

MCDB 4350/5350 Fall 2009

Learning Goals

At the end of this course, students will be able to:

- Understand and illustrate the great diversity of microbes that inhabit this planet and the distribution of life across the global biosphere.
- Prepare sequences for construction of a phylogenetic tree (identify homologous genes and align them). Understand how different algorithms affect tree shape.
- Explain the history of and problems with the procaryote/eucaryote dichotomy. Illustrate the organization and history of life as found in molecular phylogeny.
- Explain mechanisms of lateral gene transfer and assess their impact on both evolution and the human/microbe interaction.
- Propose methods to analyze different qualities of diversity, and assess the utility of different methods.

Around 100 questions you should know...

General Questions/Skills:

1. Be able to draw a polypeptide given only the letter designations for the amino acids.
2. Given a list of 6 organisms (which will be among those mentioned in class), be able to draw the big tree, labeling the three domains, the root, and relative branch lengths for the domains.
3. What are the methods used to quantify organisms? What are some pros/cons of each method?
4. What are methods used to measure diversity? What are some pros/cons of each method?
5. Define/differentiate between the three types of homologs. How could you look for each type?
6. Be able to explain dideoxynucleotide method of DNA sequencing.
7. What are the general steps in the "rRNA approach" to analyzing the diversity of the community?
8. What are the advantages to the "rRNA approach"?
9. What is wrong with "prokaryote", "protist" and "monera"? How has our understanding of these words changed?
10. What are biochemical traits are similar/different between the three domains?
11. What staining/fluorescence tools to we use to count organisms? To sort organisms (e.g., live vs. dead, or one organism from all of the rest)?
12. Given a list of compounds, be able to identify the electron donors and acceptors.
13. Be able to solve various Poisson problems: odds of no occurrence, one occurrence, of two things occurring simultaneously once each, number of passes needed for 97%, or 99% coverage.
14. Be able to sketch a phylogenetic tree given a short sequence sample

(e.g., three sequences, 1- AGGUACGUUA 2- UGCCACGGUU 3- AGGUACGGUA).

15. Have a general understanding of the various mechanisms of photosynthesis and other forms of carbon fixation and how they are distributed throughout the tree.

Definitions (be able to spell out acronyms):

1. Xenolog, Paralog, Ortholog, Homolog
2. Polytoomy
3. Clade
4. Indel
5. Chemotroph, Heterotroph, Autotroph, Phototroph
6. Microbial Community, Microbial Population, Microbial Diversity
7. Haekel: What did he do about when?
8. Woese: What did he do about when?
9. Antony von Leeuwenhoek: What did he do about when?
10. Fannie Hesse: What did she do about when?
11. Tree calculations: Distance, Parsimony
12. Cryptotendolith
13. Psychrophile, Thermophile (including operating temperatures of each)
14. Electroporation
15. FAME
16. DGGE
17. FACS
18. MPN
19. FISH
20. PCR
21. RT-PCR, Q-PCR
22. Sequence Space
23. Stramenopile (example?)
24. Choanoflagellate
25. Testate Amoebae
26. Chytrid

Specific Questions:

1. How is the Big Tree rooted?
2. What makes a homolog?
3. Chemiosmosis has been around since the beginning of life. What is chemiosmosis (a cartoon will help your answer) and how do we know about its antiquity?
4. Why use ribosomal RNA for determining the phylogeny of all life?
5. Around how many genes are in the E. coli genome? How many base pairs?

6. In a pairwise nucleotide sequence comparison, the number of observed differences (30%) is lower than the actual number of changes. Why would there be more changes than what is observed? About how many changes actually occurred on the evolutionary path between these sequences?
7. What are the basic steps to making a polyacrylamide gel? Draw relevant chemical steps.
8. What are Koch's postulates? How is our understanding of their application changing, and what changes could be made to the postulates?
9. What are the characteristics of a credible bio-warfare agent?
10. What is a physiological island? What are some examples?
11. What are two examples of genomic rearrangements that result in a differentiated state in bacteria? Show cartoons.
12. How would you count viruses in environmental samples? About what is the "typical" virus / cell ratio in environmental samples, e.g. marine water?
13. What is an integron (show cartoon)? Why are they health menaces?
14. What is a metagenomic library? How would you make it?
15. What is syntrophy? What is "interspecies hydrogen transfer" and how does it work?
16. What is a biofilm? What are the advantages to life in a biofilm?
17. Describe the general features of a two component response regulator. Why is one component always membrane associated?
18. What are fimbriae? What is their significance to pathogenicity?
19. What is a Type III secretion apparatus? What is one specific example of the role in pathogenesis?
20. From what type of bacteria did chloroplasts come? What are the reasons to believe that?
21. The cyanobacterial photosynthetic mechanism evolved after the photosynthetic mechanisms used by other bacteria. How do we know that?
22. From what type of bacteria did mitochondria come? What are the reasons to believe that?
23. Short line segments in phylogenetic trees is sometimes interpreted as indicating a "primitive" line. What is an alternate explanation?
24. Why is the use of honey as a sweetening agent good for your teeth compared to cane sugar?
25. How do commensal bacteria contribute to our well-being?
26. Microbial diversity in anoxic environments is probably significantly richer than in oxic environments. Why is that?
27. What are three known pathways for carbon fixation?
28. What is a heterocyst? How does one differentiate? What does it do?
29. What about a sequence would indicate that it might come from an unusual organism?
30. What are the three known ways in which DNA is transferred between cells?

31. About when was the origin of life? What are two pieces of evidence for that?
32. The tetrapyrrol chemical group is fundamental to bioenergetics. Draw the basic tetrapyrrol chemical structure and list three sorts of specific molecules with which it is associated.
33. What are two molecular strategies that life uses to render high-temperature proteins more stable to thermal denaturation than homologous low-temperature proteins?
34. What are some forms of microbial motility?

Examples of “thought questions”:

1. You are trying to use PCR to monitor the relative proportions of two bacteria that occur in mixed cultures of the two organisms. You have one primer set specific for organism A and another set specific for organism B. PCR with the two primer sets results in different product sizes, so in principle you can do mixed PCR and detect the two products (organisms) in the same gel electrophoresis tract. Unbeknownst to you is that the two primer sets do not have exactly the same efficiency: the efficiency of primer set A is only 99% the efficiency of primer set B. If you start the PCR with equal amounts of DNA from the two organisms, what would be the proportions of PCR products after 30 cycles? 40 cycles? What would be an alternative way to score the proportions of the two organisms in your cultures?
2. Despite popular attention, for thermodynamic reasons there probably isn't life in the ocean postulate to lie below the ice crust of the jovian moon Europa. Why is that?
3. Recent submersible dives into a deep trench off the west coast of South America have revealed an unusual sludge that by rRNA analysis is composed of ~80% bacteria and ~20% various crenarchaeotes. You are interested in determining whether the bacteria are making a living through primary productivity. They can't be cultured, so you are forced to analyze the natural community. What would you do?
4. You are working with an EPA bioremediation team. At a strategy meeting a colleague brings up that he thinks it might be quicker and cheaper to use “FAME” to characterize the makeup of biodegrading microbial communities. You explain why this is not a good idea, and why an rRNA approach is superior. What is FAME and why is it not a good idea for community characterization? What is the rRNA approach and why is it a better idea?
5. A colleague of yours, a physicist and mass spec jock, knows not one whit about biology. He (no woman would be this dumb) thinks that he has discovered a way that life uses nuclear fuel! He thinks that because of the following experiment: He grew up some *Synechococcus* (a cyanobacterium) with bicarbonate as the sole carbon source. Then, using his mass spectrometer, he measured the amounts of the stable isotopes

C12 and C13 in the microbes and in the bicarbonate he fed them with. He observes that the ratio C12/C13 is higher in the biomass than in the bicarbonate C-source, and concludes that the organisms are using the energy from the neutron that is missing in some of the biomass carbon. You explain why he is wrong, and suggest an experiment to prove that to him. How do you explain the isotopic fractionation, and what experiment do you suggest?

6. Organisms of the Aquificales phylogenetic division of Bacteria all use molecular hydrogen as an energy source, and all detected so far live at extremely high temperatures. It is possible, however, that organisms in this relatedness group could live at low temperatures. How would you hunt for them? What kind of sample would you use?

7. A recent outbreak of water-borne gastrointestinal disease has been traced to water fountains in the Porter Biosciences Building. Culture of both water samples and swabs has failed to isolate a pathogen. Describe the specific steps that you would use to hunt for the pathogen.