SACRAMENTO STATE
Leadership begins here.

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

Dr. Amy Rogers

Fall 2006
Lectures: MW Noon
Office Hours: Wednesdays 9:00 AM

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)

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!

★ 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.

Early work: Francesco Redi
17th century

Conclusion: No spontaneous generation

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.

Spontaneous Generation of Microbes?

<u>Evidence *against:*</u>

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

<u>Rebuttal</u>:

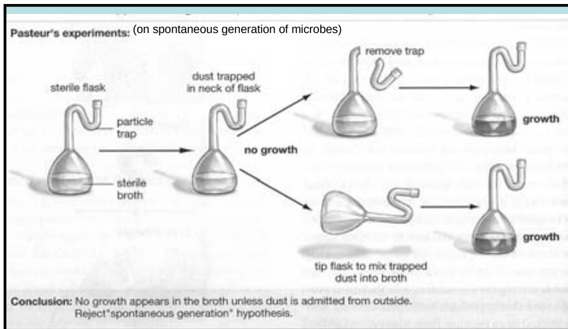
- Methods used to sterilize the broth "altered" the <u>air</u> (e.g., by heating), and then fresh, unaltered air was kept out
- "Altered" air couldn't interact with the "vital force" in the food

Enter the giant:

Louis Pasteur (1822-1895)

19th century French scientist. Accomplishments include:

- Famous experiment refuting <u>spontaneous generation</u> of microbes
- <u>Isolated *specific* organisms</u> involved in wine fermentation, and disease
- Developed first <u>rabies vaccine</u>
- ***<u>Pasteurization</u>*** technique to kill unwanted microbes



- **Unaltered** fresh air has access to the broth but broth remains sterile because microbial particles are caught in the "swan neck" flask
- Broth was not "altered" by boiling either; it can still support growth

Actual Pasteur "swan-neck" flask

Figure 1-7 Microbiology, 6/e
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Robert Koch (1843-1910)

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

<u>The Germ Theory of Disease</u>:

microorganisms can invade other organisms and cause disease

Idea formulated in mid-19th century but **<u>not</u>** widely accepted

<u>Koch's Postulates</u>

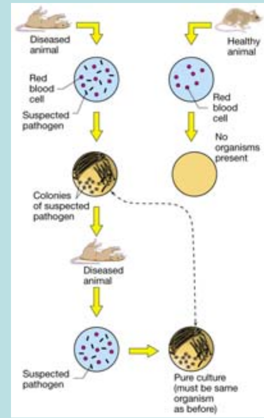
- provided a way to establish this theory
- indirectly refute spontaneous generation

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)

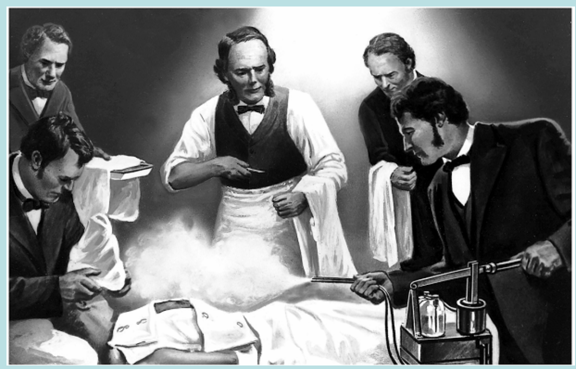
1. **Suspect pathogenic organism should be present in all cases of the disease and absent from healthy animals**
2. **Suspect organism should be grown in pure culture**
3. **Cells from a pure culture of suspect organism should cause disease in healthy animal**
4. **Organism should be re-isolated and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



Other names to know & respect…

- Edward Jenner (18th C.)
  - <u>1st vaccine:</u> <u>smallpox</u>
    - 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 **<u>Aseptic technique</u>**

Joseph Lister & Antiseptic Surgery (1869)



Carbolic acid

- Alexander Fleming
  - Discovered ***<u>penicillin</u>*** (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

Sir Alexander Fleming (1881-1955)

Classification of Microbes (**Taxonomy**)

Taxonomy began with Linnaeus in 1700's

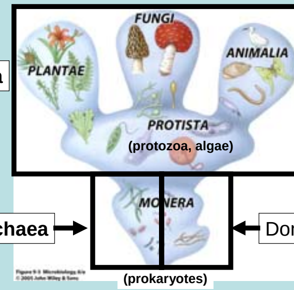
The Linnean system:

- 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

Classification of microorganisms presents unique challenges

One currently used taxonomic system: 5 Kingdoms, 3 Domains



ABLE 9.2

The Five-Kingdom System of Classification

	Monera (Prokaryotae)	Protista	Fungi	Plantae	Animalia
Cell type	Prokaryotic	Eukaryotic	Eukaryotic	Eukaryotic	Eukaryotic
Cell organization	Unicellular; occasionally grouped	Unicellular; occasionally multicellular	Unicellular or multicellular	Multicellular	Multicellular

Evidence accumulated that the archaeobacteria are fundamentally different from other bacteria. Kingdom Monera was divided into two domains.

- Domain **Bacteria**
  - Prokaryotes (kingdom Monera)
    - eubacteria (*eu*, true)
    - cyanobacteria (formerly called blue-green algae)
- Domain **Archaea** (archaeobacteria; *archae*, ancient)
  - Prokaryotes (kingdom Monera)
  - Many are extremophiles
- Domain **Eukarya**
  - All eukaryotes (kingdoms Protista, Fungi, Plantae, Animalia)

Archaea

- *Very* ancient origin
- Many live in **extreme** environments

3 major groups:

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 & can catalyze unusual reactions, under unusual conditions
  - •Some (e.g. DNA polymerases *Taq* & *Pfu*) are extremely valuable in molecular biology (PCR)

Vent is 3000 m below sea level. 360°C. Sulfurous volcanic gas

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)

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 O157*

Bacteria of the same species which, when grown in pure culture, have unique characteristic(s)

(that are not significant enough to make them a different species)

What features make one species of bacteria different from anot

(what kinds of criteria are used to classify bacteria?)

TABLE 9.5

Criteria for Classifying Bacteria

Criteria we will use to identify bacteria in lab this semester → Morphology, Staining, Growth, Nutrition, Physiology, Biochemistry

Criteria	Examples
Morphology	Size and shape of cells; arrangements in pairs, clusters, or filaments; presence of flagella, pili, endospores, capsules
Staining	Gram-positive, Gram-negative, acid-fast
Growth	Characteristics in liquid and solid cultures, colony morphology, development of pigment
Nutrition	Autotrophic, heterotrophic, fermentative with different products; energy sources, carbon sources, nitrogen sources, needs for special nutrients
Physiology	Temperature (optimum and range); pH (optimum and range), oxygen requirements, salt requirements, osmotic tolerance, antibiotic sensitivities and resistances
Biochemistry	Nature of cellular components such as cell wall, RNA molecules, ribosomes, storage inclusions, pigments, antigens; biochemical tests
Genetics	Percentage of DNA bases (G + C ratio); DNA hybridization
Serology	Slide agglutination, fluorescent-labeled antibodies
Phage typing	Susceptibility to a group of bacteriophages
Sequence of bases in rRNA	rRNA sequencing
Protein profiles	Separate proteins by two-dimensional PAGE (electrophoresis)

Table 9-5 Microbiology, 6/e

Bacterial Growth

"Biology is the only science in which multiplication means the same thing as division."
http://members.fortunecity.com/alienbunny/

- 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***

Binary Fission

DNA has replicated

Septum forms

Cell divides

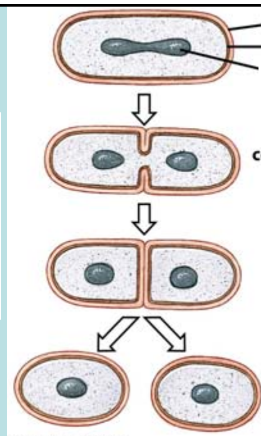
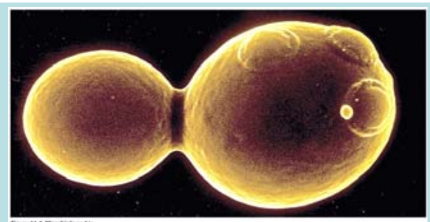


Figure 6-1a Microbiology 6/e

★ 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 O_2

For ANY of these factors, you can find certain bacteria that thrive at high or low extremes

Bacterial growth requirements example:

Temperature

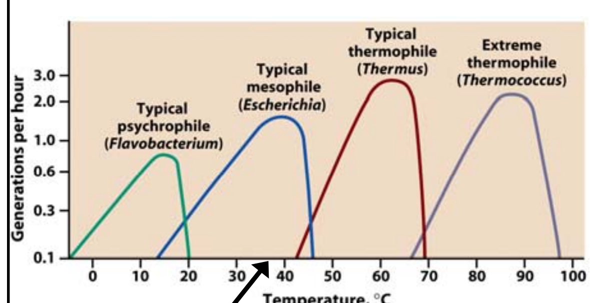
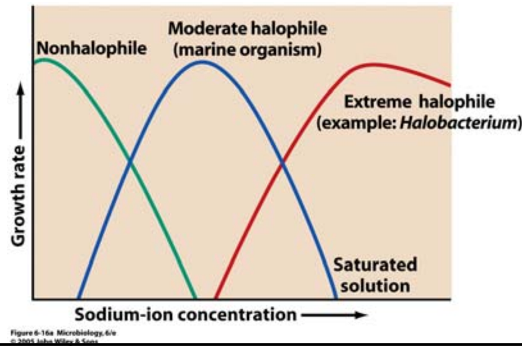


Figure 6-14 Microbiology, 6/e

Bacterial growth requirements example: Osmolarity



Bacterial growth requirements example: **Oxygen**

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

O_2 requirement is related to type of metabolism the bacteria perform.

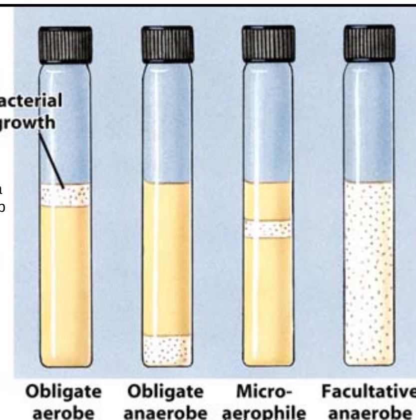
★**Obligate aerobe:** *must* have free oxygen (O_2) to grow

★**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.

Solid media
Air at the top

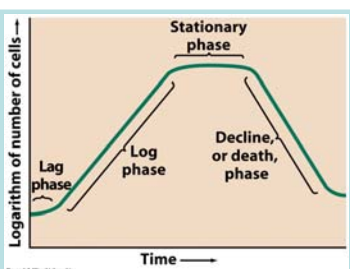


(prefers a low O_2 concentration)

(will grow ***best*** at the top)

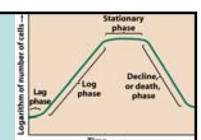
★ Bacterial Growth Curve

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



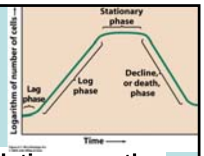
Axes: **Living** cells vs time

Lag phase



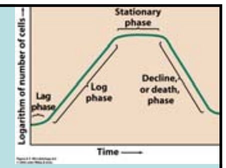
- Bacteria “wake up” to their happy surroundings:
  - Increase in metabolic activity
    - Enzyme synthesis
    - ATP production
    - Increase in cell size
  - Preparing for cell division but **no increase in number** of bacteria yet

Log phase



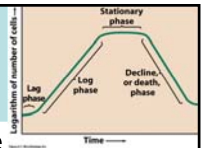
- **Exponential (logarithmic) population growth**
- 2^n cells present after *n* doublings
  2 4 8 16 32 64…
- ***Generation time:*** time required for each doubling
  – Under ideal conditions, this time is genetically determined (varies with species)
  – Generally between 20 minutes & 20 hours
    - 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 occuring), and death at the center.

Figure 6-19b Microbiology, 6/e
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Agar plate (solid media) streaked for isolation of individual colonies

Relevant reading in Black's Microbiology:

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