Chapters 1, 9 & 6: Microbiology History; Microbial Taxonomy; Growth & Culturing of Bacteria

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Lectures: MW Noon
Office Hours: Wednesdays 9:00 AM

Biological chemistry

• Remember to review chapter 2 of Black for Wednesday’s Chemistry Quiz

• If you have not taken an organic chemistry course (CHEM 6B, 20, 24) you do NOT have the necessary prerequisites for this class!

First “news” articles are on my website. Please read and submit very brief answers. Articles will be posted weekly.

Plan to turn in your answers the following Wednesday (but I will accept answers up to two weeks from date article is posted).

Visit my website frequently!

Chemistry review quiz Wednesday. 20 points

Prepare on your own! Use textbook chapter 2, my study guide, my Powerpoint slides (at website)

Spontaneous Generation: Life from nonliving matter

Observation: Every year, the Nile River floods, leaving behind nutrient-rich mud. However, along with the muddy soil, large numbers of frogs appear.

Conclusion: Muddy soil gave rise to the frogs.

Observation: Before there were refrigerators, a trip to the butcher shop meant battling the flies around the carcasses.

Conclusion: Rotting meat was the source of the flies.

Spontaneous Generation: What about microorganisms?

Microbes seemed to arise spontaneously from broth, etc.

• Think about old food in your refrigerator! Does it spontaneously generate life?

This was a widely held belief in 1800’s.

Hampered the development of microbiology as a science, and slowed our understanding of infectious disease

• If microbes come from nonliving things, then infections don’t necessarily “spread” from one source to another; they can arise spontaneously.

Early work: Francesco Redi
17th century

Conclusion: No spontaneous generation
Spontaneous Generation of Microbes?

**Evidence against:**
- Boil broth, and seal the flask: broth does *not* produce life (broth does not become cloudy)

**Rebuttal:**
- Methods used to sterilize the broth “altered” the air (e.g., by heating), and then fresh, unaltered air was kept out
- “Altered” air couldn’t interact with the “vital force” in the food

Enter the giant: Louis Pasteur (1822-1895)

19th century French scientist. Accomplishments include:
- Famous experiment refuting *spontaneous generation* of microbes
- Isolated *specific* organisms involved in wine fermentation, and disease
- Developed first rabies vaccine
- **Pasteurization** technique to kill unwanted microbes

Robert Koch (1843-1910)

- German contemporary of Pasteur
- *Many* contributions:
  - Isolated anthrax, tuberculosis, other organisms
  - **Pure cultures**: Technique for obtaining bacterial cultures containing *only one kind* of organism
  - Koch’s postulates

The Germ Theory of Disease:

- *Microorganisms can invade other organisms and cause disease*

Idea formulated in mid-19th century but *not* widely accepted

**Koch’s Postulates**
- provided a way to establish this theory
- indirectly refute spontaneous generation
Koch's Postulates

1. The microbe must be present in every case of the disease but absent from healthy organisms.
2. The suspected microbe must be isolated and grown in a pure culture.
3. The same disease must result when the isolated microbe is inoculated into a healthy host.
4. The same microbe must be isolated again from the diseased host.

Koch's Postulates (1884)

1. Suspect pathogenic organism should be present in all cases of the disease and absent from healthy animals.
2. Suspect organism should be grown in pure culture.
3. Cells from a pure culture of suspect organism should cause disease in healthy animal.
4. Organism should be re-isolated and shown to be same as the original.

Other names to know & respect...

- **Edward Jenner** (18th C.)
  - 1st vaccine: smallpox
  - Observation: Milkmaids who got cowpox didn’t get smallpox
  - Inject fluid from a cowpox blister: protected
  - Cow = vacca (Latin) → vaccine

- **Joseph Lister** (late 19th C.)
  - Surgeon
  - Introduced Aseptic technique

- **Alexander Fleming**
  - Discovered penicillin (1928)
  - Observed a zone of inhibition around a contaminating fungus, where bacteria did not grow
  - The mold, of the genus Penicillium, secretes an antibacterial agent

- **Florey & Chain**
  - figured out how to produce it (1940's)
  - "The Third Man" starring Orson Welles

To learn more about remarkable human achievements in the emergence of microbiology, read Microbe Hunters by Paul de Kruif.
Classification of Microbes

(Taxonomy)

Taxonomy began with Linnaeus in 1700's

The Linnean system:
- Categorizes living things into smaller and smaller groups which share characteristics
  - Kingdom, phylum, class, order, family, genus, species
  - Linnaeus divided all life into 2 kingdoms: Plant & Animal
  - This has since proved to be inadequate
- Gives each organism a binomial (two word) species name
  - e.g. Homo sapiens (genus + species)

Our classification of living things changes over time as our understanding of "relatedness" improves

One currently used taxonomic system: 5 Kingdoms, 3 Domains

Domain Eukarya
- Protists
- Fungi
- Plants
- Animals

Domain Archaea
- Bacteria
- Protozoa
- Algae

Domain Bacteria
- Photosynthetic
- Some are an excellent food source (e.g. Spirulina, shown at right)
- Others produce toxins that can poison water
- Some can fix nitrogen

Cyanobacteria
- Domain Bacteria (along with eubacteria)
  - Photosynthetic
  - Some are an excellent food source (e.g. Spirulina, shown at right)
  - Others produce toxins that can poison water
  - Some can fix nitrogen
- Chloroplasts are likely remnants of endosymbiotic cyanobacteria (who took up residence inside a eukaryotic cell)

Archaea
- Very ancient origin
- Many live in extreme environments
  - 1. Methanogens
    - Produce methane in absence of oxygen
  - 2. Extreme halophiles
    - Live in very salty places
  - 3. Extreme thermoacidophiles
    - Live in hot springs, submarine vents, etc.

Bioprospecting: searching the genomes of extremophiles for commercial uses
- Enzymes produced by extremophiles have unique properties and can catalyze unusual reactions under unusual conditions
- Some (e.g. DNA polymerases Taq & Pfu) are extremely valuable in molecular biology (PCR)

The next few weeks: Classification and properties of Eubacteria (true bacteria)

Pure culture: a culture of bacteria containing only one species
- The entire culture will display characteristics distinctive for that species only, making identification possible
- Mixed cultures contain more than one species
- Strain: subspecies e.g. E. coli 0157
  - Bacteria of the same species which, when grown in pure culture, have unique characteristics
  - What features make one species of bacteria different from another?
Criteria we will use to identify bacteria in lab this semester

Bacterial Growth

- Growth is cell division
  - Binary fission

- No cell cycle; DNA synthesis is continuous in continuously dividing cells
- Chromosome is attached to the cell membrane, which grows & separates the replicated chromosomes to opposite sides of the septum

Budding

- Yeast and a few bacteria reproduce by budding instead of binary fission
- Budding & binary fission are asexual (no genetic diversity is created)

Bacterial Growth Requirements

- Each species or strain of bacteria has unique needs
- To grow bacteria in a lab, you must artificially replicate the necessary conditions

- Nutrients (elements, carbon sources, energy source, etc.)
- pH
- Temperature
- Moisture
- Hydrostatic pressure
- Osmotic pressure (tonicity of environment)
- Radiation
- Oxygen / no O₂

For ANY of these factors, you can find certain bacteria that thrive at high or low extremes
Bacterial growth requirements example: Osmolarity

General categories:
- **Aerobes**: require oxygen to grow
- **Anaerobes**: do not require oxygen

To grow bacteria in culture, must provide the appropriate oxygen level in the environment

O₂ requirement is related to type of metabolism the bacteria perform.

★ **Obligate aerobe**: must have free oxygen (O₂) to grow
★ **Obligate anaerobe**: killed by free oxygen
★ **Aerotolerant or indifferent**: do not use oxygen but are not harmed by it, so they grow equally well in the presence or absence of air
★ **Facultative anaerobe**: organisms that can respire aerobically but will shift to anaerobic metabolism if oxygen is absent. Grow better in air because aerobic respiration is much more efficient.

★★ **Bacterial Growth Curve**
- Describes how bacterial population expands over time
- Applies to bacteria freshly added to a nutrient-rich liquid medium

- **Lag phase**
  - Bacteria “wake up” to their happy surroundings:
    - Increase in metabolic activity
      - Enzyme synthesis
      - ATP production
      - Increase in cell size
    - Preparing for cell division but **no increase in number** of bacteria yet
Log phase

- Exponential (logarithmic) population growth
- \(2^n\) cells present after \(n\) doublings
- Generation time: time required for each doubling
  - Under ideal conditions, this time is genetically determined (varies with species)
  - Generally between 20 minutes & 20 hours
    - Typically less than 1 hour

Stationary phase

- Cell division slows as the environment changes (fewer nutrients, pH and oxygen level change)
- Some cells dying, some cells dividing
  - Number of living cells stays about the same

Decline phase

- Think New Orleans Superdome
  - Food runs out
  - Wastes accumulate
  - The medium can no longer support healthy cell division
  - Cells die
- Population of live cells in the culture decreases
  - Can maintain stationary phase in a device called a chemostat
  - Human cities are a kind of chemostat: fresh medium is continuously added as old medium is withdrawn, maintaining the log phase

Bacterial growth in a lab: Colonies

- Colony: pile of bacteria growing on solid media
  - all cells are descendents of one original cell
  - Clones
  - Use to get a pure culture

  In a colony, you see all phases of the growth curve simultaneously, with early phases at the edges (where rapid growth is occurring), and death at the center.

Relevant reading in Black’s Microbiology:

- Chapter 1 History
- Chapter 9 Taxonomy
  - p. 232-244; p. 252
- Chapter 6 Growth & Culturing of Bacteria