Fundamental Food Microbiology

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Dedication

To my parents, Hem and Kiron, and my family.

The Author

Bibek Ray, Ph.D., is professor of food microbiology in the Department of Animal Science at the University of Wyoming, Laramie. Professor Ray obtained a B.S. and M.S. in Veterinary Science from the University of Calcutta and University of Madras, in India, respectively. He received his Ph.D. in Food Science from the University of Minnesota in 1970 and joined the faculty in the Department of Food Science, North Carolina State University, and then the Department of Biology at Shaw University, both at Raleigh, North Carolina. He joined the University of Wyoming, Laramie, in 1981. At the University of Wyoming, he expanded his research to intestinal beneficial bacteria and bacteriocins of lactic acid bacteria along with his previous research activities in the area of microbial sublethal injury. He also teaches courses in Food Microbiology, Food Fermentation, and Food Safety. His laboratory has been involved in extensive and thorough studies in both basic and applied areas of bacteriocin, pediocin AcH from *Pediococcus acidilactici* H. In addition, his group is studying various aspects of bacteriocins produced by *Lactococcus, Leuconostoc, Lactobacillus,* and *Pediococcus*, as well as *Bacillus* and *Staphylococcus* spp. He has received research funding from the National Science Foundation, American Public Health Association, National Live Stock and Meat Board, U.S. Department of Agriculture, North Atlantic Treaty Organization (with Turkey), and Binational Agriculture Research Development Agency (with Israel), Wyoming Development Fund, and industry funds. At present, he is studying the combined effect of bacteriocins, ultrahigh hydrostatic pressure and pulse field electricity, and sublethal injury for the destruction of microbial cells and spores and its application in food preservation. This project is funded by the U.S. Army. In addition, Dr. Ray has been engaged in collaborative research programs with research institutes/universities in Turkey, Israel, India, and France.

Professor Ray has published over 100 research articles, reviews, book chapters, proceedings articles, and popular articles in the area of Food Microbiology. He has also edited two books: *Injured Index and Pathogenic Bacteria* (1989) and *Food Biopreservatives of Microbial Origin* (1992 with Dr. M. A. Daeschel), both published by CRC Press, Boca Raton, Florida. He is a member of the American Society for Microbiology, Institute of Food Technologists, and International Association of Milk Food and Environmental Sanitation. He is a Fellow of the American Academy of Microbiology and on the Editorial Board of the *Journal of Food Protection*. In 1994, Professor Ray was awarded the University of Wyoming Presidential Achievement Award in recognition of his excellence in academic performance.

Preface

Between the time I first studied food microbiology as an undergraduate student and now the discipline has undergone a radical change. This change is well expressed by Dr. David Mossel of the Netherlands in his letter published in *ASM News* (59(10), 1993): from "no challenge in plate count and coliform scouting" to "linkage of molecular biology to food safety (also food bioprocessing and food stability) strategies — proclaim a new era in Food Microbiology." This transition was necessary to meet the changes that occurred in the food industry, especially in the United States and other developed countries. The necessary knowledge, techniques, and expertise for this transition were all available. This book reflects this transition from the traditional approach to an approach to meet the needs of those who are directly or indirectly interested in Food Microbiology.

Introductory Food Microbiology is a required course for undergraduates majoring in Food Science. In some form it is also taught in several other programs, namely Microbiology, Public Health, Nutrition and Dietetics, Veterinary Science, and others. For the majority of food scientists, except those majoring in Food Microbiology, this single course forms the basis of the study microorganisms and their interactions to food. Similarly, for the latter groups, Food Microbiology is probably the only course that provides with information on the interaction of food and microorganisms. This book was written with the major objective of relating interaction of microorganisms and food in relation to food bioprocessing, food spoilage, and foodborne diseases. Thus, it will be useful as a text in the introductory food microbiology courses taught under various programs and disciplines. In addition, it will be a valuable reference book for those who directly and indirectly are involved in food and microbiology, which includes individuals in academic institutions, research institutions, federal, state, and local government agencies, food industries, food consultants, and even food lobbyists.

The subject matter has been divided into seven sections. For undergraduate teaching, the first six sections can be taught as a semester or a quarter course; Section VII (Appendices) can be used as advanced information for an undergraduate course that contains materials that are either taught in other courses, such as advanced food microbiology or food safety courses and laboratory courses. The first section contains four chapters that describe the history of food microbiology, characteristics of microorganisms important in foods, their sources, and significance. The second section also has four chapters that deal with microbial growth and metabolism in food and the significance of microbial sublethal injury and sporulation in foods. In the third section, the different beneficial uses of microorganisms, which include bioprocessing, biopreservation, and probiotics, are explained in eight chapters. Section IV deals with spoilage of foods by microorganisms and their enzymes and methods used to determine food spoilage. In addition, a chapter is included to discuss some of the emerging spoilage bacteria. Section V has seven chapters and deals with foodborne pathogens associated with intoxications, infections, and toxico-infections, as well as those that are considered opportunistic pathogens and pathogenic parasites and algae. In addition, a chapter has been included on emerging pathogens and chapter on indicators of pathogens. In Section VI, different methods used to control undesirable microorganisms for the safety and stability of food are discussed. A chapter on new nonthermal methods and a chapter on hurdle concept in food preservation are included.

The materials in each chapter are arranged in logical, systematic and concise sequences. Tables, figures, and data have been used only when their presentation is necessary for better understanding. At the end of each chapter, a limited list of selected references and suggested questions have been included. To reduce confusion, especially for those who are not familiar with constant changes in microbial genera, three first letters have been used to identify genus of a species. The index has been prepared carefully so that the materials in the text can be easily found.

I thank Mrs. Deb Rogers for an excellent performance in typing the manuscript. Finally, I thank my students, who, over a period of the last 20 years, have suggested what they would like to have in a food microbiology course. Those suggestions have been followed in writing this book.

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SECTION I

INTRODUCTION TO MICROBES IN FOODS

Microorganisms are living entities of microscopic size and include bacteria, viruses, yeasts and molds (together designated as fungi), algae, and protozoa. While bacteria are classified as procaryotes (cells without definite nuclei), the fungi, algae, and protozoa are eucaryotes (cells with nuclei); viruses do not have regular cell structures and are classified separately. Microorganisms are present everywhere on earth, which includes humans, animals, plants and other living creatures, soil, water, and atmosphere, and they can multiply everywhere except in the atmosphere. Together, their numbers far exceed all other living cells on this planet. They were the first living cells to inhabit the earth over 3 billion years ago and since then have played important roles, many of which are beneficial to the other living systems.

Among the microorganisms, some molds, yeasts, bacteria, and viruses have both desirable and undesirable roles in our food. In this section, their importance in food, predominant microorganisms associated with food, and the sources from which they get in the food and their significance are presented in the following chapters:

Chapter 1: History and Development of Food Microbiology
Chapter 2: Characteristics of Predominant Microorganisms in Food
Chapter 3: Sources of Microorganisms in Food
Chapter 4: Significance of Microorganisms in Food

CHAPTER 1

History and Development of Food Microbiology

CONTENTS

I. INTRODUCTION

Except for a few sterile foods, all foods harbor one or more types of microorganism. Some of them have desirable roles in food, such as in the production of fermented food, while others cause food spoilage and foodborne diseases. To study the role of microorganisms in food and to control them when necessary, it is important to isolate them in pure culture and study their characteristics. Some of the most simple techniques in use today for these studies were developed over the last 300 years; a brief description is included here.

II. DISCOVERY OF MICROORGANISMS

The discovery of microorganisms[1-4] ran parallel with the invention and improvement of the microscope. Around 1658, Athanasius Kircher reported that, using a microscope, he had seen minute living worms in putrid meat and milk. The magnification power of his microscope was so low that he could not have seen bacteria. In 1664, Robert Hooke described the structure of molds. However, probably the first person to see different types of microorganisms, especially bacteria, was the Dutch businessman turned naturalist Anton van Leeuwenhoek, using a microscope that probably had not above 300× magnification power. He observed bacteria in saliva, rainwater, vinegar, and other materials, sketched the three morphological groups (spheroids or cocci, cylindrical rods or bacilli, and spiral or spirilla), and also described some to be motile. He called them animalcules and in 1675 reported his observations to the newly formed leading scientific organization, The Royal Society of London, where his observations were read with fascination. As fairly good microscopes were not easily available at the time, during the course of the next 100 years, other interested individuals and scientists only confirmed Leeuwenhoek's observations. In the 19th century, as a result of the Industrial Revolution, improved microscopes became more easily available, and that stimulated many inquisitive minds to see and describe creatures they discovered under a microscope. By 1838, Ehrenberg (who introduced the term *bacteria*) had proposed at least 16 species in four genera and by 1875 Cohn had developed the preliminary classification system of bacteria. Cohn also was the first to discover that some bacteria produced spores. Although, like bacteria, the existence of submicroscopic viruses was recognized in the mid-19th century, they were observed only after the invention of the electron microscope in the 1940s.

III. WHERE ARE THEY COMING FROM?

Following Leeuwenhoek's discovery, although there were no bursts of activity, some scientific minds did have the curiosity to determine where the animalcules, found to be present in many different objects, were coming from.[1-4] Society had just emerged from the Renaissance period and science, known as experimental philosophy, was in its infancy. The theory of spontaneous generation, i.e., the generation of some form of life from nonliving objects, had many strong followers among the educated and elite class. Since the time of the Greeks, the emergence of maggots from dead bodies and spoiled flesh was thought to be due to spontaneous generation. But around 1665, Redi disproved that theory by showing that the maggots in spoiled meat and fish could only appear if flies were allowed to contaminate them. The advocates of the spontaneous generation theory argued that the animalcules could not regenerate by themselves (biogenesis), but that they were present in different things only through abiogenesis (spontaneous generation). In 1749, Needham showed that boiled meat and meat broth, following storage in covered flasks, showed the presence of animalcules within a short time. This was used to prove the appearance of these animalcules by spontaneous generation. Spallanzani (1765) showed that boiling meat infusion in broth in a flask and sealing the flask immediately prevented the appearance of these microscopic organisms and thus disproved Needham's theory. This was the time when Antoine-Laurent Lavoisier and his co-workers showed the need of oxygen for life. The believers of abiogenesis rejected Spallanzani's observation, suggesting that there was not enough vital force (oxygen) present in the sealed flask for animalcules to appear through spontaneous generation. Later, Schulze (1830; by passing air through acid), Theodor Schwann (1838; by passing air through red hot tubes), and Schroeder (1854; by passing air through cotton) showed that bacteria failed to appear in boiled meat infusion even in the presence of air. Finally, in 1864, Louis Pasteur demonstrated that, in boiled infusion, bacteria could grow only if the infusions were contaminated with bacteria carried by dust particles in air. His careful and controlled studies proved that bacteria were able to reproduce (biogenesis) and life could not originate by spontaneous generation. John Tyndall, in 1870, showed that in a dust-free box, boiled infusion could be stored in dust-free air without microbial growth.

IV. WHAT ARE THEIR FUNCTIONS?

The involvement of invisible organisms in many diseases in humans was suspected as early as the 13th century by Roger Bacon. In the 16th century, Francostro of Verona suggested that many human diseases were transmitted by small creatures from person to person. This was also indicated by Kircher in 1658. In 1762, von Plenciz of Vienna suggested that different invisible organisms were responsible for different diseases. Schawnn (1837) and Hermann Helmholtz (1843) pointed out that putrefaction and fermentation were connected with the presence of the organisms derived from the air. Finally, Pasteur, in 1875, showed that wine fermentation from grapes and souring of wine were caused by microorganisms. He also proved that spoilage of meat and milk was associated with the growth of microorganisms. Later, he showed the association of microorganisms with several diseases in humans, cattle, and sheep, and later developed vaccines against several human and animal diseases, including the rabies virus. Robert Koch, in Germany (in the 1880s and 1890s), isolated bacteria in pure cultures responsible for anthrax, cholera, and tuberculosis. He also developed the famous Koch's postulate to associate a specific bacterium as a causative agent for a specific disease. Along with his associates, he also developed techniques of agar plating methods to isolate bacteria in pure cultures, the petri dish (by Petri in his laboratory), and staining methods for better microscopic observation of bacteria.

With time, the importance of microorganisms in human and animal diseases, soil fertility, plant diseases, fermentation, food spoilage and foodborne diseases, and other areas was recognized, and microbiology was developed as a separate discipline. Later, it was divided into several disciplines, such as medical microbiology, soil microbiology, plant pathology, and food microbiology.[1-4]

V. EARLY DEVELOPMENTS IN FOOD MICROBIOLOGY (PRIOR TO 1900 A.D.)

It is not known exactly when our ancestors recognized the importance of the invisible creatures, now designated as microorganisms, in food. But it had to be around 8000 B.C. in the Near East after they developed agriculture and animal husbandry.[3-6] They produced more foods than they could consume within the short growing season, and a portion of the produce was lost due to spoilage. They solved the problems and secured uniform food supplies throughout the year by developing different preservation techniques. Between 8000 and 2000 B.C., they used drying, cooking, smoking, salting, low temperature, baking, modified atmosphere, fermentation, spices, and honey to extend the storage life of different types of raw and processed foods. Although we are not sure if they had perceptions about the cause of foodborne diseases, they definitely associated food spoilage with some invisible factors and developed successful preventative measures.

From the time of the Greeks until the discovery of biogenesis, spoilage of foods, especially of meat and fish, was thought to be due to spontaneous generation, such as the development of maggots. When the presence of different types of bacteria in many foods was discovered, their appearance through spontaneous generation was explained to be the cause of food spoilage. Schawnn (1837) and Helmholtz (1843) associated the presence of microorganisms (bacteria) in food with both putrefactive and fermentative changes of foods. However, they did not believe in spontaneous generation, but they could not explain how microorganisms could bring about those changes. Finally, Pasteur resolved the mystery by explaining that contamination of foods with microorganisms from the environment and their subsequent metabolic activities and growth were the causes of fermentation of grapes, souring of milk, and putrefaction of meat.

Diseases caused by the consumption of certain foods (foodborne disease) was recognized at least during the Middle Ages. Ergot poisoning in Europe was related to the consumption of grains (infested with molds) in the 12th century. In 1857, consumption of raw milk was suspected to be the cause of typhoid fever. In 1870, Selmi related certain food poisoning with ptomaine (histamine). Gaertner was the first to isolate *Salmonella* from a meat implicated in a foodborne disease in 1888. Denys, in 1894, was able to establish *Staphylococcus aureus* with food poisoning and, in 1896, Ermengem isolated *Clostridium botulinum* from food. The association of many other pathogenic bacteria and viruses to foodborne diseases was established after 1900 A.D. This aspect is discussed in Section V.

Pasteur, in the 1860s, recognized the role of yeasts in alcohol fermentation. He also showed that souring of wine was due to growth of acetic acid–producing bacteria (*Acetobacter aceti*), and developed the pasteurization process (heating at 145°F for 30 min) to selectively eliminate these undesirable bacteria from wine. Like fermentation, cheese ripening was suggested by Martin in 1867 to be of microbial origin. John Lister, in 1873, was able to isolate milk-souring bacteria (*Lactococcus lactis*) by the serial dilution (dilution to extinction) procedure. Cienkowski, in 1878, isolated the bacteria (*Leuconostoc mesenteroides*) associated with slime formation in sugar