Topics

- Nitrogen Cycle
- Communities, symbiosis, genome reduction
- Horizontal Gene Transfer
- Biotechnology
Nitrogen Cycle

Important Reactions

✓ Nitrogen Assimilation Incorporation of to NH₃ into organic molecules (R-NH₂)

✓ Deamination Deamination of organic molecules releasing NH₃

✓ Nitrification

✓ Denitrification

✓ N₂ Fixation
Nitrogen Cycle: Nitrification

Oxidation of \( \text{NH}_3 \) to \( \text{NO}_2^- \) then \( \text{NO}_3^- \)

Microbial Nitrification

![Diagram of Nitrogen Cycle]

- Only carried out by microbes
  - Soil and aquatic bacteria
  - Aerobic chemolithoautotrophic two step process
    - Ammonia oxidizers (Nitrosomonas, Nitrosocococcus, Nitrosospira, archaea)
    - Nitrite oxidizers (Nitrobacter, Nitrospira)
  - Enzyme is ammonia monooxygenase
Nitrogen Cycle: Denitrification

Nitrate reduction, conversion of $\text{NO}_3^-$ to $\text{N}_2$ gas

Only carried out by microbes
- Anaerobic chemoheterotrophic process
- Facultative anaerobes in sediment and oxygen-limited water
- Anaerobic respiration (using Nitrate and Nitrite as TEAP)
- Oxidation of organic matter for energy
- Several enzyme systems---dissimilatory nitrate reductase
- Loss of Nitrogen from system
- Nitrate to ammonia NOT denitrification

Plants perform assimilatory nitrate reduction
- Convert $\text{NO}_3^-$ to organic nitrogen ($\text{R-NH}_2$)
Nitrogen Cycle: $\text{N}_2$ Fixation
Molecular conversion of $\text{N}_2$ gas to $\text{NH}_3$
(exist in solution as $\text{NH}_4^+$)

- **ANAEROBIC**
- Proteobacteria, Cyanobacteria, Archaea
- Requires nitrogenase enzyme
  - 21 genes
  - 8 subunits/accessory proteins
  - Molybdenum and iron cofactors
- High energy cost (16 ATP per $\text{N}_2$)

$$\text{N}_2 + 8\text{H}^+ + 8\text{e}^- + 16 \text{MgATP} \rightarrow 2\text{NH}_3 + \text{H}_2 + 16 \text{MgADP} + 16 \text{P}_i$$
Rhizobium

• Free-living are aerobic, not N₂ fixers
• When symbiotic
  – *Rhizobium* turn on plasmid-based *Nod* genes
  – Become anaerobic N₂-fixing, bacteroid form
  – Legumes form nodules to control symbiotic relationship

Images of free-living Rhizobium and bacterioids in nodule removed due to copyright restrictions.
Symbiosis and Genome Reduction

- **Nanoarchaeum equitans**
- **Mycoplasma genitalium**
- **Wigglesworthia glossinidia**
- **Rickettsia conorii**
- **Pelagibacter ubique**
- **Ehrlichia ruminantium**
- **Bartonella quintana**
- **Thermoplasma acidophilum**
- **Bartonella henselae**
- **Coxiella burnetii**
- **Silicibacter pomeroyi**
- **Rhodopirellula baltica**
- **Streptomyces coelicolor**
- **Prochlorococcus marinus**
  - MIT9313
  - SS120
  - MED4
- **Synechococcus**
  - sp.WH8102
- **Mesoplasma florum**
- **Wigglesworthia glossinidia**
- **Nanoarchaeum equitans**

**Legend:**
- **Host-associated**
- **Free-living**
- **Obligate symbionts/parasites**
- **Obligate symbionts/parasites**
- **Pelagibacter ubique**

Figure by MIT OCW.
Symbiosis and Genome Reduction

- *Buchnera aphidicola* from two aphids *Schizaphus graminum* (Sg) and *Acyrthosiphon pisum* (Ap)
- 70 million years
  - No chromosomal rearrangements
  - Sequence divergence (9⁻⁹ synonymous substitutions/yr
  - 1.65⁻⁹ non-synonymous substitutions/yr
- *E. coli* and *Salmonella* spp. (closest free-living relatives) 2000x more liable

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**50 Million Years of Genomic Stasis in Endosymbiotic Bacteria**

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*SCIENCE* 296:2376 (2002)

Figure by MIT OCW.
Genome Dynamics in *Buchnera*

Obligate endosymbiont
- Substantial sequence divergence
- Prominence of pseudogenes
- Loss of DNA repair mechanisms
- Stable genome architecture HOW??
  - Gene transfer elements reduced/eliminated
    - Reduced phage
    - Reduced exchange w/other genomes
    - Fewer repeat sequences
    - Fewer transposons
  - Lack of recombination mechanisms (no recA, recF)
  - Lower frequency of recombination
**Argobacterium**

- **Ti plasmid & crown gall disease**
  - A portion of the Ti plasmid is inserted into the plant chromosome causing the formation of the tumor or gall.

Figure by MIT OCW.
Horizontal Gene Transfer: Transformation

- Release of DNA by growing or decaying cells
  - Stabilization
  - Exposure to bacteria
  - Inactivation
  - Degradation
  - Expression of competence

- Uptake
  - Restriction
  - Recircularization
  - Degradation
  - Recircularization

- Recombination
  - Expression
  - Selection
  - Negative, Neutral, Positive

Figure by MIT OCW.
Horizontal Gene Transfer: Conjugation and Transduction

Chromosome

Prophage

Mobilizable plasmid

Transposon

Conjugative plasmid

Mobile gene cassettes

IncX

IncY

IncZ

Integron

Specialized Transduction

Generalized Transduction

Pilus

Donor cell

Recipient cell

Figure by MIT OCW.
DNA Technologies

• Basics of Sequencing
  – Sanger method
  – Shotgun sequencing

• Cloning (cloning vectors)
  – Plasmids
  – Phage
  – Bacterial Artificial Chromosomes (BAC)
  – Yeast Artificial Chromosomes (YAC)