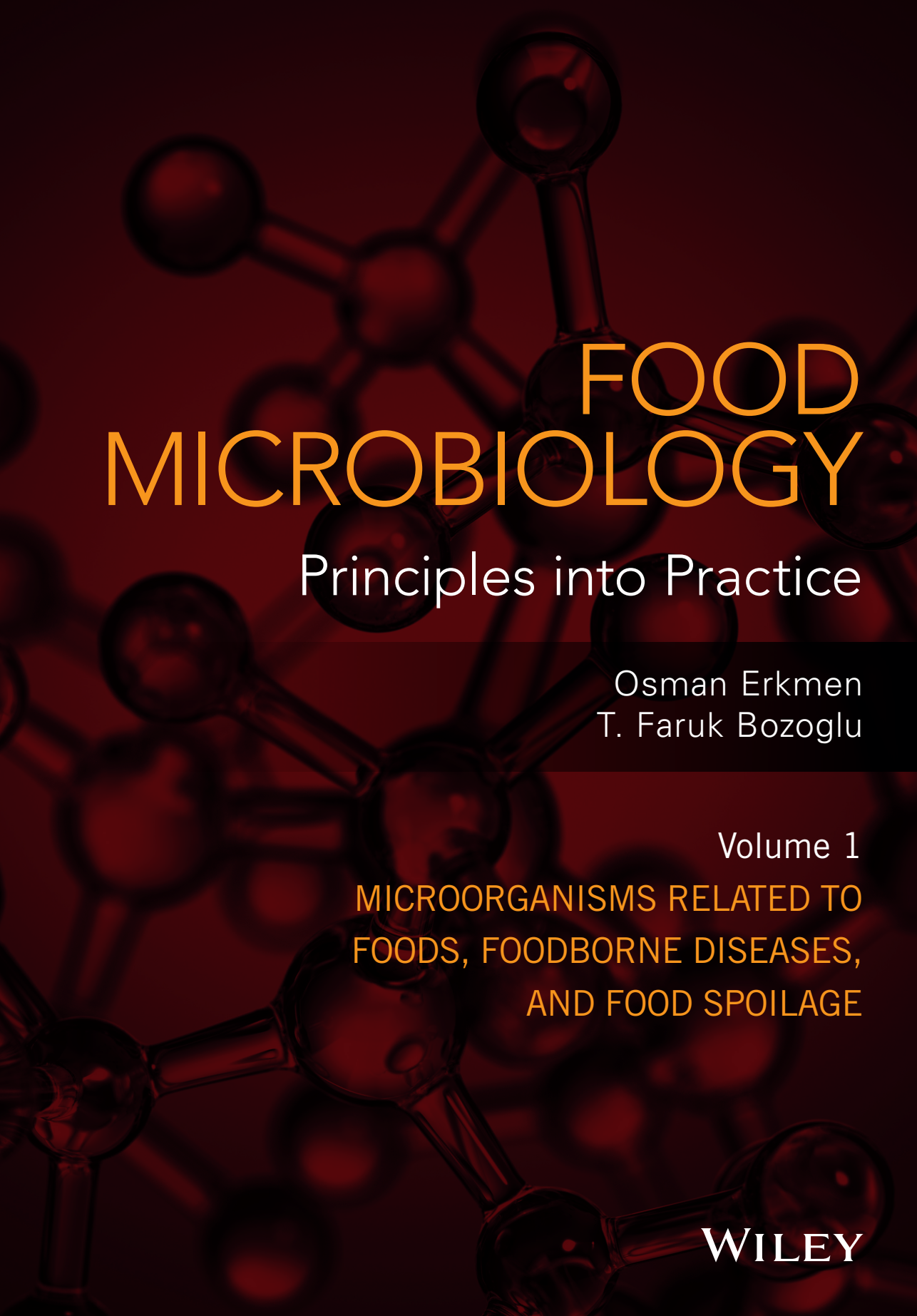

FOOD MICROBIOLOGY

Principles into Practice

Osman Erkmen
T. Faruk Bozoglu

Volume 1

MICROORGANISMS RELATED TO FOODS, FOODBORNE DISEASES, AND FOOD SPOILAGE

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Volume 1: Microorganisms Related to Foods, Foodborne Diseases, and Food Spoilage

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Born in 1955 in Konya, Turkey, Osman Erkmen is professor of food microbiology in the Department of Food Engineering under the University of Gaziantep (Gaziantep, Turkey) since 2004. He received his BS degree in Biology (1985) and MS degree in Food Microbiology (1987) from the Middle East Technical University (Ankara, Turkey). He did his PhD in General Microbiology from the Department of Microbiology under the University of Gaziantep in 1994. He started his career as a research assistant at the Department of Food Engineering in 1985 and later became assistant professor in 1994 and associate professor of Food Microbiology in 1999. Since 2004 he is working as professor in this department. At the Department of Food Engineering, he expanded his research to the use of nonthermal processes and natural antimicrobials in food preservation; in the production of fermented foods; in the microbial production of thiamin, alcohol, and citric acid from industrial wastes; and in the microbial inactivation kinetics and modeling. He received funding for research from the University of Gaziantep Foundation, the Scientific and Technological Research Council, and the Republic of Turkey State Planning Organization. He has been studying the combined effect of nonthermal processes and natural antimicrobials in the destruction of microbial cells and spores, its application in food preservation, and in the microbial production of lycopene from industrial wastes. He teaches courses in Food Microbiology, General Microbiology, Food Sanitation, and Food Toxicology.

Professor Erkmen has published over 100 research articles, reviews, book chapters, proceeding articles, and popular articles in the fields of Food Microbiology, Food Toxicology, Food Sanitation, and General Microbiology with more than 1500 citations. He is the editor of the book *Gıda Mikrobiyolojisi* (Food Microbiology) in Turkish language and is author of two books: *A Laboratory Manual in General Microbiology* and *Basic Methods for the Microbiological Analysis of Foods*.

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Born in 1950 at Ankara, Turkey, Professor Dr. T. Faruk Bozoglu received his BS degree in Chemistry (1973) and MS degree in Organic Chemistry (1977) from the Middle East Technical University (METU), Ankara, Turkey. He did his PhD in Food Microbiology from the Department of Food Science under the North Carolina State University, Raleigh, NC (1982). He joined the Department of Food Engineering at METU and is working as full-time Professor since 1992. He has carried out many collaborative researches with American and European Universities, especially on nonthermal processes. He has to his credit more than 60 SCI publications (BOZOGLU F* and BOZOGLU TF*) and more than 1100 citations. He is the advisor of 21 PhDs and more than 30 MS graduates. He has conducted two NATO ASI and participated in more than 70 international symposiums. He is also the chairman of METU Sport Club and Vice President of Turkish Dance Sports Federation.

Preface

This book deals with microorganisms affecting foods, foodborne diseases, and food safety, and it is intended as a reference source for academic institutions and food industry. A main characteristic of this book is that it is fundamental and comprehensive, not requiring any background knowledge of microbiology. Therefore, its usage is not bound to a particular time. It is hoped that the book will serve varied departments such as Food Engineering, Faculty of Health Science, Agricultural Engineering, Food Technology, and Nutrition and Dietetic Department, as well as anyone interested in different fields of food study. An enormous food industry exists, producing different food products ranging from milk, meat, eggs, and poultry to cereals. Therefore, many communities, including engineers, food producers, and people from other fields, deal with the relationships between microorganisms and food. Food safety and application of food standards greatly depend on the awareness of microorganisms in foods. Actually, this book aims to give food producers and other related people valuable information on this field and help them to gain new perspectives. Thus, it will be a valuable source informing the reader about the importance of microorganisms in food industry, protection of foods against microbial hazards, and solutions to problems such as foodborne diseases, food spoilage, and toxin formation. In addition, its readily comprehensible language and the concise explanation of concepts make this book all the more appropriate and useful for the people who have an interest in the field.

Due to the diverse relations between food materials and microorganisms, the authors have designed this volume primarily for students who lack in knowledge of microorganisms. Sections I and II concentrate on organism's habitats, their activities, and the factors that affect their growth and death. Section III focuses on foodborne diseases, the topic that is believed to be the most important as well as troublesome. Section IV presents the principles for the detection of unwanted microorganisms in food and their toxins. Finally, Section V covers food spoilage that occurs as a consequence of either microbial growth in food or the release of enzymes during their growth in the food environment. Numerous references have been recommended in this volume for those who are interested in having an in-depth knowledge of microbiology.

Osman Erkmen and T. Faruk Bozoglu
Gaziantep, 2016

SECTION I
Microbiology and Microbial Behavior in Foods

There are microbiological, chemical, and physical hazards in foods. Microorganisms are living microscopic sized organisms and include bacteria, viruses, yeasts and molds (named together as fungi), algae, and protozoa. They play important roles in other living organisms and in ecosystems. Microorganisms have both desirable and undesirable roles in foods. The use of microorganisms in foods and their isolation involve use of specific methods. Some of the simplest techniques in use today in food microbiology have been developed over the last 300 years. Food microbiologists must understand the basic principles of microbiology, have knowledge of food systems, and be able to solve the microbiological problems that occur in complex food ecosystems. Different types and numbers of microorganisms in raw and processed foods are important with respect to foodborne diseases, food spoilage, and food bioprocesses. Microorganisms metabolize some food components to provide needed energy and cellular materials. This section presents discovery of microorganisms, food microbiology subjects, and microbial growth characteristics in foods.

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CHAPTER 1
History and Development of Food Microbiology

1.1 Introduction

Microbiology is the branch of biological science that deals with microorganisms and agents (prions, viroid, etc.) that are invisible to the naked eye. It helps to understand the smallest of all biological life. With time, the importance of microorganisms in human and animal diseases, soil fertility, plant diseases, fermentation, food spoilages, and foodborne diseases was recognized, and microbiology was developed as a specific discipline. Later, microbiology was divided into several subdisciplines, such as medical microbiology, mycology, soil microbiology, plant pathology, and food microbiology. Except for a few sterile foods, all foods contain one or more types of microorganisms. Some of them have desirable roles in food, such as in the production of fermented food, whereas 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 indicate their morphological, physiological, biochemical, and genetic characteristics. Some of the simplest techniques in use today for these studies have been developed over the last 300 years.

The Earth is about 4.6 billion years old. The surface area of Earth was cooled, and oceans and atmosphere were formed about 3.8 billion years ago. The first living simplest cells from simple molecules evolved in the Earth's vest oceans between 3.8 and 3.5 billion years ago. This primitive life form on the Earth is known as the universal ancestor. The oldest known fossils from sedimentary rocks are prokaryotic cells, 3.5 billion years in age. They were found in Western Australia and South Africa. The nature of these fossils and the chemical composition of the rocks indicate that they have lithotrophic and fermentative modes of metabolism and they first evolved prokaryotic Archaea cells. Photosynthetic microorganisms known as cyanobacteria evolved about 3 billion years ago. Photosynthesis arose and oxygen was accumulated by the atmosphere. They were prokaryotic cells and lack from membrane-bound organelles (such as mitochondria, nucleus, and golgi apparatus). For approximately 2 billion years ago, prokaryotic cells were the only form of life on the Earth. The larger, more

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complicated eukaryotic cells (fungi) appeared much later, between 1.5 and 2.1 billion years ago. Sexual reproduction evolved about 1.2 billion years ago and this initiated a rapid increase in the evolution of organisms. Sexual reproduction from two parent organisms resulted in increasing of genetic variations and biological evolution.

1.2 History of Microorganisms in Foods

1.2.1 Early Development on Foods

During the last ice age, 10 000–20 000 BC, nomadic populations of humans used crops beside wild animals. The barley was flourished in Nile from around 18 000 BC. Around 8000 BC, as agriculture and animal husbandry, they were adopted by the early civilizations and food supply, especially agricultural products became available during the growing seasons. Preservation of foods became important for uniform supply of food around the year. The first animals to be domesticated were goats and sheep in Near East in about 9000 BC. The first evidence of beer manufacture has been traced to ancient Babylonian in 7000 BC. The first fermented milk has been used in diet between 6100 and 5800 BC in Anatolia after the cow was domesticated. Wines have been prepared by Assyrians in 3500 BC. Milk, butter, and cheese were used by the Egyptians as early as 3000 BC. Fermented sausages were prepared by the ancient Babylonians and Chine as far back as 1500 BC. By 3000 BC, the people of summer (now Iraq) had developed an agricultural economy and livestock breeding. They constructed irrigation canals. They could move their livestock during their migration and slaughtered when needed. Between 8000 and 1000 BC, many food preservation methods, such as drying, cooking, baking, smoking, salting, sugaring (with honey), low-temperature storage (in ice), storage without air (in pits), fermentation (with fruits, grains, and milk), pickling, and spicing, were used, probably mainly to reduce spoilage.

1.2.2 Discovery of Microorganisms

From the time of Renaissance period until the late nineteenth century, it was generally accepted that some life forms arose spontaneously from nonliving matter. Such "spontaneous generation" appeared to occur primarily in decaying matter. The spontaneous generation theory argued that animalcules (an older term for a microscopic life) could not generate by themselves (biogenesis), but they were present in different matters only through abiogenesis (spontaneous generation). Some scientific minds were curious to determine where do animalcules come from, they observed them in many different matters that were emanating. The earliest attempt in spontaneous generation from air and matter was proved by Francesco Redi. In 1668, he placed meat in several dishes, half of these were covered with gauze and an empty dish was served as controls. After several days, the uncovered meat dishes were covered with maggots, but neither

the covered meat, nor the empty dishes had similar infestations. Thus, the spontaneous generation of maggots in spoiled meat resulted from the presence of flies in air (nonliving matter). John Turberville Needham (1745) boiled broth and then tightly sealed to exclude exterior air. When the containers were opened, they were found to be full of animalcule. After repeating the experiment with several other broths, Needham concluded that spontaneous generation actually did occur from nonliving matter.

In 1768, Lazzaro Spallanzani repeated the experiments of Needham and Redi, but removed air from the flask by vacuum. Days later, the unsealed bottle seemed with small living things. The sealed bottle showed no signs of life. He proved that spontaneous generation could not occur without air and the air was a source of contaminants but nonliving matter was not generating life. Thereby, he disproved Needham's theory. Anthonie van Leeuwenhoak (1676–1683) observed different types of animalcules under microscope up to 300x magnification. He observed them in saliva, rainwater, vinegar, and other materials. He sketched three morphological groups (cocci, bacilli, and spiral) and also described some to be motile. Francois Nicholoas Appert, in 1804, developed methods to preserve foods in sealed glass bottles (canning) by heat in boiling water. He credited to Spallanzani's research. Schulze (1830), Theodor Schwann (1838), and Schroeder (1854) passed air through a filter and they showed that bacteria failed to appear in boiled meat infusion even in the presence of air. They also credited to Spallanzani's research. In 1859, Louis Pasteur placed nutrient solutions in flasks that had necks bent into S-shaped curves. He then boiled the solution for a few minutes and allowed them to cool. Growth was not taking place in the contents of the flasks because dust and living things had been trapped on the walls of the curved necks. To prove his assumptions were correct, he simply broke the necks of the flask and then solutions became cloudy with the growth of organisms. He demonstrated that bacteria could grow only in the infusion that was contaminated from dust particles in air. He proved that bacteria were able to reproduce (biogenesis), the contamination come from life forms in the air and life could not originate by spontaneous generation (abiogenesis). John Tyndall, in 1870, also showed that boiled infusion could be stored in dust-free air in a box without microbial growth.

1.2.3 Development of Food Microbiology

In 1664, Robert Hook described the structure of molds. Theodor Schwann (in 1837) proved that yeast cells were responsible for the conversion of sugars to alcohol, a process they called alcoholic fermentation. In 1838, Ehrenberg introduced the term bacteria and has reported at least 16 bacterial species in four genera. In 1875, Ferdinand Cohn developed the preliminary classification system of bacteria. He also discovered that some bacteria produced spores. Louis Pasteur studied on milk souring (1857), causes of diseases (1862), and defects in wine (1866). He showed how to keep solutions sterile. Pasteur's discoveries led to the

development of aseptic techniques to prevent contamination of microorganisms. He found that yeast ferments sugars to alcohol and bacteria can oxidize the alcohol to acetic acid. He demonstrated that all fermentations were due to the activities of specific yeasts and bacteria (1857). He reported that some fermentative microorganisms were anaerobic and could live only in the absence of oxygen, whereas others were able to grow either aerobically or anaerobically. In 1870, Pasteur placed heat preservation methods of foods on a scientific basis. He heated the wine (at 60 °C for 30 min) to destroy undesirable microorganisms, known as "pasteurization." He developed an anthrax vaccine by using heat-treated (inactivated) bacterial cells. He later used vaccination to fowl cholera and anthrax, both diseases caused by bacteria. He also made many discoveries including food spoilage, food preservation, diseases, and immunity. Microbiology and food microbiology become sciences by the studies of Pasteur.

John Tyndall (1877) realized that some bacteria had the ability to form resistant structures known as spores. Through a series of boiling and cooling steps, he inactivated these structures. He first allowed spores to germinate (by incubation) and then killed the new cells that arose from spores. He repeated this experiment on three successive days. He produced sterile broths. This technique was given the name "tyndallization" in his honor.

Robert Koch (1890) isolated bacteria in pure cultures from diseased cattle with anthrax. He developed techniques of agar plating methods to isolate bacteria in pure cultures and staining methods for better microscopic observation of bacteria. He introduced germ theories (Koch's postulates) from his research including for criteria to identify the causative agent of disease.

1 The pathogen must be present in all diseased animals.
2 The pathogen can be isolated from diseased animal and grown in pure culture.
3 The pathogen from the pure culture must cause the disease when it is injected into a healthy animal.
4 The pathogen must be reisolated from the new diseased animal and shown to be the same symptoms as the originally inoculated pathogen.

Sergei N. Winogradsky (1907) and Martinus W. Beijerinck prepared the enrichment culture technique. Paul Ehrlich (1915) found that some chemical agents have the ability to inhibit or kill microorganisms without damaging the animals. Alexander Fleming (1928) recognized that some microorganisms exhibit antibiosis; they are able to produce natural compounds that inhibit the growth of competitors. He showed that the bacterium (*Staphylococcus aureus*) was inhibited by the mold (*Penicillium notatum*). Later, Howard Florey and Ernst Chain (1940) cultivated *Penicillium* and purified the first widely available antibiotic, penicillin G.

1.2.4 Modern Microbiology

The use of lenses and lens systems to increase the apparent size of an object is the most important fact in the development of microbiology as a true science. The Italian astronomer Galilei (1564–1642) was the first scientist to use a lens to

magnify the image of a small object. The first microscope was constructed by a Dutch scientist Anthonie van Leeuwenhoek (1676) to examine different matters using microscope. He drew three bacterial shapes (rods, cocci, and spirals). These shapes are very good approximations of actual forms known today.

In 1838, Matthias Schleiden proposed that all plants are composed of cells. One year later, Theodor Schwann (1837) would extend this concept to animals and vegetables. He also proposed that tissues originate from cells. Rudolf Virchow (1843) indicated the idea of self-replication. This leads Virchow to purpose "every cell from a cell." In time, the combined works of Schleiden, Schwann, and Virchow purposed the cell theory that says (1) all living things are composed of cells and (2) all cells arise from other cells. This theory is universally accepted today.

Since the 1940s, knowledge of microbiology has expanded with increasing advances in microscopy, biochemistry, and genetic research. In 1953, James D. Watson and Francis H.C. Crick defined the structure of the DNA molecule. In 1956, F. Jacob and E.L. Wollman discovered the circular structure of the bacterial chromosome. Two years later, M. Meselson and F. W. Stahl described the DNA replication. In 1970s, discoveries in microbiology led to the development of recombinant DNA technology and genetic engineering. In 1980s, phylogenetic "tree of life" (three domain system; Bacteria, Archae, and Eukaryote) was proposed from similarities and dissimilarities of nucleotides sequenced rRNA.

1.3 Fields of Food Microbiology

1.3.1 Importance of Microorganisms in Foods

In the early twentieth century, studies continued to understand the association and importance of microorganisms in foods. Sanitation was used in the food handling to reduce contamination by microorganisms. Specific methods were studied to prevent microbial growth as well as to destroy the spoilage and pathogenic microorganisms. Specific methods were developed for the isolation and identification of microorganisms. Beneficial bacteria used in food fermentation, especially dairy fermentation, were isolated and characterized. However, after the 1950s, food microbiology entered a new era. Basic information on the physiological, biochemical, and biological characteristics of microorganisms in foods (such as microbial interactions in food environments and microbial physiology, biochemistry, genetics, and immunology) has helped open new frontiers in food microbiology. Among these are food fermentation/probiotics, food spoilage, foodborne diseases, and food safety.

1.3.1.1 Foodborne Diseases

Many pathogenic microorganisms can contaminate foods during various stages of their handling, production, storage, serving, and consumption. Foodborne illness

may result from consumption of water and foods in raw or cooked when they contain the pathogenic microorganisms or their toxins in sufficient quantity. Foodborne diseases cannot only be fatal, but they can also cause large economic losses. Foods of animal origin associate more with foodborne diseases than foods of plant origin. Mass production of foods, new processing technologies, storage of foods, changes in food consumption patterns, and the increase in imports of food from other countries have been increased the chances of higher number of outbreaks as well as the introduction of new pathogens. On the other hand, effective methods are developed to ensure the safety of consumers against foodborne diseases.

Foodborne diseases are attributed primarily to pathogenic bacteria, toxigenic molds, and enteric viruses and protozoa. Some of bacteria responsible for foodborne diseases are *Aeromonas hydrophila*, pathogenic *Escherichia coli*, *Listeria monocytogenes*, *Bacillus cereus*, *Campylobacter jejuni*, *Clostridium botulinum*, *Clostridium perfringens*, *S. aureus*, *Yersinia enterocolitica*, *Salmonella*, *Shigella*, and *Vibrio*. Some of toxigenic mold species present in the genera are *Penicillium*, *Aspergillus*, and *Byssochlamys*. Some of the viruses of concern in foods are hepatitis A virus, Norwalk virus, Norwalk-like virus, and rotavirus. *Cryptosporidium parvum*, *Cyclospora cayetanensis*, *Giardia lamblia*, and *Toxoplasma gondii* are some pathogenic parasites. Beside microorganisms, chemicals and natural toxins in foods can also cause foodborne diseases.

1.3.1.2 Food Spoilage

Spoilage is the unfitness of food for human consumption. Food may be spoiled by chemical and biological agents. Biological spoilage can result from the action of inherent enzymes, growth of microorganisms, invasion of insects, contamination with parasites, and presence of worms and the like. About one-fourth of the world's food supply is lost through action of microorganisms alone. Chemical spoilage results from purely chemical reactions, such as browning and oxidation reactions. The chance of food spoilage and association of new types of microorganisms have greatly increased due to new marketing trends, new processing techniques, extending shelf-life, and changes of temperature between production and consumption of foods. Many food materials are processed to destroy enzymes and microorganisms, thus prolong the keeping quality of foods for hours, days, months, or even years.

1.3.1.3 Food Bioprocessing

Microorganisms can play some positive role in food. They can be consumed in themselves as the edible fungi and algae. Many microorganisms are used to produce different kinds of fermented foods using raw materials from animal and plant sources. The main desirable microorganisms used in the production of fermented foods are lactic acid bacteria (LAB). LAB produce new product in milk, brined vegetables, many cereal products, and meats with added carbohydrate.

Examples to such fermented foods are cheeses, yogurt, wine, beer, pickles, sauerkraut, and sausages. In addition to being more shelf stable, all fermented foods have aroma and flavor characteristics. In some instance, the vitamin content of the fermented food is increased along with increasing digestibility of the raw foods. Consumption of these foods has increased greatly over the last 10–15 years and is expected to increase still more in the future. Genetic recombination techniques are being used to obtain better fermentative microorganisms for new products and to improve quality of foods.

1.3.1.4 Food Biopreservation

Biopreservation refers to extending storage life and enhancing safety of foods using natural microflora, starter culture, and antimicrobials. In fermented foods, beneficial microorganisms can reduce pH and produce antimicrobial agents, such as H_2O_2, organic acids, and bacteriocins. These produce are shelf-stable foods. Many food ingredients including enzymes, pigments, aromatic and flavoring compounds, and so on, may be produced by natural or engineered microorganisms. Antimicrobial metabolites of microorganisms are being used in foods to control undesirable microorganisms. LAB have a major potential for use in biopreservation because they are safe to consume and produce desirable products.

1.3.1.5 Probiotic

Probiotic means "for life" and is the live microbial cell preparation with survival in the colon. Microorganisms contributing the health and balance of the intestinal tract are referred to as the "friendly", "beneficial", or "good" microorganisms. When they are ingested, they maintain a healthy of intestinal tract, and help fight illness and disease. Many beneficial bacteria survive in the gastrointestinal tract of humans. Probiotic microorganisms are usually of the genus *Lactobacillus* and *Bifidobacterium*.

1.3.1.6 Food Safety

Total quality management can be applied from farm to fork to control microorganisms, to prevent microbial growth, and to protect foods against contamination of spoilage and pathogenic microorganisms. Food safety can be provided by application of hazard analysis and critical control points (HACCP) in food production, processing, and preservation. Microbiological characteristics of foods, such as unprocessed and low-heat-processed ready-eat foods, can be indicated for product safety. Food safety legislation provides production of foods according to the standards. It is impossible to conduct microbiological studies for each food product to ensure safety and stability of food products. Mathematical models can be used to determine the influence of combinations of several parameters on microorganisms. Although they may not be accurate, they can provide first-hand information very rapidly, and be helpful to eliminate many of hazards. Information from mathematical models can then be used to conduct a traditional study

that is feasible both experimentally and economically. They can be used to predict growth and inactivation of pathogenic and spoilage microoganisms in food products by studying microbial growth rate at different pH, a_w, temperature, preservatives, and the other factors.

1.3.1.7 Microbial Physiology and Food Preservation

Microbial physiology is cell structure, growth factors, metabolism, and genetic composition of microorganisms. Physiological characteristics of microorganisms are studied through analysis of the cellular response to different environmental conditions. Microbial physiology performs a qualitative and/or quantitative characterization of certain microbial species, such as growth on different carbon, nitrogen, and energy sources. Clearly, microbial physiology is an important research field on microbial species and in all applied aspects of microbiology, such as food microbiology, industrial microbiology, environmental microbiology, and medical microbiology.

All food preservation techniques exert their effect by manipulating one or more intrinsic and extrinsic factors with slowing or stopping microbial growth and inactivating (killing) microorganisms. Where microbial growth is slowed, shelf life of food is extended and different microorganisms may predominate with changing the character of the spoilage. Similarly, where microorganisms are inactivated or killed, the shelf life will depend on types of microorganisms surviving in the inactivation treatment whether the product is subjected to any posttreatment contamination. Though, modification of one intrinsic or extrinsic factor can often achieve an acceptable degree of preservation, this often means that the product's qualities are changed in a dramatic way. For example, to preserve a food by acidification, it may be necessary to produce a very acidic product of possibly limited acceptability. More frequently though a number of factors are adjusted less severely to achieve the overall antimicrobial effect in what is known as the hurdle concept or multiple-barrier concept of food preservation. Each factor modifies the food's sensory and other properties. For example, the hurdles of low pH, ethanol content, dissolved CO_2, and hop resins combine to restrict the range of microorganisms that can grow in spoil beer.

1.3.1.8 Microbiological Analysis of Foods

In food, microorganisms are present as mixed population. Studying the behavior of microorganisms in foods involves their isolation and enumeration. In the case of enumerating microorganisms, a food sample is generally diluted in a relatively inert liquid diluent that will not subject the microorganisms to osmotic and pH stress, and the dilutions are inoculated on to an appropriate solid or liquid medium and incubated. Several dilutions are usually inoculated in this way so that a detectable result or countable number of colonies is obtained. A reasonable count and the dilution can be related to the microbial number in the analyzed food. Identification of microorganisms can also involves isolating individual colonies