Summary

In this chapter we learn about environmental limits on microbial growth and how organisms adapt to changes in their environments. Sometimes the adaptations are temporary stress-reducing solutions, but in other cases adaptive mutations occur. The latter, of course, result in genetic change and evolution. By understanding all aspects of microbial growth and adaptation, we can better control organisms that are pathogenic to plants, animals, or humans.

5.1 Environmental Limits on Growth

It is at this point that the term *extremophile* is introduced. An extremophile is any organism that lives outside of our “normal” human range of reference.

Introduce different environmental parameters, including temperature, pH, osmolarity, oxygen, and pressure. Describe the ranges of possible conditions under each of these categories. Make sure to point out what is considered “normal.”

Discussion Points

- The environmental parameters are listed in Table 5.1 along with the classifications for each group of organisms.

- Ask students to identify the temperature category in which pathogens belong. Use Fig. 5.1 to introduce techniques that allow for global analysis of gene expression in changing environments.

5.2 Adaptation to Temperature

Microbes do not have the ability, as we do, to regulate temperature. Their temperature matches that of their environment. All organisms have a range of temperatures within which they can survive. Every organism also has an optimum temperature at which it grows best. The temperature limits on growth are dictated by the proteins in the cell and the structure of the cell membrane.

Microorganisms are classified by their optimum growth temperature. Microorganisms can be classified as psychrophiles, mesophiles, thermophiles, and hyperthermophiles, and each classification has its own set of special characteristics.

A recurring topic throughout the text is the polymerase chain reaction (PCR). Discuss the importance of the discovery of the thermoenzyme from *Thermus aquaticus* that allowed automation of this process.
Discussion Points
- Fig. 5.2A illustrates the Arrhenius equation and its relationship to growth.

- The temperature classification groups are illustrated in Fig. 5.2B. Discuss the shape and meaning of the curves.

- Psychrophiles and thermophiles can be found in some rather unique places. Figs. 5.3 and 5.4 show some of these locations and the organisms found there.

- Special Topic 5.1 describes the role of bacteria in rainmaking and ice formation.

5.3 Adaptation to Pressure
The terms barotolerant and barophilic are introduced. The barophiles are an interesting group of organisms since they contain both psychrophiles and thermophiles, many of which have been isolated from the ocean floor.

Discussion Points
- Fig. 5.5 is an interesting depiction of the topography of Earth’s surface. Point out temperature variances found at the ocean floor.

- Fig. 5.6 clearly illustrates the barosensitive, barotolerant, and barophilic classifications.

5.4 Water Activity and Salt
In this section many terms must be introduced before the concepts can be explained. In particular, it is important to explain water activity. Only after understanding water activity should discussions lead to osmolarity and ways organisms have evolved to deal with osmotic stress. Emphasis is placed on the important distinction between hypotonic and hypertonic solutions.

The concept of compatible solutes came up in a previous chapter and appears again here in the discussion about coping mechanisms under osmotic stress and for halophiles.

Discussion Points
- Aquaporins are specialized membrane channels used to transport water and to protect organisms from osmotic stress. A transverse view of an aquaporin channel is shown in Fig. 5.7.

- Halophilic salt flats can range in color from pinkish red to red, and even to a dark purple. An example of a salt flat in Nevada is shown in Fig. 5.8, along with an SEM of a halophilic bacterium.
5.5 Adaptation to pH Changes
Whereas intracellular temperature matches that of the microbe’s environment, the intracellular pH is maintained near neutrality, regardless of the external pH. This occurs because proteins operate only near neutral conditions.

There are three classes of organisms, based on the pH range within which they function best. Before these are defined, the concept of pH needs to be fully understood. The acidophiles thrive at low pH and have tetrathane membranes, which means that proton permeability is decreased. Neutralophiles thrive at neutral or near neutral pH. The alkaliphiles occupy the high end of the pH spectrum. They are commonly found in environments that not only have a high pH but high salt concentrations as well. Their membranes have diether linkages.

Also of interest is the fact that these organisms have evolved to utilize a sodium rather than a proton motive force.

Discussion Points

- Fig. 5.9 serves to illustrate pH. It not only shows how pH and pOH relate to one another and to hydrogen ion concentration, it also shows where the acidophiles, neutralophiles, and alkaliphiles fall along the pH scale.

- To help solidify the concept of pH, Fig. 5.11 lists common household items at each pH. Also note that to the left of the pH is the concentration of hydrogen ions at each pH as compared to distilled water.

- Figs. 5.12 and 5.13 illustrate how cytoplasmic pH is maintained over a wide range of external pH.

- Figs. 5.14 and 5.15 can be referenced to illustrate specialized environments. Fig. 5.15 is especially interesting because it describes why pink flamingos are pink (due to their ingestion of pink cyanobacteria, whose pigment enters the flamingos’ bloodstream, turning their feathers the same color).

- Alkaliphiles have evolved to utilize a sodium motive force versus a proton motive force. This concept is illustrated in Fig. 5.16.

- Many bacteria have developed strategies to maintain internal pH homeostasis. The mechanisms used by *E. coli* are described and diagrammed in Fig. 5.17.

5.6 Oxygen and Other Electron Acceptors
Some organisms require oxygen. At the other end of the spectrum are organisms that die in the presence of oxygen. It should be noted that they do not die from the oxygen per se, but rather that in the presence of oxygen these organisms produce reactive oxygen molecules, which kills the cells.
Although respiration and fermentation are only introduced here and will be detailed in other chapters, they are important concepts to discuss initially. Once these terms have been introduced it is possible to describe facultative aerobes, facultative anaerobes, and microaerophiles.

The methods for culturing anaerobes in the laboratory are different from methods of culturing aerobes. Typical methods used are the anaerobic jar and an anaerobic chamber with glove ports.

**Discussion Points**

- To set the stage for this section, use Fig. 5.19 to illustrate oxygen levels throughout a test tube and the categorization of types of organism found in each range.

- Use Table 5.2 to introduce organisms that fall into each of these categories.

- Use Fig. 5.18 to illustrate and briefly describe aerobic respiration with the use of oxygen as the terminal electron acceptor.

- Since anaerobic respiration also uses cytochromes and the electron transport system, examine Fig. 5.18 and simply point out that the cell would use a terminal electron acceptor other than oxygen.

- Aerobes have evolved mechanisms to deal with reactive oxygen species. The various pathways are illustrated in Fig. 5.20.

- Note that *E. coli* falls in the category of a facultative aerobe, having the capacity to both ferment and carry out aerobic respiration.

- Fig. 5.21 shows the apparatuses used in a laboratory when working with anaerobic microbes.

### 5.7 Nutrient Deprivation and Starvation

We do not generally think about organisms having to deal with nutrient deprivation and starvation, but in actuality, the natural environments they survive in rarely ever have nutrients in excess. For this reason it is essential that organisms have mechanisms in place to cope with poor nutrient levels. Many different stress survival genes can become activated. First, a cell retools and expresses genes to make transport systems for other potential nutrients, regardless of substrate availability. Cells also have the capacity to protect themselves against oxygen radical production and temperature and pH extremes.
One should recognize that in nature, in all likelihood, there is not just a single stress but multiple stresses imposed on an organism simultaneously. Sometimes, some of the genes produced under one stress situation are also expressed under another. It should be noted that protein profile studies were carried out with *Salmonella* in the laboratory under single stresses versus *Salmonella* in phagocytic vacuoles. Even though the single-stress laboratory conditions would all be present in the vacuole, the protein profile observed in the vacuole was not what was expected from the laboratory results. The point should be made that *in vivo* responses cannot always be predicted from *in vitro* studies.

Environments can be completely overturned by eutrophication, which occurs when suddenly a nutrient previously present at low levels becomes very plentiful. Eutrophication allows for an organism to grow rapidly to such high levels that it can kill off other species of organisms previously native to that environment.

*Discussion Points*

- Mention the production of the signal molecules, cyclic AMP and guanosine tetraphosphate, during the starvation response. Signal molecules will be a recurring subject in later chapters.

- An interesting adaptive response to a nutrient-poor environment is that the organism synthesizes gene products for novel transport systems even when the substrate is not available. Ask the students why this might be so.

- Introduce the fact that many oligotrophs actually require low nutrient levels to survive.

- Prothecae in some oligotrophs act to increase the surface area of the microorganism. Make sure the students understand how this relates to an increased capacity to transport nutrients.

- Fig. 5.23 illustrates microbial diversity in a hypothetical undisturbed natural environment versus an environment following phosphate eutrophication.

- Fig. 5.24 shows a photo of such environments as previously described.

- A good example of eutrophication is algal blooms that occur in high-phosphorous environments.

- In an attempt to prevent eutrophication, laundry detergents are now manufactured to be phosphate-free.
5.8 Physical, Chemical, and Biological Control of Microbes

This section introduces many terms and mechanisms concerning the control of microbial growth. Three important terms are sterilization, disinfection, and antisepsis. It is important to emphasize that sterilization is a process by which all living cells, as well as spores and viruses, are destroyed. Disinfection and antisepsis refer to the destruction of only disease-causing agents. Although disinfectants and antiseptics carry out similar tasks, disinfectants are used only on inanimate surfaces, whereas antiseptics are used on living surfaces.

Two other terms that are key in discussion of the control of growth are bacteriostatic and bactericidal. Something that is bacteriostatic inhibits growth, whereas a bactericidal agent actually kills the cells.

When microbes are treated with lethal chemicals or conditions, they do not all die immediately. Death occurs in a negative exponential fashion. Just as it was important to understand a growth curve, it is now necessary to introduce a death profile and the concept of a D-value.

This section introduces many different processes by which we can physically control microbial growth. Each mechanism is explained, an example of a microbe controlled in each fashion is given, and an illustration of the mechanism or equipment is usually also presented.

Likewise, different chemical means to control microbial growth are also presented. It is crucial to introduce the standard term for effectiveness, the phenol coefficient. Phenol is the standard to which all chemicals are compared. A higher phenol coefficient indicates higher efficacy of the disinfectant.

Antibiotics are also introduced. Some antibiotics are bacteriostatic, while others are bactericidal. Unfortunately for us, microbes have evolved mechanisms to be resistant to antibiotics. We are constantly looking for novel antibiotics or chemically redesigning existing antibiotics to sidestep the microbial resistance strategies.

Our society is becoming more and more health conscious. Many people are searching out probiotics, foods or supplements that contain living microorganisms. These are considered desirable to improve intestinal microbial balance.

Another potential biological control is the use of bacteriophages, viruses that infect bacterial cells. Each bacterial species is targeted only by specific phages. Bacteriophage treatment was prescribed to treat illness prior to the advent of antibiotics. This form of treatment, called phage therapy, is making a resurgence because of widespread antibiotic resistance.
Discussion Points

- The concept of a death curve and the calculation of D-values are illustrated in Fig. 5.25.

- Various means and equipment used to physically control microbial growth are shown in Figs. 5.26–5.28.

- When describing the concept of irradiation, the organism *Deinococcus radiodurans* is useful to discuss (Fig. 5.29).

- The chemical structures of common disinfectants and antiseptics are shown in Fig. 5.30.

- Did you know there was a U.S. postage stamp showing *Penicillium notatum* (Fig. 5.31)?

- If you choose to discuss the mechanism of action of penicillin, Fig. 5.32 affords the student the opportunity to see what physically happens to the cells.

- You may introduce the topic of antibiotic resistance and discuss why there is less resistance to disinfectants.

- Introduce the concept of probiotics and then open the floor to a discussion of sources and their specific benefits.

- The idea of phage therapy will be of particular interest to students. This may be the first time they have heard of it.

- Viruses will be detailed in the next chapter.

This chapter covered quite a bit of material, from the environmental changes all the way to physical, chemical, and biological means used to control microbial growth. We now know why pink flamingos are pink. We also learned that *Deinococcus radiodurans* is so resistant to irradiation that it could survive an atomic blast. In addition, we learned that instead of receiving antibiotics, sometime in the future we could receive bacteriophages to treat our illness. The latter is a good lead into the next chapter, which covers viruses.
Weblinks for Chapter 5
The following weblinks appear in Chapter 5, and on the Microbiology StudySpace student website at microbiology2.com/links.

Deinococcus
This link connects you to the Genome News Network and specifically Deinococcus radiodurans. From here you can learn interesting information about any microbial species.
http://www.genomenewsnetwork.org/articles/07_02/deinococcus.shtml

Penicillin in Jmol
A 3D picture of penicillin is rendered using the Jmol application.
http://www.bio.davidson.edu/misc/RasMol/penicillinG.html

Recommended Readings for Chapter 5
The following readings are presented at the end of the textbook chapter as resources for further exploration of the topics discussed in Chapter 5.


**Answers to Review Questions (p. 179)**

1. Explain the nature of extremophiles and discuss why these organisms are important.

   **ANS:** Extremophiles live in extreme environments. Some examples of extreme environments are high temperature, extremely high or low pH, and even high salt. These organisms have adapted to these conditions and even require them. They are important because we can use their enzymes, and they help us understand how cells work.

2. What are the parameters that define any growth environment?

   **ANS:** The parameters are temperature, pH, osmolarity, oxygen, and pressure, as well as nutrient availability.
3. List and define the classifications used to describe microbes that grow in different physical growth conditions.

**ANS:**

**Temperature:** Classification is based on optimum temperature for growth. Above 80°C—hyperthermophile; 50–80°C—thermophile; 20–40°C—mesophile; less than 20°C—psychrophile

**pH:** Optimum pH for growth: pH > 9—alkaliphile; pH 5–8—neutralophile; pH < 5—acidophile

**Osmolarity:** The concentration and type of solute molecules required for growth: halophile (> 2M NaCl)

**Oxygen:** growth only in oxygen—aerobe, growth with or without oxygen—facultative, growth only in reduced oxygen concentrations—microaerophile, growth only without oxygen—anaerobe

**Pressure:** growth at pressures above 380 atm—barophile

4. What do thermophiles have to do with the PCR reaction?

**ANS:** It was from the thermophile *Thermus aquaticus* that Taq polymerase was isolated. Taq polymerase is used in the PCR reaction since it can withstand the high temperatures required repeatedly during the cycling steps.

5. Why is water activity important to microbial growth? What changes water activity?

**ANS:** Water activity relates to how much water is available to the organism. The more solutes there are in a solution, the less water is available for microbes to use for growth.

6. How do cells protect themselves from osmotic stress?

**ANS:** Simple diffusion is not the primary means for movement across the cell membrane. Aquaporins are special membrane channels that transport water much faster than could simple diffusion. This alleviates the osmotic stress caused by differences in osmolarity across the membrane. In a hypertonic medium cells also have the capacity to synthesize or import compatible solutes to increase the internal osmolarity.

When cells are immersed in a hypotonic medium, the internal pressure increases and they need to lower the internal osmolarity to compensate. Pressure-sensitive (mechanosensitive) channels are activated at these increased pressures, and solutes are allowed to escape, thereby lowering internal osmolarity.
7. Why do changes in H⁺ concentration affect cell growth?

ANS: Enzymes, regardless of the pH at which the organism from which they were isolated thrives, tend to operate best at a pH between 5.0 and 8.5. The cells must have a mechanism to maintain a relatively neutral intracellular pH, even when the extracellular environment is well outside that range.

8. How do acidophiles and alkaliphiles manage to grow at the extremes of pH?

ANS: Acidophiles have very different membrane lipids, containing high levels of tetraether lipids. This feature decreases proton permeability of the membrane. They also have mechanisms to pump protons out of the cell.

Alkaliphiles possess a membrane composed of diether lipids, which add to its stability and prevent protons from leaking out of the cell. Most alkaliphiles have evolved to utilize a sodium motive force versus the traditional proton motive force, since there is a shortage of available protons.

9. If an organism can live in an oxygenated environment, does that mean that the organism uses oxygen to grow? If an organism can live in an anaerobic environment, does that mean it cannot use oxygen as an electron acceptor? Why or why not?

ANS: A strict aerobe is an organism that not only is able to grow in the presence of oxygen, but uses it as a terminal electron acceptor. Some organisms can exist in oxygenated environments, but do not need it to grow.

A strict anaerobe does not have the capacity to utilize oxygen as a terminal electron acceptor and are killed by reactive oxygen species that form when they are exposed to oxygen. *E. coli*, however, is a facultative aerobe. It has the capacity to use oxygen as a terminal electron acceptor when it is in an aerobic environment, but also can undergo fermentative growth if it should find itself in an oxygen-poor, or anaerobic, environment.

10. What happens when a cell exhausts its available nutrients?

ANS: One of the first things that happens is that signal molecules are produced. These molecules are involved in global control of gene expression. Genes are turned on to produce transport systems for alternative nutrients. This happens even if the matching substrate is not present. The organism may also begin to store glycogen as a potential backup reserve. As conditions get worse, the organism will proceed to ready itself for other stresses, like temperature or pH extremes.
11. List and briefly explain the various means by which humans control microbial growth. What is a D-value? What is a phenol coefficient?

**ANS:** There are physical, chemical, and biological methods to control microbial growth.

*Physical methods:* The steam autoclave utilizes high temperature created by increased pressure to effectively sterilize liquids and solids. Pasteurization is performed by heating liquids to a temperature that kills pathogens but does not destroy the food product. Filtration can be used to remove cells from solutions or air. Filters with pore sizes of 0.2 micrometers can remove microbial cells, but not viruses. Irradiation is a strategy for sterilizing food after harvesting.

*Chemical methods:* Various chemicals can be used to control growth. Phenol is the benchmark against which other disinfectants are measured in a phenol coefficient test. The results are compared to phenol and a phenol coefficient is determined. The higher the value, the more effective is the chemical.

*Biological methods:* Microbes make chemical compounds that control growth of other microbes. These compounds are referred to as antibiotics.

The D-value is obtained from a microbial death curve. The D-value corresponds to the time it takes to kill 90% of the microbial population.

12. How do microbes prevent the growth of other microbes?

**ANS:** Some microbes prevent the growth of others by producing antibiotics. The simple presence of some organisms may be enough to slow the growth of others. Sometimes this is simply accomplished by competition for nutrients, other times it is a bit more complex. Sometimes organisms make changes to the environment that retard the growth of other organisms.

Phage therapy was also introduced. A bacteriophage can slow growth or kill its target bacterium, depending upon the mode of infection it employs.
Answers to End-of-Chapter Thought Questions (p. 179)

1. Given a natural lake environment with 100 species of bacteria, why does the species with the fastest generation time not overwhelm the others? Or does it?

ANS: Lakes contain many different nutrients. Left undisturbed, the nutrient-supporting growth of the most rapidly growing bacterium will become limiting and growth will slow. Other organisms using other nutrient sources can continue to grow and maintain a presence. However, if two organisms use the same nutrient, the one that grows more rapidly will overgrow the slower one, but probably will not eliminate it completely.

2. *Escherichia coli* is a facultative species, able to grow with or without oxygen. What would it take to make this organism an anaerobe?

ANS: One would have to eliminate, by mutation, genes encoding the superoxide dismutases (it has three). Knocking out catalases wouldn’t work because the major contributor to oxidative damage is superoxide. Knocking out the aerobic cytochromes wouldn’t work. Because the mutant organism would retain superoxide dismutase, it could still grow with or without oxygen—it just couldn’t use oxygen as a terminal electron acceptor.

3. Two spore formers, *Bacillus stearothermophilus* and *Bacillus coagulans*, have D-values at 121°C of 5 minutes and 0.07 minutes, respectively. How could the spores from these organisms have such different D-values? Hint: Find the optimum growth temperatures for these organisms.

ANS: The optimum growth temperatures for *B. stearothermophilus* and *B. coagulans* are 55°C and 37°C, respectively. Because *B. stearothermophilus* is a thermophile, its enzymes are more heat stable than those of *B. coagulans*; thus, it will take longer for heat to kill *B. stearothermophilus*.

4. Phage therapy is touted by some as a solution to antibiotic resistance. Explain why this may not be true.

ANS: It is true that cells resistant to an antibiotic will remain sensitive to lytic bacteriophages. However, bacteria can also become resistant to phages, usually through mutation of a cell-surface receptor molecule (protein or carbohydrate). The use of two different phages that attach to different receptors on the target pathogen would reduce the occurrence of phage-resistant mutants.