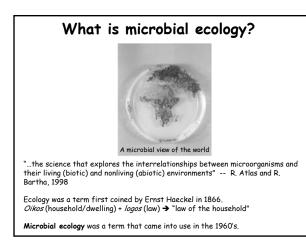
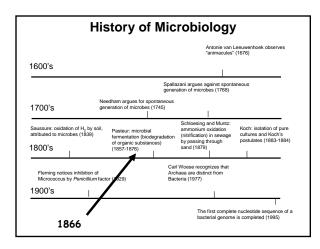
# Class Business:

Reading materials on course website

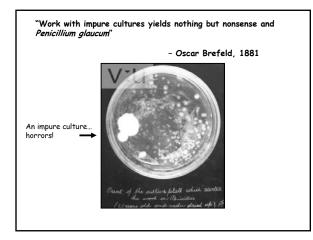
http://fire.biol.www.edu/cmoyer/cmoyer.BI405.html

Field trips (e.g.  $\rm H_2S$  scrubber at VRI and water treatment plant) - arrive early and stay late

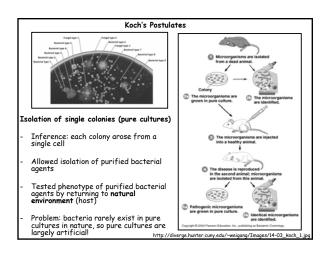




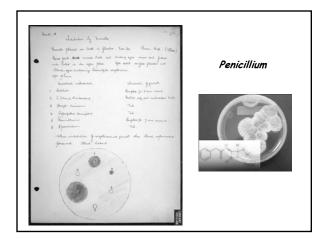












## Sergei Winogradsky (1856-1953) Microbes in mixed communities

Fundamental contribution to microbial ecology: showed that bacteria are biogeochemical agents.

- Proposed concept of chemoautotrophy
- Showed that bacteria can oxidize iron, sulfur, and ammonia to obtain energy; showed that bacteria can incorporate CO<sub>2</sub> into organic matter as do plants



# Sergei Winogradsky (1856-1953) Microbes in mixed communities

• Proposed concept of **chemoautotrophy** 

 Showed that bacteria can oxidize iron, sulfur, and ammonia to obtain energy; showed that bacteria can incorporate CO<sub>2</sub> into organic matter as do plants

 $\begin{array}{l} \textit{Beggiatoa:} filamentous, gliding, sulfur-oxidizing bacteria. Found in habitats rich in H_2S: sulfur springs, decaying seaweed beds, rhizosphere of swamp plants, lake sediments, sewage, hydrothermal vents. \end{array}$ 



Filaments of Beggiatoa; the golden granules within the tubes are sulfur.

$$\begin{split} & 2H_2S\,+\,O_2\rightarrow 2S^0\,+\,2H_2O\\ & 2.7H_2S\,+\,O_2\rightarrow 2.7S^0\,+\,2H_2O\,+\,1.4H^* \end{split}$$

# Sergei Winogradsky (1856-1953) Microbes in mixed communities

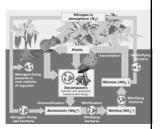
Fundamental contribution to microbial ecology:

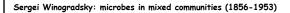
Studied the nitrogen cycle

•Nitrification,  $NH_4 \rightarrow NO_3$ •Two-step process: *Nitrosomonas*,

then Nitrobacter (isolated both)

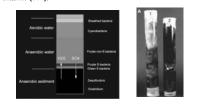
Described anaerobic bacterial N<sub>2</sub>fixation (*Clostridium pasteurianum*)

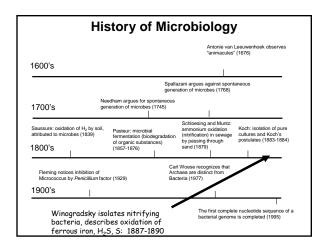




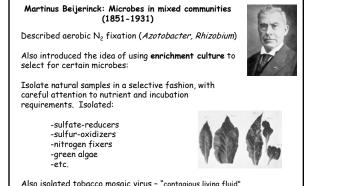
Winogradsky Column: device for culturing a large diversity of microbes; pond sediment + carbon source + sulfur source + light; gradients of  $O_2$  and sulfide form selecting for various metabolic abilities.

Winogradsky believed soil microbes should be studied in their  $\ensuremath{\mathsf{natural}}$ environment (soil).

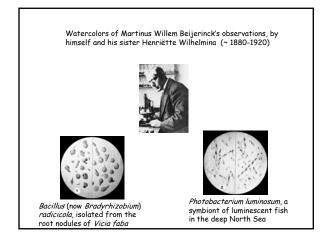




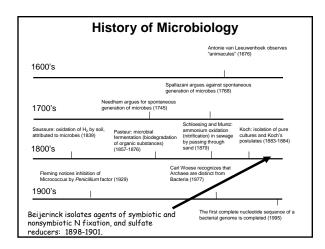




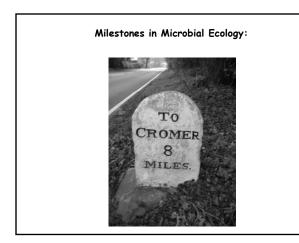
Also isolated tobacco mosaic virus - "contagious living fluid"









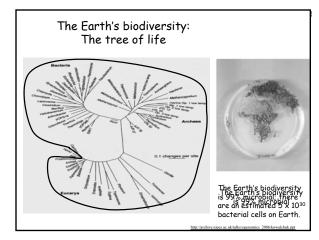


- 1887 Sergei Winogradsky studies Beggiatoa and establishes the concept of autotrophy.
- 1888 Martinus Beijerinck develops the technique of enrichment culture
- 1891 Winogradsky discovers the organisms responsible for nitrification in soil, which is of great importance in agriculture because nitrogen is a limiting nutrient in the soil.
- 1904
   Martinus Beijerinck obtains the first pure culture of sulfur-oxidizing bacterium, Thiobacillus denitrificans.
- 1904 Cornelius Johan Koning suggests that **fungi** are critical for the **decomposition** of organic matter.
- Sigurd Orla-Jensen proposes the use of **physiological characteristics** (vs. tiny round specks and tiny rod-shaped specks) for the classification of bacteria. He later 1909 publishes a monograph on lactic acid bacteria that establishes the criteria for assignment.
- The Society of American Bacteriologists presents a report on the characterization 1920 and classification of bacterial types that becomes the basis for **Bergey's manual** in 1923.

http://www.microbiologytext.com/index.php?module=Book&func=displayarticle&art\_id=

- Emile Zuckerkandl and Linus Pauling publish "Molecules as documents of evolutionary history", making a compelling case for the use of molecular sequences of biological molecules to determine evolutionary relationships.
- Don Brenner and colleagues establish a more reliable basis for the classification of clinical isolates among members of the *Enterobacteriaceae*. They use **nucleic acid reassociation**, where DNA of one organism is allowed to hybridize with another organism. This technique is used to help define a species.
- Carl Woese uses ribosomal RNA analysis to identify a third form of life, the 1977 Archea, whose genetic makeup is distinct from but related to both Bacteria and Eucarya.
- Discovery of abundant life at the bottom of the ocean near deep sea **hydrothermal** 1977 **vents**. The entire system is dependent upon sulfur oxidizing microorganisms. Light and photosynthesis do not drive the process.
- Karl Stetter isolates hyperthermophilic microbes (Archaea) that can grow at 105°C. The discovery redefines the upper temperature at which life can exist.
- Gary Olsen, Carl Woese and Ross Overbeek summarize the state of phylogeny in prokaryotes. This causes scientists to rethink the **classification of life** and emphasizes the importance of microbes.

http://www.microbiologytext.com/index.php?module=Book&func=displayarticle&ar



# Leeuwenhoek and microscopy (1674)

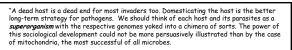


 1st observation of microorganisms (protozoa, bacteria)

•Plaque from teeth: "so many that I believe them to exceed the number of men in a kingdom" – AvL



A \_\_\_\_ D

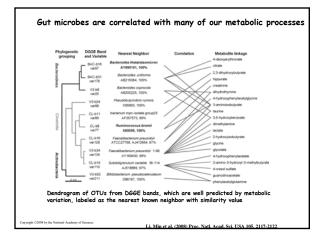


Perhaps one of the most important changes we can make is to supercede the 20thcentury metaphor of war for describing the relationship between people and infectious agents. A more ecologically informed metaphor, which includes the germs'-eye view of infection, might be more fruitful. Consider that microbes accupy all of our body surfaces. Besides the disease-engendering colonizers of our skin, gut, and mucous membranes, we are host to a poorly catalogued ensemble of symbionts to which we pay scant attention. Yet they are equally part of the superorganism genome with which we engage the rest of the biosphere.

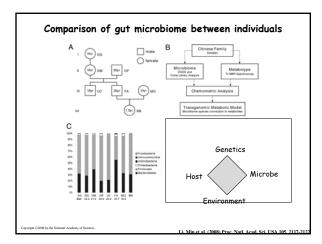
The protective role of our own microbial flora is attested to by the superinfections that often attend specific antibiotic therapy: The temporary decimation of our home-team microbes provides entriee for competitors. Understanding these phenomena affords openings for our advantage, akin to the ultimate exploitation by Dubos and Selman Waksman of intermicrobial competition in the soil for seeking early antibiotics. Research into the microbial ecology of our own bodies will undoubtedly yield similar fruit."

-- Joshua Lederberg, 2000. Infectious History. Science 288: 287 - 293

Joshua Lederberg heads the Laboratory of Molecular Genetics and Informatics at The Rockefeller University in New York City, and a Nobel laureate (1988) for his research on genetic mechanisms in bateriar. He has worked closely with the Institute of Medicine and the Centers for Disease Control and Prevention on analytical and policy studies on emerging infections.









Proposal (Amer. Acad. Microbiology):

# "Koch's Postulates for microbial communities"

- 1. Gain an understanding of the environment, including **chemical** processes and members
- 2. Acquire the isolates that carry out these processes
- 3. Return isolates to the environment and demonstrate that they are responsible for the processes of interest
- Deconstructing the community this way from constituent key players should **retain key characteristics and process rates** of the original.

# What is a microbe's environment?

Key features: liquid water, nutrients, and an energy producing system (including an electron donor and electron acceptor).

#### Sources of energy:

Photons (photosynthesis) Electrons (oxidation of reduced molecules, e.g. sugars, proteins, sulfur, iron,  $H_{2^{\rm cu}})$ 

Acceptable e- acceptors: Gaseous oxygen (like us), sulfate, nitrate, nitrite, carbon dioxide, carbon monoxide, iron and magnesium...

Desirable microbial real estate is varied, reflecting the diverse "tastes" of these wee fellow  ${\sf Earthlings}$ 

Inside of or associated with eukaryotic cells (animals, plants, fungi) At extreme temperatures (>100°C and as low as 0°C) At extreme pH (<2.0 and <11.0) Deep subsurface (3.5 km below the earth's surface) In the presence of toxic metals like copper or mercury In saturated salt solutions Adapted from Dr. R. E. Hutltert (WSU),

8

# Below is an abbreviated list of the roles microbes play in our lives:

They maintain soil fertility and soil tilth.

They clean up all the dead organic material; without them we would be up to our ears in dead things, like our ancestors.

They fix gaseous nitrogen into forms that can be used by plants to maintain the fertility of soils .

They can be used to extract minerals from ores.

They are the prime food for all the marine and freshwater life; even whales depend on them directly or indirectly for their nutrition.

Adapted from Dr. R. E. Hurlbert (WSU), 1

# Why study microbial ecology?

## The philosopher:

"Microbial ecology can show us our place in the cosmos -- how life originated and how it evolved, and how we are related to the great diversity of all other organisms."

#### The altruist:

"The study of microbial ecology can help us improve our lives via the use of microbes in environmental restoration, food production, bioengineering of useful products such as antibiotics, food supplements, and chemicals."

#### The eternal student:

"We likely know fewer than 1% of the microbial species on Earth. Yet microbes surround us everywhere -- air, water, soil. An average gram of soil contains one billion (1,000,000,000) microbes representing probably several thousand species. WOW!!! There is so much to learn..."

Adapted from http://w

 Microbial ecology is a broad umbrella encompassing a huge variety of skills and disciplines.

 Image: A straight of the straigh

## Microbial ecology is the study of life itself...

How did we get here? The study of the origin and early evolution of microorganisms may provide information on the critical early stages of life. Microbes were the first living creatures on Earth, first appearing over 3.7 billion years ago - long before multicellular plants and animals, which evolved 600 million years ago. Understanding the biology of microbes that live in conditions that we think might reflect those of a very young Planet Earth may provide insight into the origin of all life on Earth.

Studies of microbes also are fundamental in our current and ongoing understanding of the unifying principles of life (biochemistry, genetics, bioenergetics, evolution, ecology).

Work with microbial extremophiles gives us an idea about the actual chemical, physical, and genetic constraints beyond which life is not possible.

Adapted from http://www.isi

Exobiology

Does life exist elsewhere in the universe? If there is life elsewhere in the universe, it is probably microbial. While we may never know in our own lifetimes whether life exists elsewhere in the universe, microbes will probably play a key role in helping us explore and colonize space.

# Biodiversity

Many years of evolution have created a stunning diversity of microbes. But of the more than one million species of bacteria suspected to exist, only about 4200 species of bacteria are described. Major reason: failure to culture, perhaps due to:

-narrow habitat/niche of many micobes -presence of trace contaminants -presence of lysogenic phage -not grown long enough -lack of correct nutrients or incorrect levels thereof -absence of signal molecules -absence of microbe-microbe or host-microbe interactions

The infant science of microbial systematics has only recently acquired the sophisticated tools required to assess this tremendous biodiversity. It is estimated that we know less than 1% of the microbial species on Earth.

#### Ecology

Using microbes to study ecology may provide clues to how complex ecosystems operate because bacteric are small so large populations can be studied and quick to reproduce compared to larger organisms (*e.g. Richard Lenski*). A better understanding of ecology may help us better manage Earth and its dwindling renewable resources, and to protect global ecological systems.

Global Ecology - Microbes play important global roles:

Global Biogeochemical Cycles: C, N, Fe, S

Human Ecology - The study of simple microbial communities may shed light on one of the more complex (and for most of us, fascinating) communities - the microbial community within a human being. We are hosts to billions of microbes which colonize our intestinal tracts, our skin, hair, teeth. Sometimes microbial colonization causes us problems when disease results. Some scientists have joked that humans are microbes' invention for getting around.

rom http://www.isme-microbes.org/whatis/

#### Interdomain interactions

Microbes interact with eucaryotic hosts (e.g. plants and animals) in myriad ways. Plants and animals host hundreds of associations with different microbial species.

Examples:

*Rhizobium* converts dinitrogen to ammonium, a form of nitrogen that is available to plants.

Beneficial gut microflora (> 300 species)

Lichens

#### Bioremediation

Environmental degradation is one of the worst threats facing humanity. Many environmental problems may be cured by microbes.

Bioremediation of toxic wastes: Microbes are the focus of many studies because of their metabolic diversity. They can degrade, and utilize, a huge variety of compounds. This ability is harnessed for cleanup of toxic wastes. Microbes are used to clean up the following:

•Oil spills •Pesticides •Industrial wastes

# Recycling

Microorganisms recycle most organic wastes in nature into reusable resources. If not for microbes, we'd be up to our ears (and more) in dead stuff...

Humans have harnessed the power of microbes to recycle in the systems below:

Waste treatment: Microbes are used in sewage treatment for nutrient recycling, methane recovery and disease control.

 $\ensuremath{\textbf{Animal}}$  waste treatment: Microbes transform waste produced by farm animals.

**Composting:** Composting is the now-fashionable practice of converting kitchen, yard, and municipal wastes into a rich soil amendment. Although worms have gotten good press for their contribution to composting, the real workhorses of composting are microbes.

## Food Microbiology

Fine line between food spoilage and fermentation... many bacterially fermented foods were likely discovered by ancestors who were truly hungry (couldn't throw out food).

Many of our foods are prepared with the aid of microbes, such as yeast in bread, and lactobacilli in yogurt. Culturally specific - pickled herring vs. fermented seal flipper, sauerkraut vs. kimchi.

Much of the protein that we eat is the result of bacterial fixation of nitrogen from the air from microbes such as *Rhizobium*.

Many of our food supplements, from vitamins, amino acids to flavor enhancers and preservatives, come from microbes.

Although not a food, microbes are used as probiotics, a digestion supplement that colonizes the intestines, preventing the colonization of bad, disease-causing microbes.

## Biotechnology

Microbes are used as workhorses in the production of many compounds, from fuel, to pharmaceuticals, to chemicals. They are also used in mining, insect and disease control, genetic engineering and some are even used to make computer biochips.

**Fuel Production** 

- digestion of corn and sugarcane polymers in the manufacture of ethanol for automobile fuel.

- microbial fuel cells

Mining

leaching of metals from ore-bearing rocks by microbes.
 5% of world's copper ore is produced by bio-leaching.
 Uranium is mined with help of bacteria.

Adapted from http://www.isme-microbes.org/wha

**Biocontrol** - Using microbes to combat pests is called biocontrol. One of the most popular forms of biocontrol is the use of *Bacillus thuriengensis*, a bacterium that produces a toxin that kills over 40 problem pests such as the gypsy moth.

 $\ensuremath{\textit{Genetic}}$  Engineering - Some microorganisms are used to carry genes into other organisms.

-The bacterium *Agrobacterium tumefaciens* plays a key role in genetically engineering plants.

-The gene encoding the delta-endotoxin from *Bacillus thuringiensis* has been widely inserted into many crops to confer pest resistance

Adapted from http://www.isme-microbe

# The first question in exploring a habitat is often "Who's Out There"?

16 S rRNA, classical methods

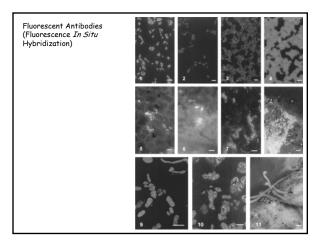
How Many?

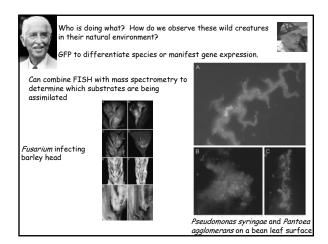
direct count (microscopic evaluation) viable count (what can be grown in lab)

How many of the direct count are viable?

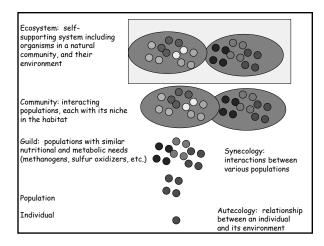
Nalidixic acid assay, causes actively respiring cells to enlarge

Propidium monoazide: leaks into dead cell membranes and binds  $\mathsf{DNA}$  – can't do  $\mathsf{PCR}$ 











# Microbes: single celled, but not isolated

Most bacteria do not normally live in isolation from one another (Exception: *Vibrio* in squid light organ = pure culture)

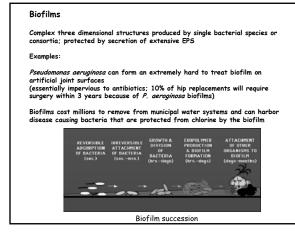
 ${\it Consortia:}\ groups of bacteria that live together and often depend upon other consortia members to provide some or all of their nutrients$ 

 $\clubsuit$  Is the "consortia effect" one reason we can grow such a relatively low number of bacteria as pure cultures in the lab?

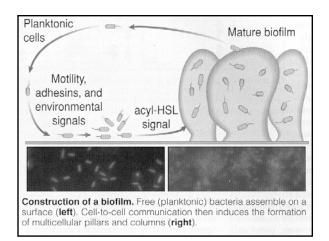
 $\mbox{Guilds:}\ groups of bacteria that have similar nutritional and metabolic needs (methanogens, sulfur oxidizers, etc.)$ 

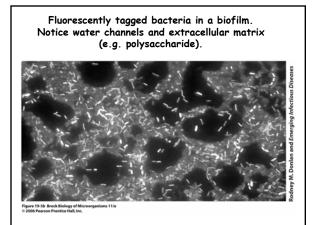
**Indicator Species:** easily identified species that suggests the presence of its consortia – e.g., *E. coli* often serves as an indicator of fecal waste; in coastal waters, toxic algal blooms can also indicate *Vibrio cholerae*.

sets and international transmission of the Set of United Set of S

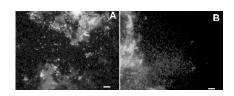








You've never seen a biofilm before? Yes you have... the plaque on your teeth, the sludge in your bathroom sink drain, the slime on your shower curtain...



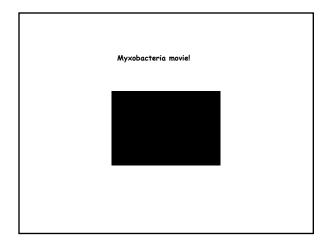
Epifluorescence microscopy of biofilm samples taken from shower curtains. These communities can contain diverse populations of microbes. (Micrograph Tom Appl. Environ. Microbal, 70:4187.4192.)

Cooperation among cells in a population: myxobacteria.

The myxobacteria are Gram-negative, ubiquitous, soil-dwelling bacteria that are capable of multicellular, social behaviour. In the presence of nutrients, "swarms" of myxobacteria feed cooperatively by sharing extracellular digestive enzymes, and can prey on other bacteria. When the food supply runs low, they initiate a complex developmental program that culminates in the production of a fruiting body composed of hundreds of thousands of cells. The myxobacteria communicate with each other, and coordinate their movements through a cell-contact-dependent signal.

--Adapted from Dale Kaiser, 2003. Nat. Rev. Microbiol. 1:45





#### Myxococcus xanthus signals starvation with diffusible A-factor, resulting in aggregation

Cells sense that nutrient density is getting low, and release A-factor



-mix of 6 amino acids (result of proteolysis): trp, pro, phe, tyr,leu, ile -amino acids are 10-fold less than concentration necessary to support growth -only released by starving cells

-each cell only releases a fixed amount of A-factor -A-factor is diffusible

If enough cells release A-factor, the population "agrees" to commit to fruiting body development, and begins the first stages of aggregation.

There's a time limit for fruiting body formation. During starvation, cells "sense impending doom" and seem to act proactively to fruit and disperse. (Why?).

Cells sense actual starvation as the lack of one or more amino acylated tRNAs, which triggers the "stringent response" during which protein synthesis is temporarily shut down so no fruiting body could be formed.

#### Myxococcus xanthus coordinates cell movement with C-factor, nondiffusible

2. C-factor, another signal, helps organize the movement\* of cells: rippling aggregation

end-to-end packing in "rafts" inside spores.



N

C-factor is a small (20 kD), membrane-bound protein C-factor is NOT diffusible C-factor requires cell-cell contact

C-factor autoinduces, and there are thresholds of C-factor for each subsequent developmental stage

\*Gliding motility in *M. xanthus* involves two different "gliding machines", one at each cell pole: 1. the S-machine, which depends on type IV pili 2. the A-machine, which seems to involve a slime extrusion mechanism. C-signal induces cells to move with increased gliding speeds, in longer gliding intervals and with decreased stop and reversal frequencies... increasing travel rates of cells.

## Microbes are useful for studying ecological interactions and evolution...

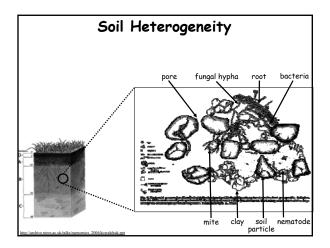
Myxobacteria share characteristics of uni- and multi-cellular organisms. They grow and divide as do all Gram-negative bacteria. In abundant nutrients, they don't form fruiting bodies.

In vitro natural selection: grew 12 "lines" of Myxococcus in test tube without starving, for 1000 generations. More than half of these developed mutants that lost fruiting body development and sporulation efficiency. (Velicer, Kroos, Lenski, 1998. PNA5 95: 12376)

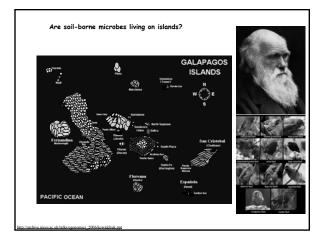
Why is social behavior selected for, in the wild (in the soil)?

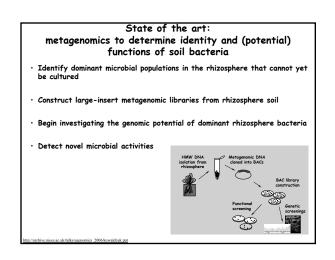
1. Spore is bigger than a cell: 0.2 mm in diameter. Can adhere to passing animal (e.g. worm, mite) and be carried to new food source.

2. Wolf pack feeding: once spores germinate in new spot, the cells secrete extracellular enzymes *en masse*, thus degrading food much more efficiently











# Good web sites:

Microbe Magazine: http://www.asm.org/microbe/

International Society for Microbial Ecology: http://www.isme-microbes.org/

Micobe Zoo: http://commtechlab.msu.edu/sites/dlc-me/zoo/index.html

Microbe World: http://www.microbeworld.org/

Kenneth Todar's Online Textbook of Bacteriology: http://www.textbookofbacteriology.net/

Small Things Considered: http://schaechter.asmblog.org/schaechter/

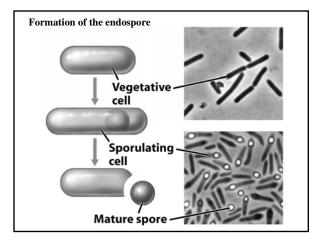
Dimensions of Microbiology: http://www.centres.ex.ac.uk/egenis/microbiology/index.htm

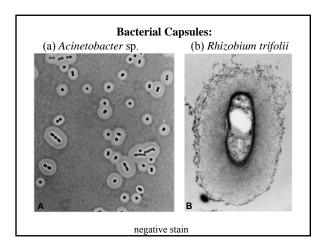
Dr. Moyer's research: http://earthref.org/FEMO/cruises/2007/index.html

> Departmental Seminar Wednesday, April 2 4 p.m.

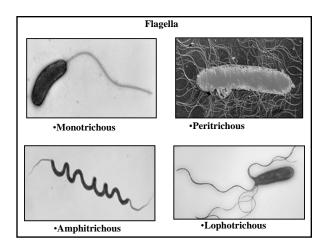
Dr. Reid Harris, James Madison University, VA

Microbial ecology of amphibian skin and mechanisms of disease prevention by mutualistic bacteria

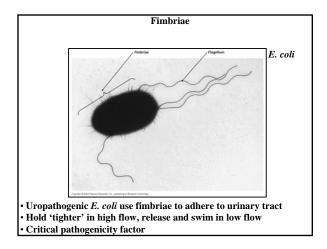




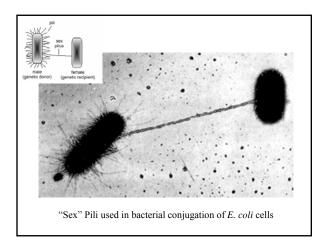




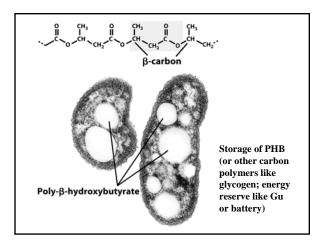


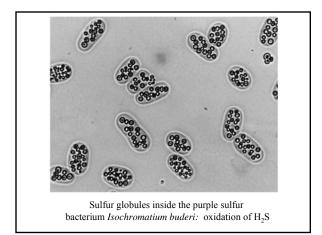




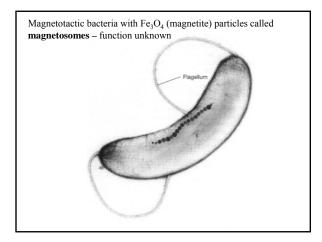




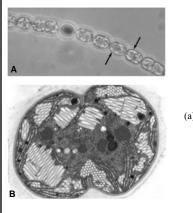












# Gas Vesicles

(a) Anabaena flos-aquae (b) Microcystis sp.

# What about the ecology of human microbes?

http://www.sciencemag.org.offcampus.lib.washington.edu/cgi/content/full/288/5464/287?ijkev=24bb1901e27ef8c3c0a1caeb6246b f2f9a4f6776&kevtype2=if\_ipsecsha

http://www.pnas.org.offcampus.lib.washington.edu/cgi/content/full/105/6/2117

http://jn.nutrition.org.offcampus.lib.washington.edu/cgi/reprint/134/2/459

http://www.nature.com.offcampus.lib.washington.edu/nrmicro/journal/v3/n5/abs/nrmicro1152.html

ttp://www.nature.com.offcampus.lib.washington.edu/nm/journal/v12/n7/abs/nm0706-736.html

http://www.nature.com.offcampus.lib.washington.edu/nature/journal/v444/n7122/abs/nature05414.html

- Big questions, small worlds: microbial model systems in ecology
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