Section 2: Organization of Life

TOPICS

Chemicals of life
Soil composition
Three domains of life
Prokaryote vs. eukaryote cell structure
Antibiotic targets

SUMMARY

We introduce soil as the sustenance for life, providing context for introducing elements and macromolecules critical to living organisms. We discuss the main cellular structures and functions required for life and begin to focus on the differences between prokaryotes and eukaryotes and how these differences affect the specificity of antibiotics. These discussions will be placed within the context of the universal tree of life.

We introduce a conundrum: If antibiotics target essential functions and essential functions are conserved, how do they work and still exhibit prokaryotic specificity? The topic provides an opportunity to reinforce key differences between the prokaryotes and eukaryotes, while extending our discussion of universal conservation to highlight how many critical functions are conserved. Toward the end of the section, we will introduce the mechanisms of action of the antibiotic classes with in-depth discussion to follow in subsequent sections.

LEARNING GOALS

- Know the elements and macromolecules required for life.
- Know the three domains, how they are related and provide examples of each.
- Compare and contrast key prokaryotic and eukaryotic cell anatomical structures.
- Be able to draw a prokaryotic cell, identifying key elements.
- Know the main cellular targets for the different antibiotic classes.

PRE-CLASS PREPARATION

Prior to class, students should read a brief description of amino acid, carbohydrate, fatty acid, and nucleotide structure. Students should be familiar with covalent bonds and electron arrangements with emphasis on valence electron structure and how it aligns with Periodic Table organization. Read Section 2 of the Laboratory Manual, Soil Environments Affecting Microbes.

PRE-CLASS ASSESSMENT

1. Name the 6 elements that compose the four macromolecules of life.
   Carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur
2. Name the 4 macromolecules of life.
   Proteins, lipids, nucleic acids, carbohydrates
3. Why do you think most anti-fungal compounds have serious side effects in humans?

Because humans and fungi are both eukaryotes, many features essential for life between the two organisms are conserved. This makes it difficult to find a drug that has specificity for fungi but doesn't affect human cells.

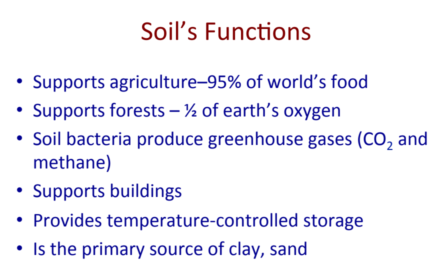
GUIDE TO THE POWERPOINT SLIDES

Outline

- Soil as the sustenance of life
- Requirements for life
- Macromolecule structure (brief introduction)
- Three domain system
- Prokaryotes vs. eukaryotes
- Cellular targets of antibiotics

Soil as the sustenance of life

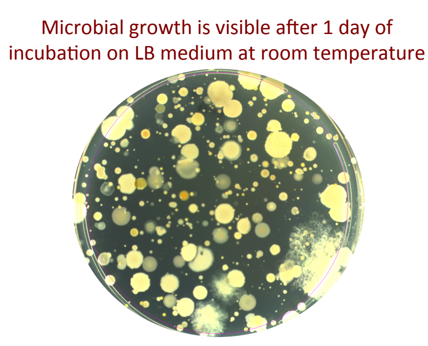
The instructor may wish to provide an overview of the characteristics of soil (refer to the lab manual reading). Soil is the source of nutrients for microbial and plant life as well as a source of structural material for our homes. We provide a few slides but leave it to the instructor to elaborate to the extent appropriate for their class. Students might find it interesting to learn that each state has a state soil. In the past, we have come to class prepared with a short description of the state soils representative of students' home states.



In order to culture microbes present in the soil, we need to provide the essential nutrients present in the soil. To encourage students to think about what the minimal requirements for life are, we suggest the following guiding questions.

<u>GUIDING QUESTIONS:</u>

- Can we expect to isolate microbes from our soil samples on standard lab media?
- What components must be present in the laboratory growth medium if we expect to isolate microbes?
- Do you expect all organisms present in the soil to grow on your Petri plate? Why or why not?



Active Learning

Activity type: Think-Pair-Share or Shout Out

We turn the guiding questions above into an active learning exercise. The Think-Pair-Share activity or Shout Out approach can be used to address the following questions.

Question: Consider the chemicals present in soil that enable microbial life to thrive.

- If you were going to design your own culture medium, list five chemicals would you be sure to include?
- Do you predict that all organisms present in your soil sample will grow on your medium? Explain.

Some students may not have the background to answer this question, but at the end of this segment, all students should be able to refer back to the collective or individual lists to determine if there are:
a) Items that should be removed
b) Items that should be added
c) Items that should be expanded

At the end of this section, if the student responses are displayed again, students can visualize their own acquisition of knowledge by reviewing and modifying the list. For example, if students list "proteins" the instructor might ask if it is necessary for *proteins* to be present or if amino acids would be sufficient? Similarly, must amino acids be present or is it sufficient to provide water, sugar, nitrate, magnesium sulfate and other salts? Ultimately, the answer depends on the biosynthetic capabilities of the organisms and this is an important distinction to make. For example, humans ingest nitrogen in the plants and animals that we eat. Plants assimilate nitrogen from nitrate or nitrite in the soil (and a major constituent of fertilizer), while different bacteria have diverse mechanisms of obtaining nitrogen. Remarkably, some can break the triple bond of nitrogen gas, reducing it to ammonia; others can absorb ammonium ions for use as building blocks to synthesize critical molecules required for life. This sets the stage for addressing the question of whether we expect all organisms present in the soil to grow on our particular medium.

It may be helpful to distinguish defined vs. undefined microbiological media. In undefined media, the exact chemical composition is unknown; items such as yeast, beef extract, or other plant/animal tissue are used. In defined media, the exact chemical composition is known and usually contains a sugar and salts.

Question: Do you expect all organisms present in the soil to grow on your Petri plate? Why or why not?

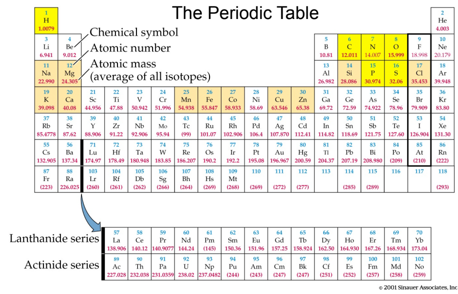
This is an opportunity to address the differences in ability to synthesize required molecules (often due to the presence or absence of key biosynthetic enzymes). In addition, requirements for oxygen, pH, and other factors can contribute to the growth of organisms on lab media. While all living organisms share a basic set of required macromolecules, they differ considerably in their biosynthetic potential. Choice of medium and growth conditions for isolation will dictate which organisms are able to grow.

For laboratory growth of microbes, the culture medium must contain:

- Water
- Carbon source (often a carbohydrate)
- Salts (provide essential elements such as phosphorus, nitrogen, sulfur, magnesium)
  - Nucleic acid requires phosphorus and nitrogen
  - Proteins require nitrogen and sulfur
  - Ions such as magnesium are important for function of many enzymes

Note: Some microbes require O_2 while others cannot tolerate it

Key concept: All life is constructed from the same chemical elements



Requirements for life

General points to make:

- Life is composed of a small set of elements.
- The majority of cellular constituents can be categorized as one of four macromolecules.
- Each macromolecule consists of a general chemical composition, repeated.

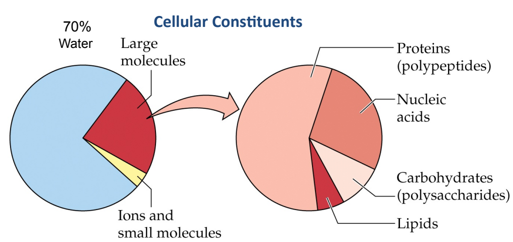
Macromolecular structure

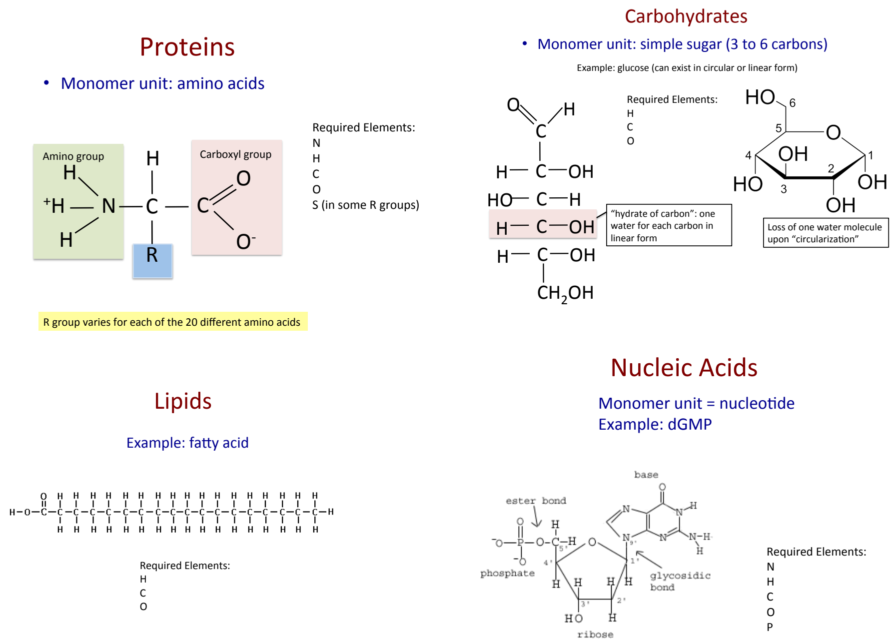
We convey that all life is constructed from the same chemical elements, then ask, “What do cells do with those elements?”

At this stage we only introduce macromolecules briefly. We will focus on each individually within upcoming sections. For example, students learn about proteins and carbohydrates by studying peptidoglycan structure, lipid and membrane structure in the context of the antibiotic gramicidin, and nucleic acid structure is covered in the context of gene expression. Emphasis should be placed on the concept that all life is constructed from the same macromolecules.

Key concept: All life is constructed from the same macromolecules

Cells use conserved biosynthetic pathways to construct required macromolecules from smaller chemical building blocks





Active Learning

Here, we remind instructors to revisit the initial active learning exercise if they wish. Students should be encouraged to review their original answers; instructors can ask students if there are any:

a) Items that should be removed
b) Items that should be added
c) Items that should be expanded or clarified

Consider the chemicals present in soil that enable microbial life to thrive.

- If you were going to design your own culture medium, list five chemicals would you be sure to include?
- Do you predict that all organisms present in your soil sample will grow on your medium? Explain.

Refer to your original list. Is there anything you'd like to change?

This is a good time to distinguish between macromolecules, the elements used to construct the various macromolecules, the molecular form in which those elements are most often found in nature, and so on (e.g. nitrogen is often obtained in the form of nitrates or from ingesting amino acids in the form of plant or animal material.

At this point, students should be able to meet the learning goal:

✓ Know the elements and macromolecules required for life.

Three domain system

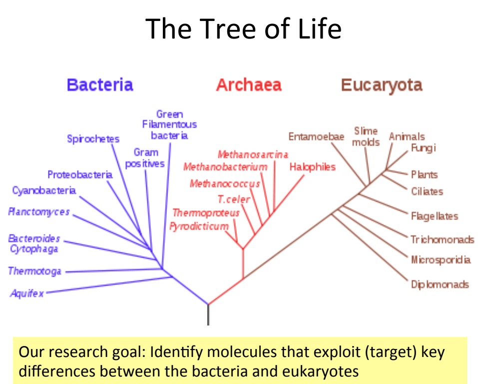
While individual species each have their own particular growth requirements, we have also stressed that many molecules are conserved throughout all living organisms. We ask a fundamental question that we will return to throughout the course, "If so many of the requirements for life are universal, how do we kill prokaryotes and not eukaryotes?"

The prokaryotes are a very divergent group, so if we want to find a drug that can target a "broad spectrum" of prokaryotes, it will probably need to target some conserved molecule. The chemistry we just discussed is **common** across all life.

GUIDING QUESTION:

- If so many of the requirements for life are universal, how do we kill prokaryotes and not eukaryotes?

An answer is not required at this point, but this provides a good moment to introduce the universal (three domain) tree of life.

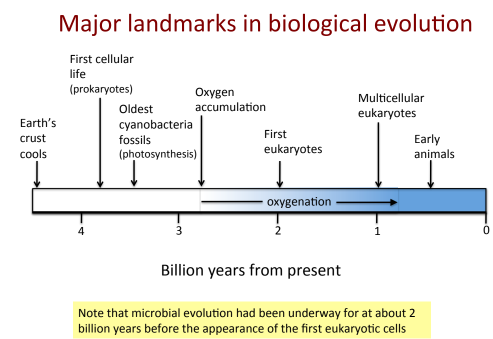


Students may be familiar with phylogenetic trees, but they often misinterpret them. We discuss them in more detail in Section 9.
Some key points:

- All life is grouped into three domains
- The tree represents the relationship between living organisms based on shared features; organisms with more shared features are grouped together.
- Shorter distance between two groups is indicative of more shared features, closer evolutionary relatedness.
- Microbes represent the vast majority of life on earth

This visual demonstration of the vast diversity of microbial life (and the relative insignificance of multicellular life) is always impressive. Taxonomic groups with a greater degree of separation, indicative of greater evolutionary divergence, still have several features in common such as the use of ATP as energy; DNA as an informational molecule; protein as structural, enzymatic or regulatory molecules, cell membrane composed of phospholipids; and so on. **But our course goal is to find molecules that can exploit (target) key differences between prokaryotes and eukaryotes.**

Instructors may wish to insert a discussion of the role of the microbes in oxygenating the earth (endosymbiosis is covered in Section 9). Metabolic diversity is not covered in detail but is introduced in Section 5.

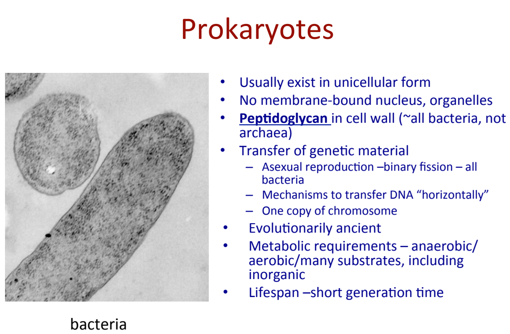


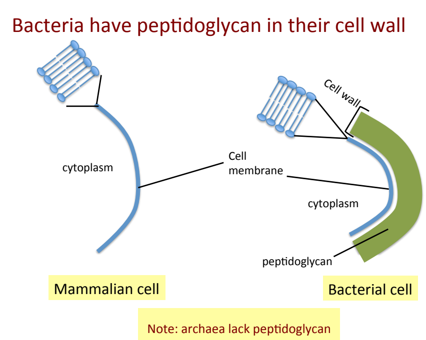
At this point, students should be able to meet the learning goal:

- ✓ Know the three domains, how they are related, and provide examples of each.

Prokaryotes vs eukaryotes

We provide slides indicating the major anatomical and biochemical differences that distinguish the prokaryotes from the eukaryotes. We will revisit many of these differences in more detail in upcoming sections. We reserve a more in-depth look at the cell wall for Sections 3 and 4. The implications of ribosomal differences are addressed in Sections 8 and metabolic differences are discussed in Section 5.





At this point, students should be able to meet the learning goal:

- ✓ Be able to draw a prokaryotic cell, identifying key elements.

Note: We will elaborate on cell wall structure in Section 3, after which students should upgrade their drawings.

Cellular targets of antibiotics

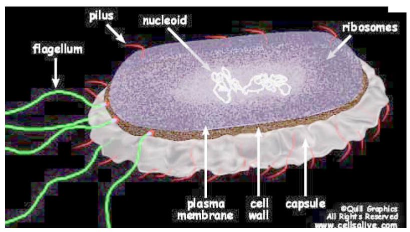
Active Learning

Activity type: Shout out

Given the slide showing bacterial anatomy:

<u>Question</u>: What functions could you target to kill bacteria?

What functions could you target to kill bacteria?

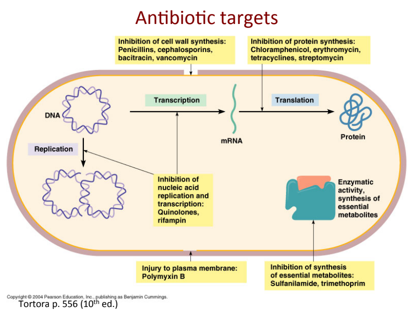


We have found it helpful to type a list of answers into the powerpoint slide, using students' exact wording then revisiting it later in the semester. The students do a remarkable job of self-correcting based on their newly acquired knowledge. The table below lists initial student answers and revised answers from a previous course.

Initial answer	**Revised answer (a few weeks later)**
Take apart cell wall	Inhibit cell wall synthesis
Flagellum	Remove from list (not essential for life)
Attack ribosomes	Bind to ribosomes to block translation
Attack pili, capsule	Remove from list (not essential for life)
Attack DNA	Inhibit DNA synthesis

Students also listed many non-specific agents such as heat and bleach.

On the slide summarizing the mechanism of action of the different classes of antibiotics, there are two interesting points to make:



1) Prokaryotic-specific features such as flagella, pili and capsule are not targets for any known antibiotics. Why might this be? *They are not essential for life of the bacterium.*

2) This is a fairly limited list of targets. Why?*To abolish an essential function in bacteria while, at the same time, leaving the host/eukaryotic cells undamaged presents a huge challenge. There are a limited number of antibiotic-target interactions that will result in this outcome.*

At this point, students should be able to meet the learning goal:

- ✓ Know the main cellular targets for the different antibiotic classes.

<u>GUIDING QUESTIONS:</u>

- If antibiotics must target essential functions, and if essential functions are conserved across the three domains, why don't antibiotics kill us?
- What cellular functions are shared between prokaryotes and eukaryotes?
- How much do those shared functions differ at the molecular level?

These questions will be addressed in upcoming sections.

POST-CLASS ASSESSMENT

1. What is your home state and what is its state soil? Indicate one property of the soil and predict how this might affect water flow or plant roots?
2. Based on the three-domain system of life, would you predict that the molecular structure of DNA is the same across all domains? Cell membranes?

Evidence, to date, indicates that the structure of DNA is universally conserved. Cell membrane structure differs in archaea (they have a phospholipid monolayer rather than a bilayer). But, in each case, the function remains the same across all groups. This is a critical point we will make again and again. Structure determines function. Generally, molecules with the same function have the same or similar structures.

Original Research Papers for Discussion

This series of articles illustrates the scientific process starting with an intriguing, yet controversial finding and subsequent data-driven methods to refute the original finding. The original result reported contradicted our notion about the requirements for life and provides an interesting opportunity to reinforce the understanding of electron distribution and arrangement of the elements within the periodic table. DNA structure and DNA as essential for life are also relevant.

Summary of the events:
Cressey, D. (2012) Controversial "Arsenic Life" Bacterium Prefers Phosphorus After All. *Scientific American* Oct. 4, 2012.

Wolfe-Simon, F., Blum, J., Kulp, T., Gordon, G. Hoeft, S., Pett-Ridge, J., Stolz, J., Webb, S., Weber, S., Peter, K., Davies, P., Anbar, A., and Oremland, R. (2010) A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus. *Science* 332:1163-1166.

Hayden, E. (2012) Study Challenges Existence of Arsenic-Based Life. *Nature* doi:10.1038/nature.2012.9861

Elias, M., Wellner, A., Goldin-Azulay, K., Chabriere, E., Vorholt, J., Erb, T., and Tawfik, D. (2012) The Molecular Basis of Phosphate Discrimination In Arsenate-Rich Environments. *Nature* 491:7422.