

MIB0 8610: Whitman literature for discussion

Fall 2009

1. Definitions of prokaryotic species: contrast four types of definition of prokaryotic species including phenotypic similarity, polyphasic taxonomy, history of lateral gene transfer, and ecological diversity. Includes a discussion of hypothetical median organisms and methods of numerical taxonomy, DNA reassociation, includes ICSB recommendations

specific references:

1. Wayne et al. 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Inter. J. System. Bacteriol.* 37: 463-464.
2. Palys, T., L.K. Nakamura, and F.M. Cohan. 1997. Discovery and classification of ecological diversity in the bacterial world: the role of DNA sequence data. *Int. J. System. Bacteriol.* 47: 1145-1156.

Some questions to consider when discussing the papers:

Wayne et al. 1987. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Inter. J. System. Bacteriol.* 37: 463-464.

Read up on the procedures of DNA:DNA hybridization. Know what the % DNA hybridization and the ΔT_m are. How are they related to similarity in the DNA sequence?

What is the ultimate standard or reference for taxonomy in prokaryotes?

Why is species the only taxonomic level with a uniform definition?

Palys, T., L.K. Nakamura, and F.M. Cohan. 1997. Discovery and classification of ecological diversity in the bacterial world: the role of DNA sequence data. *Int. J. System. Bacteriol.* 47: 1145-1156.

Compare the hypotheses of a prokaryotic species as a “ecologically distinct population” or a “sequence similarity cluster”. Is there any agreement in the goals stated here and those of Wayne et al.

What is a “domain of competitive superiority” and how does it allow for formation of a “sequence similarity cluster”. What are “housekeeping loci”?

How is equation 1 derived on p. 1146. What do each of the terms mean?

How are the numbers in Table 1 calculated?

Can ecologically distinct populations form multiple sequence similarity clusters?

Why is the 16S rRNA gene sequence not useful for delineating ecologically distinct populations?

How do sequence similarity clusters compare to DNA hybridization groups?

2. Prokaryotic evolution:

Specific references:

1. Woese, C.R. 2002. On the evolution of cells. Proc. Nat. Acad. Sci. USA 99: 8742-8747.

General references:

1. Woese, C.R. 2000. Interpreting the universal phylogenetic tree. Proc. Nat. Acad. Sci. USA 97: 8392-8396.

Some questions to consider when discussing the papers:

Woese, C.R. 2002. On the evolution of cells. Proc. Nat. Acad. Sci. USA 99: 8742-8747.

1. In simple terms what is the question being addressed here.
2. What do you think of the argument about canonical patterns and whether or not the last common ancestor was a modern cell? Are there counter arguments?
3. What is the significance of horizontal gene transfer in the evolutionary context. What might control its frequency?
4. What is the Darwinian threshold? How might it affect the root of the universal tree?
5. Why was the progenitor(s) of the modern lineages likely to be a community of organisms exchanging DNA by HGT?
6. What is your own model for how modern life might have evolved? How does your model agree with Woese's. Compare the similarities and differences, how might you resolve the differences experimentally.
7. What was your favorite thing about this paper? What was your least favorite thing?

3. Archaea

Specific references:

Makarova, K.S., A.V. Sorokin, P.S. Novichkov, Y.I. Wolf, and E.V. Koonin. 2007. Clusters of orthologous genes for 41 archaeal genomes and implications for evolutionary genomics of archaea. *Biology Direct* 2, 33.

Some questions to consider when discussing the papers:

1. What is a COG? How is it calculated and why was it used?
2. What is the difference between core, shell and ORFans? During the evolution of the archaea, do these different types of genes play different or the same role?
3. What does the tree in Figure 7 tell us about the evolution of the archaea?
4. Can you decide from this paper what it means to be an archaeon? What doesn't this paper tell you?

4. Methanogenesis

Specific reference:

Thauer, R.K., A.-K. Kaster, H. Seedorf, W. Buckel, and R. Hedderich. 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Rev. Microbiol.* 6: 579-591.

Some questions to consider when discussing the paper:

1. For methanogenesis from CO₂, what are the major coupling sites for formation (or utilization of the pmf) in methanogenesis? How do these compare with the ΔG s for individual reactions?
2. Compare the coupling in methanogens which contain cytochromes and those that do not.
3. Why (or how) do some methanogens have cytochromes and others do not?

5. Autotrophic CO₂ fixation:

Specific references:

1. Eisenreich, W., G. Strauss, U. Werz, G. Fuchs, and A. Bacher. 1993. Retrobiosynthetic analysis of carbon fixation in the phototrophic eubacterium *Chloroflexus aurantiacus*. Eur. J. Biochem. 215: 619-632.
2. Strauss, G., and G. Fuchs. 1993. Enzymes of a novel autotrophic CO₂ fixation pathway in the phototrophic bacterium *Chloroflexus aurantiacus*, the 3-hydroxypropionate cycle. Eur. J. Biochem. 215: 633-643.

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What is NMR and how does it work? What is TOCSY?

How is NMR used to identify labeling patterns in organic molecules? What are the advantages (or disadvantages) of using ¹³C-labeled tracers for ¹³C and proton NMR? Compare this methodology to using ¹⁴C-tracers.

Which amino acids are used as markers of key cellular intermediates?

In Figure 1, why were both ¹⁴C and ¹³C-labeled acetate utilized? Are these cells grown autotrophically or heterotrophically?

How is the proposed scheme in Figures 8-10 supported by the observed labeling patterns in amino acids? Are alternative pathways eliminated?

Strauss, G., and G. Fuchs. 1993. Enzymes of a novel autotrophic CO₂ fixation pathway in the phototrophic bacterium *Chloroflexus aurantiacus*, the 3-hydroxypropionate cycle. Eur. J. Biochem. 215: 633-643.

For each of the enzymes, be able to describe the reaction catalyzed and the basis for the assay.

Are all the assays convincing evidence for the specific reactions proposed? How about the specific activities— are they high enough for the proposed pathway?

How do the bioenergetics of the hydroxypropionate pathway compare to other autotrophic pathways?

What additional evidence might one collect to support (or disprove) this pathway?

6. Methanotrophy

Specific references:

Peyraud, R., P. Kiefer, P. Christen, S. Massou, J.C. Portais, and J.A. Vorholt. 2009. Demonstration of the ethylmalonyl-CoA pathway by using ¹³C metabolomics. Proc. Nat. Acad. Sci. USA 106: 4846-4851 and Supplement.

Some questions to consider when discussing the papers:

1. How does the pathway shown in Figure 1 fit into methylotrophy?
2. What is the relationship between the abundance of the potential intermediates observed in Figure 4 and their order in the pathway? Does this prove anything? Do you think a pathway of methylotrophy or a pathway of acetotrophy is being observed here?
3. How might the steady state model discussed in Table 1 and the text be generated?
4. For Table 1, please how rows 5 and 6 are calculated from rows 1-4.
5. What do you think, is their basic conclusion correct?

7. Photosynthesis:

Specific references:

1. Xiong, J., W.M. Fischer, K. Inoue, M. Nakahara, and C.E. Bauer. 2000. Molecular evidence for the early evolution of photosynthesis. *Science* 289: 1724-1730.

Some questions to consider when discussing the papers:

Xiong, J., W.M. Fischer, K. Inoue, M. Nakahara, and C.E. Bauer. 2000.

1. Why might the evolution of photosynthesis genes be different from that of the other genes in photosynthetic organisms?
2. Why do you suppose that all the photosynthesis genes are linked in some organisms but not others?
3. What is "long branch attraction"? Why are outgroups important?
4. What is the best guess for the branching order at the base of the tree for photosynthetic genes? Why might better information about this branching order be interesting?
5. What is the Granick hypothesis? How does the work reported here affect our evaluation of this hypothesis?

8. Sulfur-oxidizing bacteria:

Specific references:

1. Fossing, H., V.A. Gallardo, B.B. Jorgensen, M. Huttel, L.P. Nielsen, H. Schulz, D.E. Canfield, S. Forster, R.N. Glud, J.K. Gundersen, J. Kuver, N.B. Ramsing, A. Teske, B. Thamdrup, and O. Ulloa. 1995. Concentration and transport of nitrate by the mat-forming sulphur bacterium *Thioploca*. *Nature* 374: 713-715.
2. Schulz, H.N., T. Brinkhoff, T.G. Ferdelman, M. Hernandez Marine, A. Teske, and B.B. Jorgensen. 1999. Dense populations of a giant sulfur bacterium in Namibian shelf sediments. *Science* 284: 493-495.

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Where are the *Thioploca* mats found? Describe the water chemistry at this site.

How does *Thioploca* make a living in this habitat? What is the role of motility?

Could these organisms be found on the surface or in shallow waters?

Schulz, H.N., T. Brinkhoff, T.G. Ferdelman, M. Hernandez Marine, A. Teske, and B.B. Jorgensen. 1999. Dense populations of a giant sulfur bacterium in Namibian shelf sediments. *Science* 284: 493-495.

Compare the life style of *Thiomargarita* to that of *Thioploca*. How do they use very different strategies to achieve similar results (H₂S oxidation with nitrate as an electron acceptor)? Compare their positions in the 16S rRNA tree. What does that mean?

How slow can an organism grow? Can you think of a chemical or physiological basis for a lower limit? How would you address this experimentally.