**1 The Evolution of Microorganisms and Microbiology**

**CHAPTER OVERVIEW**

This chapter introduces the field of microbiology and discusses the importance of microorganisms not only as causative agents of disease, but also as important contributors to food production, antibiotic manufacture, vaccine development, and environmental management. It presents a brief history of the science of microbiology and an overview of the microbial world. The origin of life and microbial evolution is put in the context of microbial phylogenies.

**LEARNING OUTCOMES**

After reading this chapter students should be able to:

• differentiate the biological entities studied by microbiologists from those studied by other biologists.

• explain Carl Woese’s contributions in establishing the three domain system for classifying cellular life

• provide an example of the importance to humans of each of the major types of microbes.

• determine the type of microbe (e.g. bacterium, fungus, etc) when given a description of a newly discovered microbe

• propose a time line of the origin and history of microbial life and integrate supporting evidence into it

• design a set of experiments that could be used to place a newly discovered cellular microbe on a phylogenetic tree based on small subunit (SSU) rRNA sequences

• compare and contrast the definitions of plant and animal species, microbial species and microbial strains

• evaluate the importance of the contributions to microbiology made by Hooke, Leewenhoek, Pasteur, Koch, Cohn, Beijerinck, von Behring, Kitasato, Metchnikoff and Winogradsky

• outline a set of experiments that might be used to decide if a particular microbe is the causative agent of a disease

• predict the difficulties that might arise when using Koch’s postulates to determine if a microbe causes a disease unique to humans

• construct a concept map, table or drawing that illustrates the diverse nature of microbiology and how it has improved human conditions

• support the belief held by many microbiologists that microbiology is experiencing its second golden age

**GUIDELINES FOR ANSWERING THE RETRIEVE, INFER, APPLY QUESTIONS**

As the name of this section implies, these questions range from first-order retrieval of information from the preceding text to application of the content to open ended questions or scenarios. As such the answers are either easily obtained from the text or may have many different answers that can be considered valid. For this reason, we do not include these questions in the instructor’s manual.

**GUIDELINES FOR ANSWERING THE MICRO INQUIRY QUESTIONS**

Figure 1.1 *How would you alter this concept map so that it also distinguishes the cellular organisms from each other?*

Modify this hierarchical concept map by adding a dichotomy between prokaryotes and eukaryotes under the *cellular* classification. Bacteria (eubacteria) and archaea would be under prokaryotes and fungi and protists under eukaryotes.

Figure 1.2 *How many of the taxa listed in the figure include microbes?*

All of them.

Figure 1.6 *Why are the probionts pictured above not considered cellular life?*

All cells contain DNA, RNA, and proteins, and can self replicate.

Figure 1.8 *Why does the branch length indicate amount of evolutionary change but not the time it took for that change to occur?*

The lines on a phylogenetic tree indicate a measure of relatedness because they represent genetic sequence divergence. They are not a measure of time because it is not known how much time was needed for organisms to diverge because divergence is influenced by multiple factors.

**GUIDELINES FOR ANSWERING THE COMPARE, HYPOTHESIZE, INVENT QUESTIONS**

*1. Microscopic organisms such as rotifers are not studied by microbiologists. Why is this so?*

See Key Concepts 1.1 a. While near-microscopic in size, rotifers are complex animals, with multiple organ systems, and often over 1000 cells in their adult body.

*2. Why aren’t viruses, viroids, satellites, and prions included in the three domain system?*

Only cellular organisms are in taxonomic systems, because only they are descended from each other with vertical gene transmission. Viruses do not reproduce, they are assembled (like machines). Viroids and satellites are RNA, not organisms. Prions are proteins, not organisms.

*3. Why was the belief in spontaneous generation an obstacle to the development of microbiology as a scientific discipline?*

If true, spontaneous generation of microorganisms would make them impossible to classify and would prevent generalizations between microorganisms and larger organisms. If fact, prokaryotes are very similar at the molecular and cellular level to eukaryotes, and most of what we understand about biology at that level is from studying model bacteria, especially *E. coli*. Research surrounding the debate over spontaneous generation was foundational in the field of microbiology.

*4. Would microbiology have developed more slowly if Fanny Hesse had not suggested the use of agar? Give your reasoning.*

This is opinion, so various answers are possible. Just look for logical and factual support of their argument. Relevant issues include the intrinsic resistance of agar to degradation as compared to gelatin, and the necessity of solid media to obtain pure cultures by physical separation, which is much more difficult in liquid media (dilution to extinction).

*5. Some individuals can be infected by a pathogen yet not develop disease. In fact, some become chronic carriers of the pathogen. How does this observation affect Koch’s postulates? How might the postulates be modified to account for the existence of chronic carriers?*

In the carrier state, a host carries the pathogen without clinical symptoms. For example, approximately 20% of college students carry *Staphylococcus aureus* in their nasal passages, with no ill effects. However, it can cause disease (damage to the host) if they become immunocompromised. They can also transmit the bacteria to others. Technically, this does not relate to Koch’s postulates because they are designed to isolate the causative agent of a disease, and the carrier state is not disease. The bottom line is that while Koch’s postulates certainly do not apply in every case and they have limitations, they were of tremendous historical importance in demonstrating experimentally the germ theory of disease, which was a tremendous advance in modern medicine.

*6. Develop a list of justifications for the usefulness of microorganisms as experimental models.*

-easy to culture and manipulate, easy to store in frozen state

-genetic manipulation (adding DNA or removing DNA) is straightforward because single-celled and haploid

-large numbers easy to grow, very short generation times, inexpensive media

-all basic central cell processes (replication, transcription, translation) is conserved across all life

-no limitations based on animal safety and use rules

*7. History is full of examples in which one group of people lost a struggle against another.*

*a. Choose an example of a battle or other human activity such as exploration of new territory and determine the impact of microorganisms, either indigenous or transported to the region, on that activity.*

Tetanus and World War II, malaria and the building of the Panama canal, world trade travel and the bubonic plague spreading across Europe in the 14th century, the down-turn of Napoleon’s march into Russia; the truncation of the conquests of Alexander the Great and Attila the Hun, the success of relatively few Spanish conquistadors aided by smallpox, the casualties of the Civil War in the United States and WWI due to sepsis, and ergotism and the Salem witch trials are just some examples.

*b. Discuss the effect that the microbe(s) had on the outcome in your example.*

In most of those cases, a microbe new to an area was brought in by human activity (emerging infectious disease). For example, the Spaniards were already relatively resistant to smallpox, and they brought this virus to a naive population in Central America, thus it spread extremely rapidly.

*c. Suggest whether the advent of antibiotics, food storage and preparation technology, or sterilization technology would have made a difference in the outcome.*

Vaccines and antibiotics would have helped with the spread of tetanus, smallpox, and the plague. Antimalarial prophylaxis would have helped in the success of building the Panama Canal. Correct food storage would have reduced rats that spread the plague and ergotism. Knowledge of mold contamination would have reduced cases of ergotism.

*8. Antony van Leeuwenhoek is oft en referred to as the father of microbiology. However, many historians feel that Louis Pasteur, Robert Koch, or perhaps both, deserve that honor. Decide who should be considered the father of microbiology and justify your decision.*

This is purely opinion, so judge based on inclusion of appropriate facts and making a logical argument. Leeuwenhoek’s major contribution is the microscope and careful observations of microorganisms and cellular structures. Pasteur has numerous contributions related to vaccines, development of germ theory, disproval of spontaneous generation, development of sterilization procedures, and understanding of fermentation. Koch also had numerous contributions related to experimental demonstration of germ theory, identification of pathogens, and methods of bacterial pure culture.

*9. Consider the discoveries described in sections 1.3 and 1.4. Which do you think were the most important to the development of microbiology? Why?*

Numerous possible answers. Be sure they are factual, and make a logical argument.

*10. Support this statement: “Vaccinations against various childhood diseases have contributed to the entry of women, particularly mothers, into the full-time workplace.”*

-children spent less time being sick, thus mothers spend less time away from work

-because childhood mortality has been lowered and thus more children survive to adulthood, this may contribute to fewer children on average to each family, thus less childcare burden on the mother

-A number of sites including NORC (National Opinion Research Center) have compiled statistics comparing vaccination with employment statistics.

*11. Scientists are very interested in understanding when cyanobacteria first emerged because, as the first organisms capable of oxygenic photosynthesis, it is thought that they triggered a sharp rise in atmospheric oxygen. For many years, certain lipid biomarkers have served as “molecular fossils” to date the first appearance of cyanobacteria. However, a 2010 study questioned whether these lipids provide accurate information in light of a 2007 discovery that they also are produced by an anoxygenic phototrophic bacterium—a bacterium that does not produce oxygen as it uses light energy. The authors of the 2010 study identified genes in extant bacteria involved in synthesis of the lipid biomarkers and then constructed phylogenetic trees based on comparisons of these genes. They also identified the phyla to which the bacteria belonged (based on SSU rRNA analysis) and noted the habitats and metabolic capabilities of the bacteria used in the study. Discuss the specific challenges encountered in the study of microbial evolution. What results from the phylogenetic analysis would support their claim that 2-methylhopanoids are not reliable biomarkers? Why were habitat and metabolic characteristics also part of their analysis?*

***Read the original paper:*** *Welander, P. V., et al. 2010. Identification of a methylase required for 2-methylhopanoid production and implications for the interpretation of sedimentary hopanes. Proc. Natl. Acad. Sci. 107 (19):8537.* [*http://www.pnas.org/content/107/19/8537.full.pdf+html*](http://www.pnas.org/content/107/19/8537.full.pdf%2Bhtml)

Microbial evolution is difficult to study because events took place billions of years ago and the microbial fossil record is very sparse. Furthermore, there is the problem of contamination from nonindigenous markers in samples such as is seen with hydrocarbon biomarkers. In comparison, the use of molecular markers such as SSU rRNA nucleotide sequences in current bacterial samples are compared with relative ease. The greater the difference in sequence the greater the evolutionary divergence.

12. *It is possible to artificially create bacterial cells that completely lack a cell wall. The resulting cells, called L-forms, can be maintained and cultured. L-forms have been known for many years and have been of interest because of their unusual physiological characteristics. One of their most unusual features is that they reproduce by becoming deformed and then “falling apart,” producing two or more additional L-forms. The progeny L-forms then grow larger and reproduce by a similar method. This is done without the aid of the normal division machinery. In 2013 scientists in the United Kingdom reported their attempts to understand how L-forms “divide.” They generated mutant strains of* Bacillus subtilis *L-forms that promoted L-form proliferation. The only mutations found with this effect were those that increased*

*production of membrane lipids. They argued that these lipids were inserted into the L-form membrane and that this increased the surface area of the L-forms without increasing their volume. The increased surface-to-volume ratio caused the cells to assume unusual shapes and experience torsional stress. The torsional stress served as the biophysical force causing the L-forms to split apart. They also suggested that the study of L-form division provided a good model for how primitive cells divided before the evolution of the complex division machinery that exists in modern cells. Why did they suggest this? Do you agree? Explain your answer.*

**Read the original paper:** Mercier, R., et al. 2013. Excess membrane synthesis drives a primitive mode of cell proliferation. Cell 152:997. <http://www.cell.com/cms/attachment/2007944888/2030464522/mmc9.pdf>

The use of L-form bacteria as a model for primitive cell division is based on at least two essential facts: early cells did not have cell walls (e.g., peptidoglycan) and their division was most likely driven by purely physical forces, as observed in this experimental system. The remainer of the question asks the student to express an opinion and defend it. Students may consider the notion that if early cells divided much like L-forms, replication would not require chromosome replication or protein synthesis.