

Part I

# Review of Basic Microbiological Concepts



# Introduction to Environmental Microbiology

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### 1.1 INTRODUCTION

Working on the second edition of *Environmental Microbiology* has been challenging in that although many exciting new areas have emerged, the fundamentals of the field remain as important foundations. Great strides have been made in expanding our knowledge in the areas of genetics, evolution, and diversity and yet we still do not have a clear idea of how this knowledge can be integrated into understanding and controlling microorganisms in the environment or even how to culture many microbes. We remind the reader that while the roots of environmental microbiology are perhaps most closely related to the field of microbial ecology, which comprises the study of the interaction of microorganisms within an environment, be it air, water, or soil, the primary difference between these two fields is that environmental microbiology is an applied field in which the driving question is, *how can we use our understanding of microorganisms in the environment to benefit society?* Consequently, the subjects covered in this textbook are not the same as those covered in traditional texts on microbial ecology. Because environmental microbes can affect so many aspects of life, and are easily transported between environments, the field of environmental microbiology interfaces with a number of different subspecialties, including soil, aquatic, and aeromicrobiology, as well as bioremediation, water quality, occupational health and infection control, food safety, and industrial microbiology (Fig. 1.1).

What happened in the 1970s to cause this new field of microbiology to develop? Several events occurred simultaneously that highlighted the need for a better understanding

of environmental microorganisms. The first of these events was the emergence of a series of new waterborne and foodborne pathogens that posed a threat to both human and animal health. In the same time frame it became increasingly apparent that, as a result of past waste disposal practices, both surface water and groundwater supplies are frequently contaminated with organic and inorganic chemicals. Finally, the discovery of the structure of DNA in 1953 by James Watson and Francis Crick engendered the development of new technologies, notably the polymerase chain reaction (PCR), based on nucleic acids for measuring and analyzing microbes. The simultaneous impact of these events caused scientists to question the notion that our food and water supplies are safe and also allowed the development of tools to increase our ability to detect and identify microbes and their activities in the environment. Thus, over a relatively short period, the new field of environmental microbiology has been established.

### 1.2 AN HISTORICAL PERSPECTIVE

The initial scientific focus of the field of environmental microbiology was on water quality and the fate of pathogens in the environment in the context of protection of public health. The roots for water quality go back to the turn of the twentieth century, when the treatment of water supplies by filtration and disinfection resulted in a dramatic decrease in the incidence of typhoid fever and cholera. Application of these processes throughout the developed world has essentially eliminated waterborne bacterial disease except when treatment failures occur. Until the 1960s, it was



**FIGURE 1.1** Environmental microbiology interfaces with many other fields of microbiology.

**TABLE 1.1** Recently Discovered Microbes That Have Had a Significant Impact on Human Health

Agent	Type of microorganism	Mode of transmission	Disease/symptoms
SARS (a coronavirus)	Virus	Fomites	Respiratory
<i>Escherichia coli</i> O157:H7	Bacterium	Foodborne, waterborne	Enterohemorrhagic fever, kidney failure
Hepatitis E virus	Virus	Waterborne, foodborne	Hepatitis
Bird flu (avian influenza A (H5N1))	Virus	Aerosol, fomites, foodborne	Respiratory
Norovirus	Virus	Waterborne, foodborne	Diarrhea
<i>Helicobacter pylori</i>	Bacterium	Foodborne, waterborne	Stomach ulcers
<i>Cyclospora</i>	Protozoan	Foodborne, waterborne	Diarrhea
MRSA (antibiotic-resistant <i>Staphylococcus aureus</i> )	Bacterium	Fomites	Severe skin infections

thought that threats from waterborne disease had been eliminated. However, case studies began to accumulate suggesting that other agents, such as viruses and protozoa, were more resistant to disinfection than enteric bacteria. This concern became a reality with the discovery of waterborne outbreaks caused by the protozoan parasite *Giardia* and by *Norovirus*, both of which were found in disinfected

drinking water. Water quality continues to be a major focus in environmental microbiology because new waterborne pathogens continue to emerge. One of the best documented and largest outbreaks occurred in 1993 when more than 400,000 people became ill and over 100 died in Milwaukee, Wisconsin, during a waterborne outbreak caused by the protozoan parasite *Cryptosporidium*. Table 1.1 lists many

**Information Box 1.1 Large-Scale Food Recalls Due to Foodborne Outbreaks**

Food recall	Year	Organism	# People involved	Location
Cheese	1998	<i>Salmonella enteritidis</i>	700	Newfoundland, Canada
Turkey and chicken products	2002	<i>Listeria</i>	120	United States
Green onions	2003	Hepatitis A	600	United States
Spinach	2006	<i>E. coli</i> O157:H7	200	United States
Canned meat products	2007	<i>Clostridium botulinum</i>	4	United States

of the important pathogens that have emerged in the past 30 years. Even more worrisome, recent studies suggest that 10 to 50% of diarrhea-associated illness is caused by waterborne microbial agents we have yet to identify.

Pathogens in our food supply is a second area of immense concern. One of the difficult questions facing the food industry is that of importation of vegetables and fruits. On the one hand, there is consumer demand for a wide variety of high quality imported produce; on the other hand, there is an increased danger of importing pathogens along with the produce. One example is the protozoan pathogen *Cyclospora*. This organism gained notoriety as the causative agent of a disease outbreak affecting approximately 1000 people in the United States between early May and mid-July 1996. The *Cyclospora* outbreak was due to consumption of raspberries imported from Guatemala. Foodborne outbreaks have resulted in major recalls of various foods (see Information Box 1.1). These examples illustrate the types of questions that are addressed by environmental microbiologists. How do pathogens survive in the environment, and how can they be detected and eliminated rapidly and economically to allow both protection of human health and rapid consumption of fresh produce? Determining the presence and significance of pathogenic organisms in food, water, and air supplies is a challenge that will keep environmental microbiologists occupied in the coming decades.

As concern about chemicals in the environment emerged in the 1960s, perhaps best highlighted by Rachel Carson's landmark book *Silent Spring*, it became clear that this was a natural extension for the field of environmental microbiology. We now know that chemical pollutants in soil and groundwater have profound effects on human health and welfare, in terms of both potential disease that the intake of these chemicals can cause, and the economic impact of cleaning up contaminated environments. Health effects from chemical contaminants have been difficult to assess because the impact is not acute as for pathogens but rather

**TABLE 1.2** Common Contaminants Found in the Environment

Chemical class	Examples
Petroleum hydrocarbons (fuels)	Benzene, toluene, xylene, polycyclic aromatics
Coal tar and creosote	Aromatics and polycyclic aromatics
Chlorinated solvents	Trichloroethene (TCE), tetrachloroethene (PCE), trichloroethane, carbon tetrachloride
Munitions	Trinitrotoluene (TNT), hexanitrobenzene (HNB), 2,4,6-trinitrophenol (picric acid)
Pesticides	Organochlorines (DDT), organophosphates (chlorpyrifos), carbamates (carbaryl), triazines (atrazine), insecticides (pyrethroids), fumigants (dichloropropene)
Polychlorinated biphenyls (PCB)	Arochlor, Clophen, Phenoclor, Fenchlor, Kanechlor, Tarnol
Pharmaceuticals, food additives, and cosmetics	Endocrine disruptors (e.g., estradiol, nonylphenol, polybrominated diphenyl ethers (PBDEs)), antibiotics, surfactants, dyes
Fluorocarbons	Chlorofluorocarbons (CFCs), hydrochlorofluorocarbons (HCFCs), fluoropolymers (Teflon)
Fertilizers	NH <sub>3</sub> (gas), CO(NH <sub>2</sub> ) <sub>2</sub> (urea), (NH <sub>4</sub> ) <sub>3</sub> PO <sub>4</sub> , superphosphate
Metals	Lead, cadmium, mercury, arsenic, chromium, nickel, zinc
Radioactive contaminants	Uranium, strontium, cobalt, plutonium

cumulative, resulting in cancer many years following exposure or, more immediately, resulting in birth defects. Table 1.2 shows a partial list of the chemical contaminants that are routinely found in soil and groundwater. The cost of cleanup or remediation of the contaminated sites in the United States alone would require more than \$1 trillion. Given this price tag, it is increasingly recognized that biological cleanup alternatives, known as bioremediation, may have profound economic advantages over traditional physical and chemical remediation techniques.

Once thought unpredictable and ineffective, bioremediation is now a viable and preferred alternative for cleanup of many sites. The efficacy of bioremediation was clearly illustrated in 1989 when the *Exxon Valdez* spilled almost 11 million gallons of North Slope crude oil into Prince William Sound — the largest tanker spill in U.S. history. A combination of physical removal of free product, physical washing of the contaminated shoreline, and bioremediation has been used to slowly restore Prince William Sound.

This was the first large-scale documentation of successful use of bioremediation to clean up a contaminated site. Bioremediation is most successful when it takes advantage of naturally occurring microorganisms in the contaminated area. This realization has engendered a modern appreciation of the role of microorganisms in elemental cycling and environmental sustainability as well as the use of microorganisms for resource production and recovery.

### 1.3 MODERN ENVIRONMENTAL MICROBIOLOGY

In modern environmental microbiology, pathogens and bioremediation remain fundamental to the field, but in both cases these subject areas have been greatly enhanced through the application of molecular genetics and biotechnology tools. For example, development and assessment of new methods of detection and elimination of pathogens in our water and food supply, as well as the indoor environment we live in today, have become critical to public health with the global growth in population. Arguably, human and environmental hygiene efforts have had the greatest impact on reducing human suffering throughout the past century. This is also true today as we see the evolution of new and more virulent forms of environmentally transmitted pathogens. This has been highlighted by concerns about the spread of SARS and the potential for the rapid spread of avian bird flu. Vaccine development is often not rapid enough to guarantee protection and so the environmental microbiologist must be prepared to develop better methods to reduce or stop the spread of these agents throughout the environment. Continuing outbreaks of norovirus diarrhea on cruise ships has demonstrated how difficult an environmentally spread agent can be to control. New strategies are needed, such as the development of self-disinfecting surfaces, environmentally friendly disinfectants, more rapid methods of detecting indicators and pathogens in our environment. In addition, application of risk assessment to target the need for control where it is most effective and better products for the consumer are reducing the risk associated with environmentally transmitted infections.

The beginning of this century brought a new task for environmental microbiologists in the area of pathogen detection. The impact of just a few letters contaminated with anthrax spores demonstrated how rapidly a terrorist could disseminate a highly virulent pathogen across an entire region of a nation. It also demonstrated how little we know about the transport, survival, and methods of decontamination of lethal agents that could be used by bioterrorists. National security now depends on the development of methods for detection of select agents in the environment and models to assess their fate and control.

New challenges have similarly emerged in the area of bioremediation. New chemicals have been detected in ground

and surface waters that serve as our potable water resources. In addition, our ability to detect chemicals has gotten better and more comprehensive. For these reasons we have discovered contamination in previously “clean” sources of water. Further, as society grows, we put increasing stress on water resources. In many parts of the United States, for example, groundwater is currently being used at a faster rate than it is being recharged. Add to this increasing waste discharges containing biological and chemical contamination that enter our water resources, and it becomes clear that environmental microbiologists face large challenges in this arena. These issues also arise in contaminated soil environments. As the global population increases, land resources are becoming more valuable and communities are encroaching on contaminated sites, including landfills, mine tailings sites, and agricultural fields with years of pesticide applications. New strategies are needed in this area to treat sites that contain emerging contaminants, contaminant mixtures, and low levels of contaminants. Applications of risk assessment to target the need for control where it is most effective, and for community education to reduce risks associated with living next to contaminated sites, are also becoming more important.

A third important area of environmental microbiology has emerged over the past decade, that of molecular ecology. Molecular ecology as it relates to environmental microbiology can be defined as investigating diversity in the environment, and mining and exploiting that diversity for new natural products and activities. Molecular-based methodologies are now enabling us to detect, define, and better understand the ecology not only of natural habitats such as soil and water, but also of anthropologic environments such as households, fomites, or municipal wastes. Powerful molecular-based tools are becoming available to allow the examination of microbial communities through analysis of microbial DNA and RNA (PCR, gene probes, DNA sequencing, metagenomics) as well as proteins (proteomics). Such techniques now allow us to search for new microbes in extreme environments such as hot springs, caves, deep-sea thermal vents, and deep subsurface environments. Molecular sequence analysis of community DNA permits a new appreciation of microbial diversity, and also of how microbial communities function and communicate via quorum sensing. This new evaluation of the microbial environment is also affording innovative approaches to the discovery of high value “green” products that can be used in medicine, agriculture, and industry. Examples include new antibiotics and other natural products, plant-growth-promoting bacteria to enhance growth of crops, and new chemicals that can be used as detergents, solvents, surfactants, pesticides, and in food processing.

Holistically, these three fundamental areas of environmental microbiology enable us to pursue the goal of environmental sustainability, which we define as “the utilization of environmental resources for the benefit of human health and

welfare without deterioration of the physical environment or the biological communities contained therein.” Critical components of these biological communities are the microbial communities housed within the environment. Clearly then, the challenge for modern environmental microbiologists is to enhance our understanding of these communities in order to achieve environmental sustainability.

#### **1.4 PURPOSE AND ORGANIZATION OF THIS TEXT**

Our purpose in writing this book is to provide a text that can be used in teaching environmental microbiology as well as a general reference book for practitioners in the field of environmental microbiology. This textbook is designed for senior-level undergraduate and graduate classes in environmental microbiology. It is expected that students using this text have a background in general microbiology and organic chemistry. The overall objective of the text is to define the important microorganisms involved in environmental microbiology, the nature of the different possible environments

in which they are situated, the methodologies used to monitor microorganisms and their activities, and finally the possible effects of microorganisms on human activities. We have more fully defined and increased the number of sections in the second edition to eight: (1) a review of basic microbiological concepts; (2) a description of the various microbial environments encountered in the field including soil, water, and the atmosphere; (3) a discussion of methodologies available for detection, enumeration, and identification of microbes and their activities; (4) an examination of microbial communication, activities, and interactions with their environment and how these activities affect the cycling of nutrients; (5) a discussion of how microorganisms can be exploited for remediation of both organic and metal contaminants; (6) a description of important waterborne and foodborne pathogens as well as indicator organisms; (7) an examination of the wastewater treatment and disinfection processes used to treat our largest (in terms of volume) societal waste problem; and (8) a consideration of urban microbiology or the study of how microorganisms can impact an increasingly populated world.

