Microbial Taxonomy

1. Traditional taxonomy or the classification through **identification** and **nomenclature** of microbes, both "prokaryote" and eucaryote, is in a mess – we are stuck with it for traditional reasons.

A. A "natural" taxonomy would be based on evolutionary relatedness: Thus, organisms in same "genus" (a collection of "species") would have similar properties in a fundamental sense.

B. A natural taxonomy of macrobes has long been possible: Large organisms have many easily distinguished features (e.g., body-plans and developmental processes, that can be used to describe hierarchies of relatedness).

C. Microbes usually have few distinguishing properties that relate them, so a hierarchical taxonomy mainly has not been possible.

2. Recent advances in **molecular phylogeny** have changed this picture – we now have a relatively quantitative way to view **biodiversity**, in the context of phylogenetic maps or evolutionary trees.

A. Slowly evolving molecules (e.g. rRNA) used for large-scale structure; "fast- clock" molecules for fine-structure.

B. The literature language (e.g. "species") and formal nomenclature, however, remain solidly rooted in the tradition of Linnaeus at this time. (You have to call them something!)

3. A Bit on the Evolution of Evolutionary Thought

A. Prior to the late 19th century, the concept of evolution was on the **evolutionary ladder**. Thus, we still deal in "higher and lower" eucaryotes (I try not to use these terms – they are dumb), "missing links," and "primitive" organisms.

B. In its milieu, *E. coli* is as highly evolved as are we. *E. coli* is **simple** (~$5x10^6$ bp genome), we are **complex** (~$3x10^9$ bps); complexity has nothing to do with *evolutionary advancement*.

C. Lineages evolve by diversification, not *progression*. !!!

D. There is no such thing as a *primitive* organism alive today. **Simple**, yes, but still a finely honed product of ~ 4 billion years under the selective hammer of the niches that it and its progenitors have occupied.

4. By the late 1800s the concept of "evolutionary trees" was on the table – e.g. Ernst Haeckel, 1866 (Note that Darwin's "Origin of Species" was first presented in 1858).

A. Note "kingdoms" of Plants, Animals, Protists (non-plants and animals, mostly microbial), and "monera" (procaryotes, in retrospect) at base.

B. The conceptual basis for biological diversity was pretty much stalled at the Haeckel stage for the next century – and still is in many/most general texts of biology.

Formal Classification and Physiological Diversity

1. The phylogenetic perspective has had enormous impact on microbial taxonomy, but unfortunately the ground-rules for classification are not phylogenetic, but rooted in physiology, the properties of the organism that interact with the environment.

A. Traditional taxonomy does not correlate well with phylogenetic results, with a few exceptions (e.g., Mycoplasmas, "wall-less" bacteria, long mysterious, are one degenerate offshoot group of "Low G+C Gram Positive" bacteria).

B. For lack of distinguishing properties, microbes have been classified by most unsystematic properties:

- Cell-shape
- Staining reaction (e.g., Gram stain)
- Position of flagella (polar vs. peritrichous, tufts vs. single)
- Nutritional properties (use of different sugars, use of election acceptors, etc.)

2. By accumulating a lot of such properties, taxonomic clusters can be derived. These are collected in "Bergey's Manual of Systematic Bacteriology" (4 vols.) with the following hierarchy:

Kingdom ➜ Division ➜ Class ➜ Order (-ales suffix)

➜ Family (-eae suffix) ➜ Genus ➜ Species

A. Names below species are sometimes used –

Biovar (Biotype) - special biochemical property
Serovar (serotype) - antigenic properties
Pathovar (pathotype) - pathogenic properties like host-type
Phagovar (phagotype) - bacteriophage susceptibility
Morphovar (morphotype) - special morphological properties

B. Currently, Bergey's Manual collects organisms into "sections," in essence chapters in the Manual with no evolutionary relationships implied!

- Note lots of inconsistencies – e.g., Pseudomonas in same group as Halobacterium, an archaeon.
- Be really suspicious when you bear the term "typical Pseudomonad" (Gram - , polarly flagellated)!
- Don't use terms such as "typical Gram negative organism!" (When you hear the term, the speaker usually means a representative of the bacterial phylogenetic division *Proteobacteria*.)

D. In summary, the classical phenotypic methods consist in essence of anecdotes regarding the particular organism. If you have enough anecdotes, you can do pretty well. Hence medical diagnostics have been very successful:

- Relatively few organisms to classify (i.e., disease-related).
- Many diagnostic tests can be leveled.
- Limited number of physiologies to worry about (all are "chemoheterotrophs").

E. Note one very big caveat on the traditional approach to classification – the organism must be grown in pure culture.

- This is a problem even in medical diagnostics – there are many "cryptic" diseases that respond to antibiotic therapy.
- With environmental samples you really are in trouble: <<1% of organisms seen microscopically in environmental samples (e.g., soil, water) can be cultured using standard techniques.....

3. If you know what you are looking for (a specific organism), there are a number of techniques for identifying the presence of the organism:

A. Some specialized tests are more-or-less useful. One commonly encountered is FAME (Fatty Acid Methyl Ester) "profiles." FAs are components of membranes, esterified to e.g. glycerides.

- Unsaturated at various positions, cis/trans in various ways, but uniform in particular organism grown in particular conditions.
- Cell mass ➜ treat with NaOH to "saponify" esters ➜ HCl + CH_3OH ➜ methyl esters: Shoot gemisch through GC.
- Compare results to a database of previously determined FAME profiles – there are companies who do this & the results can be quite precise.

B. DNA "homology" tests can be useful, but are cumbersome and not generally applicable – the data cannot be used with a database.

- By comparing lots of organisms, the rule-of-thumb has emerged:
  100% hybridization – same organism
  75% "species level" relatedness
  25% "genus level"
  <20% – no information

- A lot of this has been done over the years, and you will encounter if you read on bacterial taxonomy, but is not generally applicable as a characterization tool.

- Have to pairwise compare each new organism with many other DNAs experimentally.

4. Although microbiologists had long hoped for a natural classification, by the 1950s they had abandoned the possibility, some even declaring it impossible! When molecular-phylogenetic methods became available, it is ironic that the systematists were main hold-outs in embracing the results.

In summary:

- Classical physiological descriptions of microbes constitute a taxonomy, but do not provide relationships (except as might be inferred subjectively).

- Methods such as FAME or DNA-DNA reassociation establish relationships, but only if close, i.e., they are not sufficiently general to be broadly applicable.

- All these methods require pure-cultivation of organisms for characterization, but we can't cultivate much of what is out there.