EVS 3120

Environmental Microbiology

Part I: Dr. Frances Pick
Department of Biology (GNN 307, # 6364)

email: frpick@science.uottawa.ca

Required readings for discussion in class

Jan. 15: Fulthorpe et al. 1998. High levels of endemicity in 3-chlorobenzoate-degrading soil bacteria. Applied and Envir. Microbiol. 64: 1620-1627.

Jan. 22: Wienbauer, M. & M.G. Hofle. Significance of viral lysis and flagellate grazing as factors controlling bacterioplankton production in a eutrophic lake. Applied and Envir. Microbiol. 64: 431- 438. (for Jan. 20)

Jan. 29: Kelso et al. (1997) Dissimilatory nitrate reduction in anaerobic sediments leading to river nitrite accumulation. Applied and Envir. Microbiol. 63: 4679- 4685. (for Jan

General References

- **Brock, T. et al. <u>Biology of microorganisms</u>. Sections on Microbial Ecology. 6th (1991), 7th, 8th or 9th**
- **Maier, R. M. et al. (2000) <u>Environmental Microbiology</u> Academic Press.**

Scope of environmental microbiology

- **industrial microbiology**
- **food safety & quality**
- **occupational health/infection control**
- **water quality**
- **soil microbiology and agriculture**
- **aeromicrobiology**
- **aquatic microbiology**
- **bioremediation/hazourdous waste**
- **biotechnology**

Part I: Microbial ecology

- **ECOLOGY = "law of the household" (Haeckel 1866)**
- **Study of the factors regulating the abundance and distribution of microorganisms and their interactions with the physical/chemical and biological environment.**

Microbial ecology

- **The study of ecological relationships of microbes, developed in the early 1960s along with interest in ecology and environmental quality**
- **(prior to 60s well known that several human pathogens persist in the environment and that this persistence needed to be better understood…)**

Scope of microbial ecology

1. **Interactions of microorganisms with their abiotic environment (including biogeochemical cycles). Microbes play a far more important role in natural environments than their small size would suggest.**

2. **Interactions among various microorganisms, consortia, assemblages, communities (competition, symbiosis and predation)**

Scope of microbial ecology

3. **Interactions of microorganisms with plants and animals (e.g. rhizosphere, gut flora, microbes that "jump" organisms)**

Goals of Microbial Ecology

1. **Understanding what types of microbes are where**

2. **How many there are**

3. **What they are doing**

4. **How they survive**

Goals of Microbial Ecology

5. What controls their activity and numbers

6. What role they play in the large cycles of C, trace gases and nutrients

7. How microbes chemically and physically change their environment

First, an introduction to microbes

- enormous diversity of organisms which are "microscopic" and found in virtually all habitats (air, water, soil, ice)

- enormous metabolic diversity: bacteria in particular can utilize virtually any substrate available (from various types of organic matter to high-tech. plastics…)

Classification schemes: based on function (e.g. energy source)

- autotrophy, heterotrophy and mixotrophy
  - among the autotrophs: lithotrophs (chemoautotrophes), photoautotrophs (both anoxygenic and oxygenic)
  - among heterotrophs: organotrophs use a wide variety of organic chemicals in decomposition processes (anaerobic and aerobic) using carbohydrates, proteins, lipids, hydrocarbons etc..
  - mixotrophs can switch from autotrophy to heterotrophy

Classifications based on habitat

- suspended in water = plankton (e.g. phytoplankton, bacterioplankton)
- attached communities or biofilms: periphyton, epiphyton, epilithon, epipelon, endolithon
- extreme environments: thermophiles vs psychrophiles, barophiles, halophiles, acidophiles vs alkalophiles
  (e.g. hyperthermophile from deep-sea vents that tolerate boiling water - archaebacteria)

Classifications based on size used for planktonic microbes:

- femptoplankton (< 0.2 μm) = "colloids", viruses
- picoplankton (0.2 - 2 μm) = bacteria, cyanobacteria, prochlorophytes, eukaryotes
- nanoplankton (2.0 - 20 μm) = "algae", cyanobacteria, protozoa, fungi, "marine snow"
- microplankton (20 - 200 μm) = "algae" especially colonial forms, protozoa (ciliates), rotifers
- (macroplankton (200 - 2000 μm) = large colonial algae, ciliates,rotifers, crustaceans)

Taxonomic classifications

- - viruses acellular
  - bacteria prokaryotic
  - fungi eukaryotic
  - algae "
  - protozoa "
- using Whittacker's (1969) 5 kingdom classification, microorganisms occur in 4 of the 5 kingdoms

Bergey's classification of bacteria:

The Manual is considered the authority on bacterial taxonomy

- **Vol. 1. Gram-negative bacteria of medical and commercial importance**
- **Vol. 2. Gram positive bacteria of medical and commercial importance**
- **Vol. 3. Remaining gram-negative bacteria (phototrophic, gliding,sheathed, budding and appendaged bacteria, cyanobacteria, lithotrophic, archaebacteria)**
- **Vol. 4. Filamentous actinomycetes and related bacteria**

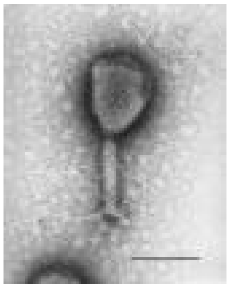
Problems in microbial taxonomy

- **the species concept**
- **the vast majority of "species" have not been isolated and are "unculturable"**
- **molecular tools are rapidly changing the situation**

1. Viruses

- **Abundant in air, water and soil.**
- **In water direct counts via TEM on vortex flow filtration concentrated water samples provide numbers 1,000 to a million more than culture plaque assays, MPNs.**
- **0.4 - 40 million per mL. TEM, Epifluorescence techniques, flow cytometry. Seem to be more in freshwater than in seawater?**

T4 phage (~ 30 X 124 nm)



- polyhedral head containing nuclear material
- rodlike tail
- tail plate with 6 prongs or spikes
- special tail structure to adhere and enable injection of nucleic acid into host bacterium

Virus ecology in natural systems

- **many viruses are bacteriophages and may be controlling bacterial and algal populations through lysis of host cell**
- **in water most are not human pathogens but some well known viruses are water born and can pose serious problems in drinking water (e.g. enteric pathogen hepatitis A virus, not effectively destroyed by chlorine)**

2. Heterotrophic bacteria

- **in water: total abundance 10,000 to 10^6 per mL from direct epifluorescence counts (DAPI, Acridine Orange, FITC).**
  - as individual free cells
  - on small particles or "marine snow", fecal pellets,
  - at the air water interphase.
  - external and internal surfaces of animals and plants
- **in sediments & in terrestrial soils (direct counts 1-60 X 10^9 cells/g dry weight)**

Bacillus infernus

- Discovered during a deep drilling project, ~2 km beneath soil and rock
- Fe (III) and Mn (IV) reducing anaerobe

Agrobacterium

- **soil bacterium, some sp. plant pathogens causing tumors or galls**
- **the Ti (= tumour inducing) plasmid infiltrates the plant DNA**
- **Ti plasmid used in genetic engineering for insertion of genes in plants (luciferase - glow in the dark plants, Bt insect toxin, Roundup resistant gene etc..)**

Heterotrophic bacteria general ecology

- **everywhere, exceptional diversity**
- **total abundance related to organic matter input (correlations with OM, algal biomass or TP...)**
- **total abundance on small time or space scales can be limited by grazing from protozoa**
- **average size in natural waters small, the more oligotrophic the smaller the average cell size (cells in culture always much bigger than natural populations)**

3. Anoxygenic photosynthetic bacteria

- **<u>Purple sulfur and green sulfur</u> photosynthetic bacteria (in low oxygen to anoxic waters with sufficient light and sulfate).**
- **use H_2S as source of electrons with S as the product deposited inside or outside the cell**

4. Oxygenic photosynthetic bacteria

- **<u>Cyanobacteria</u> (small picocyanobacteria in oceans and clear lakes up to 10^5 cells/mL, larger "bloom" forming species typical of enriched lakes, colonial forms can be visible, often N_2 fixers)**
- **<u>Prochlorophytes</u> (small picoplankton, discovered in early 90s, very abundant and common in oceans, ? freshwater)**

Chroococcus (Chroococcales)

15 um

- coccoid
- colonial
- gelatinous sheath(s)
- common in freshwater

Synechoccoccus (Chroococcales)

- **< or = 1 μm, solitary, or clumped**
- **abundant in the oceans and many lakes**
- **discovered in '79 in the oceans, responsible for a significant fraction of aquatic productivity**

Spirulina (Oscillatoriales or Nostocales)

- colonial, filamentous, no cellular differentiation
- trichome without visible cross-walls, no branching
- new "health" food product

Anabaena (Nostocales)

- **filamentous, can form macroscopic spherical colonies in eutrophic lakes**
- **cellular differentiation: heterocysts (N_2 fixation), akinetes (resting stages)**
- **some sp. produce toxins**

5. “Algae”

- in water abundance 10 -10^4 cells/mL. suspended as part of plankton or attached to surfaces (rocks, logs, higher plants, turtles...) as periphyton, or in symbiosis with protozoa, invertebrates and even some vertebrates
- size ~ 1 µm - ~ 250 µm. But macroscopic forms, multicellular seaweeds, like giant kelp 50 m long...
- A huge variety of species; many have yet to be identified or cultured

5. Some major algal groups

- Diatoms (hundreds of thousands of species characterized by unique silica frustules, which preserve well in sediments and can be used to reconstruct environmental conditions. Valuable environmental indicators in general). A few species toxic.
- Green algae (many species and a preferred food source for many invertebrates, abundant in enriched waters and rivers). No known toxic forms but may be involved in taste and odour problems

Diatoms (Bacillaryophyta)

Achnanthes

- siliceous cell walls ~ box (composed of 2 halves top & bottom)
- pennate diatom valve view epitheca with raphe, striae
  - valve view hypotheca
  - girdle view

• **SEM of pennate diatom *Navicula***

• **SEM of centric diatom with radial symmetry (in valve view)**

Bacillaryophyta (Centrales)

- *Melosira varians*
- colonial centric (2 cells stacked on top of each other- girdle view)

Flagellated green algae

- ***Chlamydomonas***
- **2 flagella (isomorphic, smooth)**
- **Lugol's dye colours starch**

Filamentous green algae

- ***Zygnema* (2 star- shaped chloroplastes)**
- ***Spirogyra* (1-16 ribbon, spiral or helical)**
- ***Mougeotia* (1-2 rectangular, parietal)**
- ***Spirogyra***

Filamentous green algae

- periphytic green algae
- freshwater streams, lakes (prominent in acidified lakes), estuaries, marine rocky shores

5. Some major algal groups

- **Chrysophytes: golden brown algae, typical of oligotrophic and coloured northern lakes). Some species toxic (marine "brown tides"), some sp. in freshwater cause taste and odour problems. Many mixotrophic phagotrophic**
- **Cryptophytes: flagellated cells with pigments similar to red algae and cyanobacteria, abundant in lakes and rivers**

Chrysophyceae

- ***Dinobryon***
- colonial, freshwater, implicated in taste and odour problems

5. Some major algal groups

- **Dinoflagellates (flagellated cells covered with organic plates like armour, several species responsible for notorious "red-tides" and paralytic shell-fish poisoning in marine coastal waters)**

Dinoflagellates

- **freshwater, marine, symbionts of coral**
- **e.g. *Ceratium hirundinella* in freshwater**
- **some sp “armored” cell wall composed of pectin plates**

Toxic dinoflagellates

- ***Pyrodinium bahamense***
- **tropical sp. often responsible for luminescent seas in Carribean**
- **implicated in paralytic shell fish poisoning (PSP)**

Primnesiophyceae

- **family with a 3rd appendage(haptonema) and 2 equal whiplash flagella**
- **marine sp. With coccoliths (Ca scales)**
- **e.g. 3 μm phytoplankton Arctic & Antarctic oceans (Prize photo Japan Electronic Microscope Academy 1997)**

6. Protozoa

- **common in water (10 - 10^4 /mL) and soils**
- **taxonomic affinity with "algal groups" (chrysophytes, cryptophytes) (algae are classified in the Kingdom Protista)**
- **bactivores, algivores**
- **form "microbial food chains" microbial food web or "loop"**
- **a few important water borne human pathogens e.g. *Giardia, Cryptosporidium*, *Entamoeba***

7. Microscopic fungi

- deuteromycetes, ascomycetes, basidiomycetes (some species unique in ability to break down lignin, e.g. white and brown, blue rot of wood)
- important particularly in terrestrial environments
- compete with bacteria for OM

7. Microscopic fungi (the good and the bad)

- positive aspects for food/beverage industry
- source of antibiotics
- mycorrhizae in agriculture and sylviculture

- negative aspects as plant pathogens (rusts, mildews, smuts)
- moulds & air quality
- moulds & food quality
- mycotoxins in food and feed (e.g. aflatoxins - carcinogens)
- cutaneous infections & opportunistic human pathogens