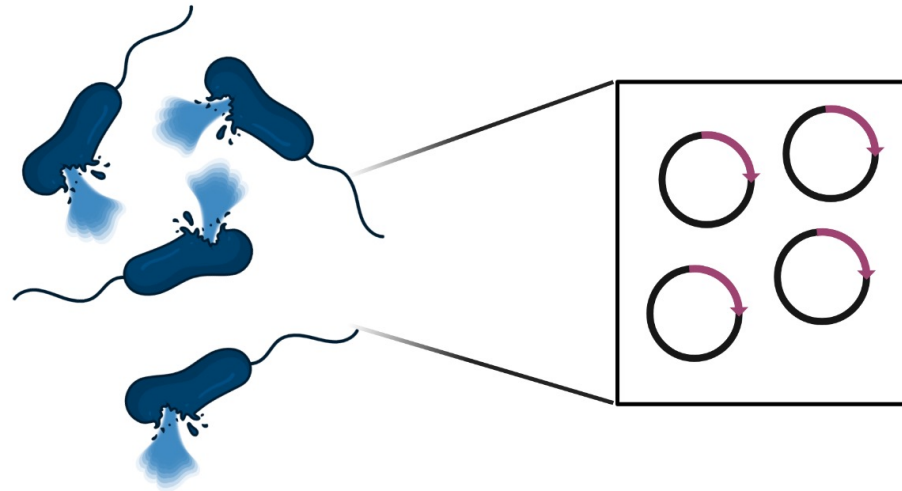
- Adsorption
- Specific metal transporters
- MT and PC
 - metal chelation
- Vacuole
 - bioaccumulation
- Some biotransformation machinery



Take home message: find the right host

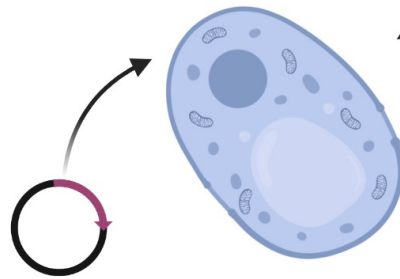
- Any of these cell types can be a chassis host for your bioremediation system
 - Depends on the environment you want to work with and the cell machinery you need
- A target for modification is selected to balance desired and undesired effects
- A chassis host needs to be able to support your genetically engineered material while also functioning in the desired environment

What are you doing in lab today



1
Purify (hopefully)
mutated plasmid
from *E. coli*

2a
Transform putative
mutants into
W303α *S. cerevisiae*



2b
Sequence plasmid
to confirm mutation

