**Study Plan: Episode 7—Microbial Diversity**

1. **Read the UNIT OVERVIEW presented in this Study Guide.**
2. **View the video "Microbial Diversity."**
3. **Read UNIT OBJECTIVES and KEY CONCEPTS sections of this study guide.**
4. **View the video a second time, this time taking notes. Pay particular attention to topics identified by the UNIT OBJECTIVES or KEY CONCEPTS as significant.**
5. **Read:**
   1. **Chapter 5 – pages 90 – 99**
   2. **Chapter 6 – pages 108 – 122**
   3. **Chapter 7 – pages 129 – 142**
   4. **Chapter 8 – pages 150 – 167**
6. **Return to the Unit Objectives and Key Concepts listed in this Study Guide. Do you feel you have achieved each objective? Review sections of the text or video pertinent to material you don't feel you have mastered.**
7. **Test your mastery of the material by answering the Review Questions at the end of this Study Guide.**
8. **Check your answers against the answer key; review material relating to any questions you missed.**
9. **Explore further! Retrieve from the library database articles listed in Suggested Further Reading that sound interesting.**

**Unit Overview**

Microorganisms are ancient; they have been accumulating, exchanging and recombining the random mutations that occur in DNA. Diverse ecological habitats have acted on this vast pool of genetic variability, selecting for divergent metabolic strategies. Organisms with new or novel metabolic capabilities fill niches created by changing conditions. Unlike animals that all obtain energy the same way, the metabolic diversity of prokaryotes has allowed them to be more successful at filling diverse niches. The morphological similarity of microorganisms obscures their metabolic diversity. It is only when we look at the genetic differences of microbes we see the wealth of diversity that characterizes the microbial world.

High-speed DNA sequencing has contributed to an explosion of genetic information, redefining our understanding of the diversity of life. Modern molecular techniques have also been used to amplify and sequence the DNA of microbes that we have been unable to study under laboratory conditions. Use of this technique has confirmed what scientists had long suspected — that only a fraction of the world’s microbes have been isolated and identified! Unseen and poorly understood, these microorganisms are thought to play an important role in maintaining the chemical balance of the biosphere. Without these microorganisms — and their better-known and more clearly understood fellows — plant and animal life would not exist!

**Unit Objectives**

* Explain the relationship between genetic diversity and metabolic diversity.
* Describe our current understanding of the “tree of life”
* Describe the contribution molecular technology has made to our understanding of the diversity of life.
* Provide an overview of DNA sequencing
* To become more familiar with the different groups of microorganisms that we have been studying – bacteria, protozoa, fungi, and protists – and their general characteristics

**Key Terms**

* + - * acyclovir
      * amoeba
      * antibodies
      * apicommplexan
      * ascospore
      * bacillus
      * basidiospore
* capsids
* capsomeres
* chitin
* ciliates
* coccus
* differential medium
* differential technique
* dinoflagellate
* enriched medium
* envelope
* flagellates
* Gram-stain
* glycocalyx
* helical virus
* hypha
* icosahedrons
* lichens
* lysogenic cycle
* lytic cycle
* mycelium
* mycorrhiza
* peptidoglycan
* protozoan
* provirus
* selective medium
* septum
* simple stain
* spikes
* spirillum
* spirochete
* spore
* trophozoite
* yeast
* zooplankton
* zygospore

**Key Concepts**

**THE ENVIRONMENT AND DIVERSITY**

* There is no doubt that microbes play a key role in life on our planet – cycling of nutrients, decomposition of organic matter, production of vitamins within our intestinal tract, and the list goes on.
* Mutations can occur randomly when DNA is replicated, but at an extremely low frequency. This is why many scientists have had to place such a long time-frame on the theory of evolution.
* Organisms with new gene sequences, a result of random mutation, may be better adapted to exploit newly available resources than other organisms, and thus, may thrive and reproduce where others cannot. Normally, such a beneficial mutation is extremely rare. It also may produce a mutation that is advantageous in one situation, but harmful in a different situation.
* As environments change, different resources become available.
* Metabolic diversity follows from genetic diversity.
* Animals all acquire energy in the same way; microbes are very diverse and can acquire their energy in many different ways – they have expanded into numerous niches.

**MICROBIAL CLASSIFICATION**

* Classical classification schemes used differences in morphology.
* Eukaryotic microbes show differences in morphology; prokaryotes appear more uniform in appearance.
* Classical methods of classifying bacteria include:
* The shape and arrangement of bacteria cells and gram-staining properties. The gram stain divides bacteria into two groups, those with (gram-negative) and those without (gram-positive) an outer membrane. Gram-positive bacteria also have a thicker cell wall (made of peptidoglycan – the component targeted by penicillin) than the gram-negative bacteria.
* Biochemical properties which reflects metabolic abilities.
* Serology, which uses antibodies (name comes from "serum", the liquid component of blood which contains human antibodies). Antibodies, which are a product of the human immune system, are highly specific and, thereby, distinguish between closely related strains and species.
* The classical methods of identifying bacteria are used in the clinical laboratory to identify causes of infection
* Genomic approaches are yielding new insights into microbes (note the expanding understanding of eukaryotic microbes too. Currently, algae and protozoa are in the kingdom Protista. Several new kingdoms are being proposed as the result of genomic data – as if we don’t already have enough to learn!)
* Genomic approaches are allowing the study of prokaryotes one cannot culture in the lab
  + Dirt samples, Yellowstone extreme environments (where at least of 100 different species are believed to have been found.
  + Based on these techniques Norm Pace estimates that 95% of the living organisms in the ocean are microbial in nature.
  + rRNA gene sequencing is used to place organisms into one of the 3 branches (domains) on the “Tree of Life”: Eukarya, Bacteria, Archaea. The “Tree of Life” is an attempt by scientists to get an idea of all the different organisms and how similar or dissimilar they are.
  + rRNA genes have DNA regions that we find common in many organisms and there are DNA regions of the gene in which we find a high variability in the sequence of the bases.
  + The more similar the DNA or rRNA sequences, the closer related the organisms might be.
  + Viruses cannot be classified by rRNA genes since they do not have it! (Remember that viruses do not have ribosomes!)

**MOLECULAR TECHNIQUES**

* Sequencing requires large amounts of DNA: Polymerase chain reaction (PCR) is a procedure that produces many copies of DNA (amplifies is the term used in the video) from a very small sample of genetic material.
* When using PCR to make DNA, you need add the nucleotides (subunits of DNA) different colored dyes (adenine = color #1, guanine = color #2, etc.)
* Analyze the sequence of bases in DNA by reading the sequence of dyes (note use fluorescent dyes and use laser to excite the dye)
* Analyze multiple small segments of the DNA – known as shotgun sequencing
* Computers used to line up sequences
* Whole genome sequence now possible due to new dyes for labeling the DNA bases and computing power
* One can also determine the size of DNA pieces on an agarose gel (agar is in the petri dishes you used in lab 3);
  + DNA is negatively charged. When a current is applied the DNA moves to the positive pole.
  + The smaller the piece the faster it moves (easier time moving through the pores in the agarose).
  + The DNA bands are then visualized by adding a dye that shows up under UV light.
* Cloning is a technique that is also used, in which copies of the DNA can be made without ever growing the bacteria in the lab. This technique and others demonstrated that there were hundreds of different bacteria growing in the hot springs in Yellowstone National Park.
* Genetic engineering is a based on the use of enzymes from various organisms that allow us to study single genes outside of the genes natural host.
  + An enzyme that cuts DNA at specific patterns can be used. These are called restriction enzymes (also restriction endonucleases).
  + An enzyme is used to glue pieces of DNA together. This enzyme is called DNA ligase (or ligase for short).
  + Plasmids are small ringlets of DNA that are found in many bacteria; these plasmids are non-essential for the growth and survival of the bacteria and can be thought of as “luxury” DNA. The genes to be studied are glued into the plasmids and then put into the bacterial cells in order to produce a large amount of protein or study other aspects of the gene. Note that plasmids are considerably smaller then the chromosomal DNA of the bacteria.

**Other topics (from the textbook)**

**Bacteria**

* As has been mentioned in previous Study Plans, bacteria are usually thought of as being one of three different shapes – rod-shaped (bacilli). spherical (cocci), and spiral (spirochetes and spirilla).
* Bacilli usually measure in the range of 0.5 m – 20 m (m = micrometer) in length.
* Cocci usually measure around 0.5 m in diameter; we find these in groups that are important diagnostically.
  + A group of two is called diplococci (causative agent of gonorrhea)
  + A group found in a chain is called streptococci (as in *Streptococcus pyogenes*, the causative agent of Strep throat)
  + A group in a grape-like cluster is called staphylococcus (as in *Staphylococcus aureus*, a pathogen)
* Staining procedures were developed to better visualize them with the microscope.
  + Simple stains – stain one color, usually positively charged to stick to the negatively charged organism
  + Gram-stain – used to distinguish between two different groups of bacteria. The gram-positive have a thick layer of peptidoglycan that is more vulnerable to penicillin, while gram-negative bacteria have a thinner layer of peptidoglycan sandwiched between two membranes.
* Glycocalyx is an outer coating found on some bacteria, often pathogens, that help the bacteria survive dryer conditions, chemicals, and other environmental conditions.
* Some bacteria can form a tough survival structure called a “spore” (short for endospore). This is seen with *Bacillus* sp. and *Clostridium* sp.
  + Can survive boiling for 2 hours or alcohol for 20 years
  + Includes the causative agent of anthrax, tetanus, and botulism
* Bacteria can divide very rapidly. Under optimal conditions in the laboratory, *E. coli* can double in number every 20 minutes.
* For those that can be grown in the laboratory we use culture media of different types:
  + selective medium – kills or inhibits some bacteria while allowing others to grow
  + differential – allows us to tell the difference between two different bacteria
  + enriched medium – medium with lots of extra nutrients to help “picky” bacteria grow

**Viruses**

* Viruses range in size from 27 nanometers (nm) up to 250 nm. About 500 viruses can fit into a single bacterium (sounds like a New York City apartment building!)
* Viruses are described as being in one of three shapes
  + Helix – twisted into a spring or spiral staircase
  + Icosahedron – a symmetrical shape composed of 20 faces and 12 points
  + Complex – a shape other than the two previous shapes
* All viruses have a protein coat surrounding the nucleic acid core.
* The nucleic acid can be either DNA or RNA
* The outer coat is called the capsids and the protein subunits that make up the capsids are called capsomeres.
* They do not have ribosomes – thus, no protein synthesis
* Some viruses have a membrane surrounding the capsids – this is called an envelope. On the envelope we can find spikes. These spikes can tip off the immune system that there is an invader!
* Viral replication:
  + Attachment – the virus finds and binds to the host cell – a highly specific occurrence (cell specific)
  + Uncoating – the viral capsids is removed
  + Synthesis – the viral nucleic acid hijacks the cells synthetic machinery and viral nucleic acid and proteins are made.
  + Assembly – all of the pieces are put together
  + Release – the new virus particles leave the cell, often killing the cell (lytic cycle)
* Our bodies produce antibodies to fend off viruses; otherwise, we might be able to use antiviral agents, such as acyclovir. Our bodies also produce interferon which signals healthy cells to produce antiviral proteins.
* Viral vaccines have been developed
  + inactivated viruses – unable to replicate and must be injected
  + attenuated viruses – able to replicate, but at a very reduced rate
  + genetically-engineered – only viral parts are used for the vaccination. Safest of the vaccine types.
* Viruses sometimes do not kill the cell, but integrate their DNA into the host cell genomes (or a DNA copy of their genome if they are an RNA virus). This is known as lysogeny or lysogenic cycle. Inside the host cell chromosome it is referred to as a provirus.
* Viruses are cultured in fertilized chicken eggs or tissue culture cells for s tudy or vaccine production.

**Protozoa**

* Protozoa are single-celled eukaryotes that lack cell walls, ingest food particles, generally move about freely, and do not produce spores.
* Many produce a survival structure called a cyst, which is fairly resistant to environmental and chemical stresses. Cysts have been considered for bioterrorism.
* The vegetative or actively-feeding form is called a trophozoite.
* Zooplankton are classified as protozoa; they are an important part of the aquatic food-chain. They feed on algae and convert them into nutrients that are used by other organisms in the ocean.
* The protozoan are broken into four groups based upon the type of motility
  + Amoebas – move by cytoplasmic extensions called pseudopodia; also known as sarcodines.
  + Flagellates – as the name implies, they use flagella for motility
  + Ciliates – use cilia, which are more like short hairs, are used for motility
  + Sporozoa – adults are non-motile but are transported by vectors from one host to another (a.k.a. apicomplexans)
* Medically-significant **amoeba** include *Entamoeba histolytica* (amoebic dysentery – bloody diarrhea) and *Acanthamoeba* (corneal infections of contact lens wearers)
* Important members of the **flagellate** group include *Trichonympha* (breakdown cellulose in the termite gut to release glucose), *Trypanosoma brucei* and *Trypanosoma cruzi* (African Sleeping sickness and South American Sleeping sickness, which is better known as Chagas disease, respectively), *Giardia lamblia* (fouler-than-usual smelling diarrhea), *Trichomonas vaginalis* (a sexually transmitted disease), and *Leishmania tropica* (causative agent of many illnesses in the Persian Gulf War).
* The **ciliates** group has very few pathogenic species. We find heterotrophic organisms that range from 10 m up to 3 mm in size. *Paramecium* are an example from this group.
* The **sporozoan** group has a number of significant pathogens in it. It includes *Plasmodium* sp., which is the causative agent of malaria (it has a fairly complex life cycle that includes the mosquito), *Toxoplasma gondii* (causes toxoplasmosis, a disease that can be very serious in immunocompromised individuals), and *Pneumocystis* *jirovecii* (a fatal pneumonia for AIDS patients).
* Dinoflagellates are an aquatic group of protozoa that produce red pigments to give us red tides. Red tides, while very colorful, are dangerous because of a toxin that is produced by these organisms. The toxin bioaccumulates, which means in ends up in ever-increasing amounts as we move up the food chain. Symptoms include lack of muscular coordination and balance, slurred speech, and difficulty in swallowing.

**Fungi**

* Members of this kingdom are eukaryotes, which means they have nucleus.
* They serve as important decomposers of organic matter.
* Fungi can be found in one of two forms – yeast-like or mold-like
* Yeasts tend to be round or oval and are not visible to the eye unless allowed to grow a sufficient amount of time.
* The molds are characterized by the formation of strands or hair-like structures that we call hyphae. These members of the fungi can thrive in acidic environments (between 5 and 6), such as bread, cheese, and sour dairy products.
  + Hyphae contain nuclei and cytoplasm. If the hyphae are a continuous stream of cytoplasm they are called nonseptate or coenocytic. If they have walls separating the nuclei and the cytoplasm they are called septate – the wall is a called a septum.
  + A mass of hyphae are referred to as mycelium – usually called vegetative hyphae.
  + Hyphae that shoot up into the air and have a fruiting body ((that produces spores) are called aerial or reproductive hyphae.
  + The cell walls have a material called chitin, whereas plants have cellulose cell walls.
  + Fungi can have a sexual reproductive cycle in order to increase genetic diversity. In this event two specialized hyphae are made that act as the male and female with each having half the amount of chromosomes as usual (haploid)
* Zygomycetes is a typical hyphae-forming member of the fungi and is often found growing on bread (While man cannot live on bread alone, zygomycetes can).
  + A diploid zygote is formed when a “male” and a “female” hyphae join and combine their genetic information. The asexual cycle is much simpler.
  + Some important members of the zygomycetes include *Rhizopus* sp., which are used to produce sake from rice and cortisone.
* Ascomycetes produce a sexual spore that is called an ascospore. Included in this group are the *Penicillium* sp. (which are responsible for certain antibiotics and is used for production of Roquefort and Camembert cheese), *Aspergillus* sp. (which produces dangerous aflatoxins or citric acid, soy sauce and vinegar), and *Claviceps purpurae* (which produces a nervous system disorder called ergot disease and chemicals to relieve migraines without killing the patient).
* Some yeasts cause human illness, such as *Candida albicans*, while others are used in food, beer, and wine production (*Saccharomyces*).
* Lichens are a mutualistic relationship (where both members benefit from the association) between a fungus and a either a green alga cell or a cyanobacterium. Lichens can be found in rather diverse environments, including deserts and Arctic regions.
* Mycorrhizae are mutualistic relationships between fungi and vascular plants. In this case the fungi supply the plant with many nutrients while the plant supplies photosynthetic products of use to the fungi.
* Medically significant fungi include Candida *albicans* (causes vaginitis and trush), *Cryptococcus neoformans* (pneumonia-like illness and meningitis), skin infections (athlete’s foot, ringworm, etc.), *Coccidiodes* *immitis* (Valley fever in California; possible bioterrorism agent), *Histoplasma capsulatum* and *Blastomyces dermititidis* (respiratory illnesses in the immunocompromised population).

**Review Questions**

**True/False**

1. When placed on an agarose gel, DNA moves because it is negatively charged.

2. When placed on an agarose gel, the bigger pieces of DNA move fastest.

3. The classical methods of identifying bacteria have no place in the modern genomic era.

4. Microbes have to be grown in the laboratory before their DNA can be sequenced.

**Multiple Choice**

**1. When using PCR to make copies of DNA for sequencing**

A) all bases have the same colored dye but the different fragments of DNA sequenced will have a different color

B) there will be 4 different colored dyes; one for each of the 4 bases in DNA

C) there will be 4 different colored dyes; each base will randomly have one color

D) there will be 2 colored dyes based upon the base pairing rule; A and T will have the same and C and G will have the same

**2. Diversity of microbial life:**

a) is less than the diversity of visible organisms

b) is greater than the diversity of visible organisms

c) is less than the number of lies made by politicians

d) is not a concern of man

**Short Answer**

* 1. Explain the abilities prokaryotes have that allow them to expand into new environments

**Discussion Questions**

1. One strain of *E. coli* is a normal inhabitant in the human. Another form of this same species, if ingested, can poison the body. What do you think makes the two forms different?

**Answers**

**True/False**

1. T 2. F 3. F 4. F

**Multiple Choice**

* 1. B 2. B

**Short Answer**

1. consider mutations coupled with rapid growth of the population and gene sharing

**Discussion**

1. Start with the consequences to the organism of making its host sick and genetic changes in the host population caused by such an illness; move to the survival rates shaping the parasite population and how they differ from forces shaping the free-living population.