Bacillus anthracis

Last Lecture:
1. Introduction
2. History
3. Koch’s Postulates

Today’s Lecture:
1. Prokaryote vs. Eukaryote
2. Classifying prokaryotes
3. Phylogenetics
I. Basic Cell structure: (Fig. 1.5)

A. All cells have

1. Barrier separating outside/inside
   CYTOPLASMIC MEMBRANE

2. CYTOPLASM
   - RIBOSOMES
   - NUCLEIC ACIDS

B. Many cells (plants, prokaryotes, fungi) have: CELL WALLS
   - PROTECTION/STRUCTURE/PERMEABLE

C. Eukaryotic cells
   1. Major feature that is not in prokaryotic cells are:
      - MITOCHONDRIA
      - CHLOROPLAST
      - NUCLEUS
   2. Generally larger and more complex than prokaryotes (Fig. 23.1)
      END = ~ 20 mm
      2 mm = Prok.

3. DNA is contained in a membrane compartment called the nucleus bound

D. Prokaryotic cells (Fig. 4.43)
   1. Two domains: ARCHAEA and BACTERIA
   2. Generally SMALLER and LESS than eukaryotes
   3. DNA is free in the cytoplasm.
      COMPLEX AGGREGATES → NUCLEOID.
## II. Characteristics of the Domains of Life

Characteristics of the primary domains *(Table 1.2, 1.3, 1.4)*

<table>
<thead>
<tr>
<th>Property</th>
<th>Bacteria</th>
<th>Archaea</th>
<th>Eukaryote</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear membrane</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Peptidoglycan cell walls</td>
<td>YES</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Membrane Lipids: glycerol-hydrocarbon linkage</td>
<td>ESTER C-O-C</td>
<td>ETHER C-O</td>
<td>Should be ester linkage NOT ether</td>
</tr>
<tr>
<td>Contain plastids</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Chloroplasts</td>
<td>NO</td>
<td>ND</td>
<td>YES</td>
</tr>
<tr>
<td>Transcription</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mRNA processing (capping, polyA tail)</td>
<td>NO</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>mRNA splicing</td>
<td>NO*</td>
<td>NO*</td>
<td>COMMON</td>
</tr>
<tr>
<td>RNA Polymerase</td>
<td>ONE TYPE 4 SUBUNITS</td>
<td>SEVERAL 8-12</td>
<td>3 TYPES 12-14 SUBUNITS</td>
</tr>
<tr>
<td>Genes in operons</td>
<td>YES</td>
<td>YES</td>
<td>&amp; NO</td>
</tr>
<tr>
<td>Transcription Factors Required</td>
<td>NO</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Ribosome size</td>
<td>70S</td>
<td>70S</td>
<td>80S</td>
</tr>
<tr>
<td>Metabolism: Nitrogen Fixation</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Chemolithotrophy</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Growth &gt; 80°C</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
III. Artificial vs. Phylogenetic Classification
   A. Artificial classification is based on: **OBSERVABLE TRAITS**.
      E.g.: GRAM STAIN, GLUCOSE METABOLISM, PHENOTYPE
   B. Phylogenetic classification is based on: **evolutionary history**.
      1. Requires: molecular marker should not change much
      2. Preferred by bacteriologists
   C. Two prokaryotic domains: **Archaea / Bacteria**

IV. Nomenclature follows the binomial system of names.
   A. Domain, Phylum, Class, Order, Family (Genus, Species (Table 17.1))
   B. Example: Genus, Species: *Escherichia coli* must be Latin endings.
      1. Genus is always capitalized and the species is lower case
      2. Always italicize or underline.
      3. Name usually has some significance.
   C. How do identify a new isolate and classify it to the species level?
      1. There are international guidelines: e.g. DNA-DNA hybridization

IV. Phylogenetic Trees
   A. Basically two ways to create a phylogenetic tree:
      1. using: **PHYSIOLOGICAL CHARACTERISTICS**
      2. using: **MOLECULAR DATA**
   B. The molecular-based system
      1. Phylogenetic Tree shown in **Fig 1.6**
         a) **ACCEPTED BY MANY BIOLOGISTS**
         b) The tree is derived from **RIBOSOMAL RNA's**
         c) Pioneered by **Carl Woese** *(Box 17.4)*
      2. This organization suggests that most of the diversity of life is in the **PROKARYOTIC** domains based on ribosomal RNA differences.
         a) Humans to corn: ~0.1% base changes/site. 10%
         b) Between bacteria and archaia: ~0.4% base changes/site.
      3. Archaea are **MORE** closely related to Eukarya than Bacteria.
   4. Eukaryotic microbes —
VI. The 16S or 18S rRNA gene is commonly used for phylogenetic classification of prokaryotes and eukaryotes:

A. General features of a molecular marker?
   1. **Found in all organisms**
   2. The function is: **Similar in all organisms**
   3. There are **variable** and **conserved** regions
   4. The gene tolerates: **Some mutation/change**

B. The gene encoding 16S ribosomal RNA (18S in eukaryotes)
   1. The 16S rRNA is part of the ribosome
      a) Occurs in **all prokaryotes**
      b) **Conserved function**
      c) Gene size: **1500 nucleotides**
      d) composed of many domains that can change independently of each other (*Figure 17.1—Sequenced by Harry Noller*)
      e) **Mutation that affect function-slow**
      f) Large databases of sequences (Ribosome Database Project)

VII. Methods for making a phylogenetic tree

A. Phylogenetic trees: *Figure 17.4 and 17.5*, PCR and sequencing discussed in Boxes 16.1 and 16.3
   1. Structure: nodes (internal vs. external), branches, and branch lengths (17.4)
   2. Two main types: rooted and unrooted (17.3)
B. Steps to make a 16S rRNA gene Phylogenetic Tree

1. **ISOLATE DNA FROM OUR STRAIN**

2. Use Polymerase Chain Reaction (PCR) to: **AMPLIFY DNA FOR THE 16S rRNA GENE**

What goes into a PCR:

1. **DNA**
2. **PRIMER (NUCLEIC ACID DNA)**
3. **DEOXY NUCLEOTIDE TRIPHOSPHATE**
4. **POLYMERASE - THERMOSTABLE - Taq**
5. **BUFFER + Mg^{2+}**

Put tubes in a thermocycler machine. What does this do?

- **Denature:** DNA → SINGLE STRANDS
- **Anneal:** PRIMERS BIND DNA
- **Extension:** POLYMERASE SYNTHESIZES NEW DNA. \( \times 30 \) 2 copies \( 2^n = 2^{30} \)

3. Analyze by agarose gel electrophoresis

- **VISUALIZE DNA**

4. Sequence the 16S rRNA gene and compare the sequence to a database of sequences to see what prokaryote you’ve got by ALIGNING yours to others.

- **USUALLY DONE w/ COMPUTERS**

5. Turn the alignments into a: **Phylogenetic Tree**

Let’s go over Fig. 17.6
### Prokaryotes vs. Eukaryotes and Diversity

#### (A) 16S rRNA sequences

<table>
<thead>
<tr>
<th>Organism (strain)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>G</td>
<td>C</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>b</td>
<td>G</td>
<td>A</td>
<td>C</td>
<td>G</td>
<td>C</td>
<td>C</td>
<td>A</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>c</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>U</td>
<td>C</td>
<td>U</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>d</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>U</td>
<td>A</td>
<td>G</td>
</tr>
</tbody>
</table>

- Calculate distance matrix
- Start an intermediate tree with most closely related strains.
- Calculate second distance matrix.
- Add other intermediate tree of closely related strains.
- Calculate distance between two intermediate trees.
- Then the average distance and draw the branches.

\[
\begin{align*}
    d(ab)(cd) & = \frac{d(ab) + d(cd)}{2} \\
    d(ab) &= \frac{1 + 2}{2} = 1.5 \\
    d(cd) &= \frac{9 + 2}{2} = 5.5 \\
    d(ab)(cd) &= \frac{1.5 + 5.5}{2} = 3.5
\end{align*}
\]
**Cell membrane**: lipid and protein layer surrounding the cytoplasm. In cells lacking cell walls (some microorganisms, all animal cells), it is the boundary between the cell and its surroundings.

**Cell wall**: rigid outer layer of the cell, of varying chemical composition. It is found in many microorganisms and all fungi and plants.

**Nuclear material**: the hereditary material, DNA. In most cells (but not typical bacteria) the DNA is contained within a membrane.

**Cytoplasm**: contains organelles, enzymes, chemicals. It is the site of most cellular metabolic activity.
Prokaryotes vs. Eukaryotes

Microbial Life 2e, Figure 4.43

Microbial Life 2e, Figure 1.6
Fig. 17.1
Box 16.1