Microbe Mission B & C

1. I want to emphasize that I will follow **modern phylogeny** in this event. I will use the more modern, three domain approach. [The Whittaker Five Kingdom concept (Animals, Plants, Fungi, Protists, and Monera) is obsolete.]

2. In brief, the molecular, cellular, and fossil evidence acquired over the last 30 years provides strong evidence that life on earth evolved from a single type of cell some 3.5 billion years ago; early on, this cell type gave rise to three separate lines of descent which are reflected in the organisms living at the present time. The phylogenetic trees that result from the analysis of nucleotide sequences in the 16S and 18S ribosomal RNAs of hundreds of different organisms are reproduced on the next page and, in outline, below. Many of the details of the new tree are controversial, and most of the taxonomic names have not been formalized. Therefore, you will find many groups of organisms given names in the venacular rather than in Latin.

MODERN PHYLOGENY

Domain Archaea (cell type: procaryote)

- Division 1. The methanogens, e.g., *Methanococcus*
- Division 2. The extreme halophiles, e.g., *Halococcus*
- Division 3. The hyperthermophiles, e.g., *Pyrodictium*

Domain Bacteria (cell type: procaryote). The Divisions (Kingdoms) are:

- The Firmicutes (Gram positive bacteria, e.g., the cocci, e.g., *Stapylococcus*; *Streptococcus*; *Enterococcus*; *Lactobacillus,* and the endospore-formers, e.g., *Bacillus, Paenibacillus, Clostridium*).
- The Actinobacteria (Gram positive bacteria, e.g., *Mycobacterium, Corynebacterium, Streptomyces*)
- The Cyanobacteria/chloroplasts/Prochlorophytes.
  - Cyanobacteria, e.g., *Spirulina*, *Oscillatoria*, *Synechococcus*
- The Proteobacteria/mitochondria
  - Alpha-Proteobacteria, e.g., *Rhizobium*, *Rickettsia rickettsii*
  - Beta-proteobacteria, e.g., *Neisseria, Nitrosomonas*
  - Gamma-proteobacteria, e.g., *Vibrio cholerae*, *Pseudomonas aeruginosa*; *Azotobacter*; *Leigionella; Azotobacter; Escherichia*
- The Spirochetes, e.g., *Treponema*; *Borrelia*
- Bacteroidetes, e.g., *Bacteroides*

Some other Divisions will NOT be on the test (*e.g., Verrucomicrobium* and Thermotogales; Aquificae; *Planctomyces* and relatives; *Deinococcus* and *Thermus*; Chlorobi; Chloroflexi).

Domain Eucarya (cell type: eucaryotic).

- Fungi, e.g., *Penicillium, Saccharomyces, Candida, Trichophyton*
- Protists, e.g., *Plasmodium*
- Algae, e.g., *Alexandrium, Scenedesmus, Dunaliella*
- Plants (are not microbes; understand why)
- Animals (are not microbes; understand why)

NOTE: One or several genera are given for each group. Learn the characteristics of each genus, especially the species within each genus that might cause disease in humans. Also learn the importance of certain genera for the environment or for human food production.

3. I will <u>not</u> be asking <u>specific</u> questions about phylogeny, but must be learned and used in a phylogenetic context. Therefore, we will assume that the students have a general knowledge of these modern ideas.

4. I will NOT be asking questions about the following diseases: common cold, dengue fever, Ebola, Mononucleosis, Viral encephalitis, Viral pneumonia, West Nile Fever, Bacterial meningitis, ~~dental caries,~~ toxic shock syndrome, typhus, histoplasmosis, estuary-associated syndrome.

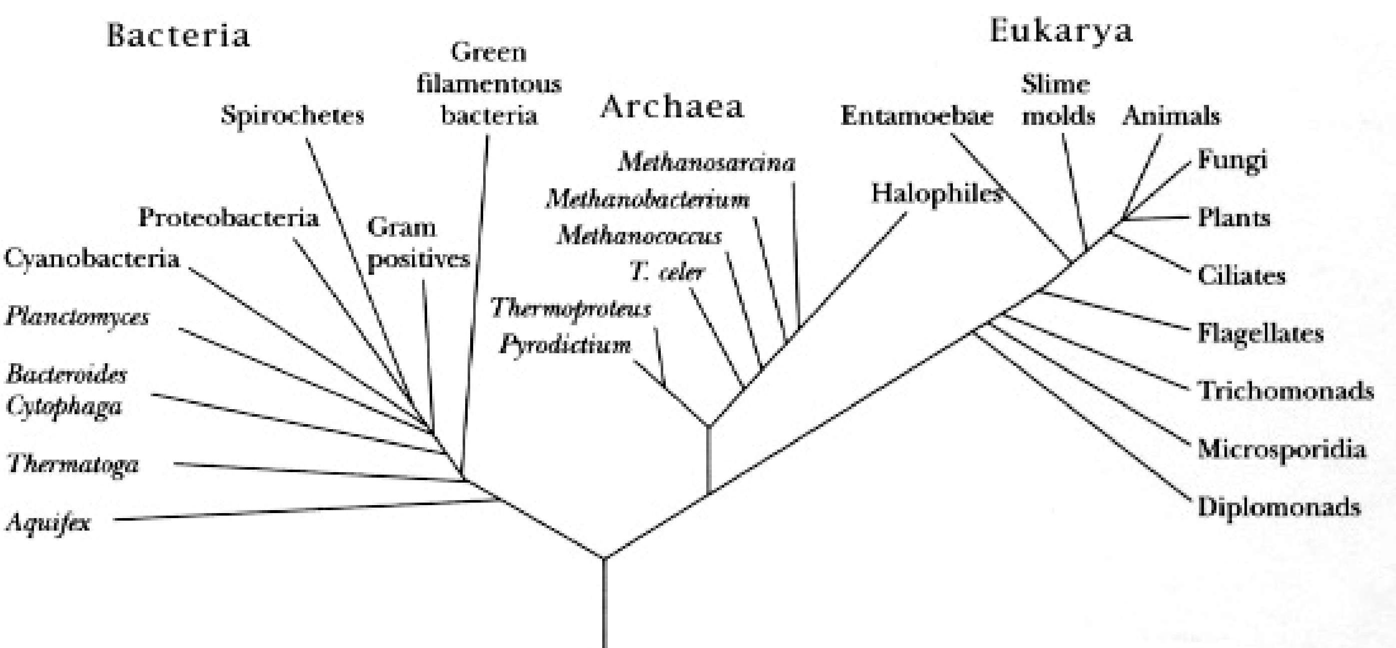
5. Please make sure your students know the name of their team and how to use their simple scientific calculator (programmable calculators are NOT permitted). Goggles are <u>not</u> needed.

6. Do not contact me directly. If you or your students have questions about this event, please email the questions to your head coach who will forward them to Liz Jablecki. She will send the questions to me and my answers to you and everybody.

7. CALL FOR FEED BACK: It would be very helpful to me if you would tell me how your students liked this event. Were the questions on the Regional exam too easy? too hard? about right? Was enough time allowed for each question? How can I improve the questions? Other comments? Please reply to your head coach who will send your remarks to Liz Jablecki who will pass on your comments to me.

The Phylogenetic Tree of Life based on comparative ssRNA Sequencing is below. The Tree shows the procaryotes in two Domains, Archaea and Bacteria. At a taxonomic level, most organisms at the tips of the Archaeal branches represent a unique Order; most organisms a the tips of the bacterial branches are classified into a unique Phylum. On the Archaeal limb, the three physiological groups are evident in the names: "thermo" and "pyro" for the extreme thermophiles; "methano" for the methanogens; and "halo" for the extreme halophiles. The most important, best known, and diverse groups (phyla) branching off of the Bacterial limb are the Cyanobacteria, Proteobacteria and Gram positives.

<u>Below are questions from an old Division **B** event for which I was the Event Captain.</u>



From: www.textbookofbacteriology.net/kt_toc.html

Station #1: Before you is a beaker.

USE the pH strips to measure the pH of the liquid.
WRITE the pH value you measure on your answer sheet.

Microorganism are called acidophiles, or alkalinophiles, or neutrophiles depending on the pH values at which they grow.

PREDICT which kind of "-phile" a microorganism would have to be to grow in this beaker and write the correct answer on your answer sheet.

WHERE in nature would you look for a microorganism that could grow at the pH you measured?

Station #2: Eight days after the students, staff and parents at an elementary school in Maine attended an agricultural fair, 160 became ill with diarrhea, abdominal cramps, nausea, vomiting, body aches and fever. These symptoms lasted from 10 hours to 13 days with median duration of illness of 6 days.

Alarmed by this outbreak, the state health department undertook an investigation. All 611 people who attended the fair were asked about the kinds of food they ate at the fair and whether or not they got sick (see table).

Food item	number of sick people who ate the food	% of sick people who ate the food	number of healthy people who ate the food	% of healthy people who ate the food
hot dog		65		65
hamburger		55		51
lettuce		50		48
popcorn		74		82
milk		25		32
apple juice		95		2
total	160	-----	451	-----

Make the calculations and **FILL IN** the missing blanks in the table above.

WHICH food or drink is the most likely source of the microorganism that caused the 161 people to get so sick?

WHAT is the reason for your answer?

The parasite *Cryptosporidium* was found in the sick people’s feces; this parasite is known to cause the symptoms listed above. The parasite was also found in the feces of cows grazing under apple trees. Apples were shaken off these trees the day before the fair, washed with clean water and squeezed for juice the day of the fair.

SUGGEST a reasonable hypothesis to explain how the parasite got from the cows to the people.

SUGGEST a way to reduce the chances of people getting sick from ingesting *Cryptosporidium* with apple juice.

Station #1: Some scientists claim to have discovered "nanobacteria", spherical free-living cells that range in diameter from 0.05 to 0.5 µm (50-500 nanometers) with an average of 0.1 µm. Other scientists are skeptical of these claims. The skeptics point out that the smallest known bacterium is a spherical mycoplasma with a diameter of 0.3 µm. Mycoplasmas have a genome that consists of 800,000 nucleotide pairs on one chromosome that takes up a volume in the cell of about 0.003 μm^3. The mycoplasma chromosome contains about 500 genes, the smallest number known for a cellular organism.

MAKE the calculations and fill in the table below.

	"nanobacteria"	mycoplasma
diameter of cell	0.1 µm	0.3 µm
volume of cell ($V = 4/3\pi r^3$)		
number of nucleotide pairs in	unknown; assume 500,000	800,000
volume of DNA		0.003 μm^3
% of cell volume taken up by		

If "nanobacteria" really exist and can live independently like mycoplasmas in a rich nutrient environment, then they would likely have about the same numbers of genes and nucleotides in their DNA as mycoplasmas.

ANSWER this question: Is there room in the "nanobacterial" cell for such a genome?

Examples of questions from old Bioprocess Lab exams: Division C

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volume of DNA		0.003 μm^3
% of cell volume taken up by DNA		

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ANSWER this question: Is there room in the "nanobacterial" cell for such a genome?

STATION 10 and 11 (15 points) . Many species of bacteria are able to swim in water by means of rotating flagella. Such bacteria show chemotaxis – the movement of an organism toward or away from a chemical. Chemicals that induce positive chemotaxis are called attractants, and chemicals that induce negative chemotaxis are called repellents. Chemotaxis is studied in bacteria as diagrammed in Figure 1. Bacterial cells are evenly suspended in buffer. A capillary tube containing the same buffer and the chemical of interest is lowered into the bacterial suspension. After a time interval has passed, the number of bacteria in each capillary is measured.

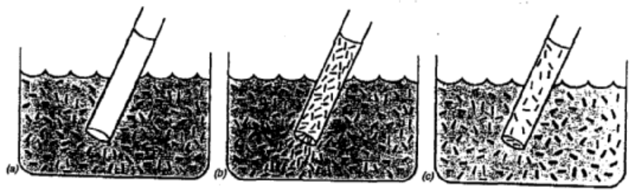

Figure 1. Diagram of the capillary technique for studying chemotaxis in bacteria. The bacteria (the dark small lines) are suspended in buffer in each of three beakers. Capillaries, each with a different chemical (a) acetate, (b) glucose, or (c) only plain buffer, are lowered into the beakers and left there for specific lengths of time. At each time interval, the capillaries are removed, their contents expelled into separate tubes, the the number of bacteria per ml in each tube determined.

Data from a typical experiment are shown in Figure 2 on the next page. **EXAMINE** the figure and answer the questions.

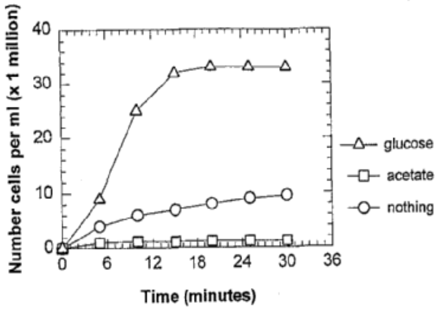

Figure 2. Time course showing numbers of bacteria in three capillaries each filled with buffer and the indicated chemical.

Answer these questions.
1. After 30 min approximately how many bacteria per ml are in the capillary containing glucose?
2. Which chemical is an attractant for these bacteria?
3. Which chemical is a repellent for these bacteria?
4. Which is the control capillary?
5. Why are there bacteria in this capillary?
6. Suggest a way to determine the number of bacteria in each capillary.