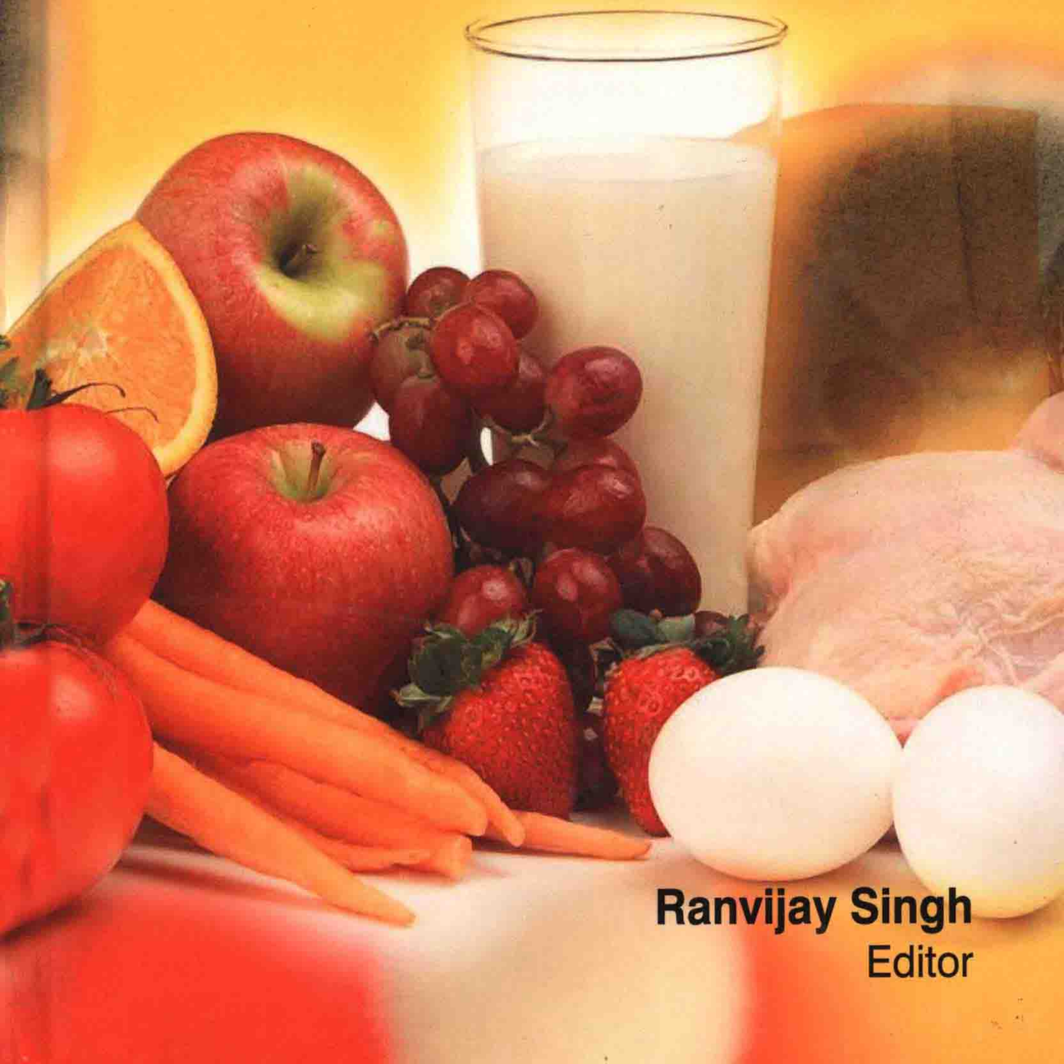
Basics of Food Microbiology



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Editor

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Food Microbiology

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Preface

Microbes are single-cell organisms so tiny that millions can fit into the eye of a needle. They are the oldest form of life on earth. Microbe fossils date back more than 3.5 billion years to a time when the Earth was covered with oceans that regularly reached the boiling point, hundreds of millions of years before dinosaurs roamed the earth. The field of food microbiology is a very broad one, encompassing the study of microorganisms which have both beneficial and deleterious effects on the quality and safety of raw and processed foods. Food science is a discipline concerned with all aspects of food-beginning after harvesting, and ending with consumption by the consumer. It is considered one of the agricultural sciences, and it is a field which is entirely distinct from the field of nutrition. In the U.S., food science is typically studied at land-grant universities. Examples of the activities of food scientists include the development of new food products, design of processes to produce these foods, choice of packaging materials, shelf-life studies, sensory evaluation of the product with potential consumers, microbiological and chemical testing. Food scientists in universities may study more fundamental phenomena that are directly linked to the production of a particular food product.

Food scientists are generally not directly involved with the creation of genetically modified foods. Some of the subdisciplines of food science: Food safety, Food engineering, Product development, Sensory analysis, Food chemistry. The primary tool of microbiologists is the ability to identify and quantitate food-borne microorganisms; however, the inherent inaccuracies in enumeration processess, and the natural variation found in all bacterial populations complicate the microbiologists job. Without microbes, we couldn't eat or breathe. Without us, they'd probably be just fine. Understanding microbes is vital to understanding the past and the future of ourselves and our planet. Archaea look and act a lot like bacteria. So much so that until the late 1970s, scientists assumed they were a kind of "weird" bacteria.

Then microbiologist Carl Woese devised an ingenious method of comparing genetic information showing that they could not rightly be called bacteria at all. Their genetic recipe is too different. So different

Woese decided they deserved their own special branch on the great family tree of life, a branch he dubbed the Archaea. Archaea comes from the Greek word meaning "ancient." An appropriate name, because many archaea thrive in conditions mimicking those found more than 3.5 billion years ago. Back then, the earth was still covered by oceans that regularly reached the boiling point—an extreme condition not unlike the hydrothermal vents and sulfuric waters where archaea are found today. Some scientists consider archaea living fossils that may provide hints about what the earliest life forms on Earth were like, and how life evolved on our planet. In addition to superheated waters, archaea have been found in acid-laden streams around old mines, in frigid Antarctic ice and in the super-salty waters of the Dead Sea. A number of other extreme-living bacterial species also enjoy these conditions, too, such as the community of cyanobacteria and bacteria shown top right.

The book has been written keeping in mind for the graduate and post graduate level bio-sciences and interdisciplinary courses.

—Ranvijay Singh

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Chapter 1

Food Microbiology

Introduction

Food microbiology is the study of the microorganisms that inhabit, create, or contaminate food. Of major importance is the study of microorganisms causing food spoilage. "Good" bacteria, however, such as probiotics, are becoming increasingly important in food science.

In addition, microorganisms are essential for the production of foods such as cheese, yogurt, other fermented foods, bread, beer and wine.

Food Safety

Food safety is a major focus of food microbiology. Pathogenic bacteria, viruses and toxins produced by microorganisms are all possible contaminants of food. However, microorganisms and their products can also be used to combat these pathogenic microbes. Probiotic bacteria, including those that produce bacteriocins, can kill and inhibit pathogens. Alternatively, purified bacteriocins such as nisin can be added directly to food products. Finally, bacteriophages, viruses that only infect bacteria, can be used to kill bacterial pathogens.

Thorough preparation of food, including proper cooking, eliminates most bacteria and viruses. However, toxins produced by contaminants may not be heat-labile, and some are not eliminated by cooking. Source of Contamination should be identified and have eradicate the problem and much more precautions should be taken to eliminate the contamination in foods.

Fermentation

Fermentation is one way microorganisms can change a food. Yeast, especially Saccharomyces cerevisiae, is used to leaven bread, brew beer and make wine. Certain bacteria, including lactic acid bacteria, are used to make yogurt, cheese, hot sauce, pickles, fermented sausages and dishes such as kimchi. A common effect of these fermentations is that the food product is less hospitable to other microorganisms, including pathogens and spoilage-causing microorganisms, thus extending the food's shelf-life.

Food fermentations are ancient technologies that harness microorganisms and their enzymes to improve the human diet. Fermented foods keep better, have enhanced flavours, textures and aromas, and may also possess certain health benefits, including superior digestibility. For vegetarians, fermented foods serve as palatable, protein-rich meat substitutes. Some cheese varieties also require molds to ripen and develop their characteristic flavours. Asian cuisines rely on a large repertoire of fermented foods. In particular, Aspergillus oryzae and A. sojae, sometimes called koji molds, are employed in many ways. Their hydrolytic enzymes suit them for growth on starch and other carbohydrate-rich substrates. In the koji process, fungal enzymes perform the same function as the malting enzymes used in the beer fermentations of western cultures.

The koji molds release amylases that break down rice starch, which in turn can be fermented to make rice wine. Fermented rice beverages have numerous local variations and names, depending on country and region. Rice wine is called shaoshing in parts of China, sake in Japan, takj or yakju in Korea, as well as by many other names across Asia. The koji molds are also effective in a variety of legume fermentations, of which miso and soy sauce are best known.

Miso is a mixture of soybeans and cereals usually used to flavour soups. Soy sauce is a flavourful, salty liquid sauce made from soybeans that have been fermented by koji molds, yeasts, as well as several halophilic bacteria. Other names for soy sauce include jiangyou (China), makjang and kanjang (Korea), toyo (Philippines) and siiu (Thailand).

Probiotics

Probiotics are living organisms that, when consumed, have beneficial health benefits outside their inherent nutritional effects. There is a growing body of evidence for the role of probiotics in gastrointestinal infections, irritable bowel syndrome and inflammatory bowel disease. Lactobacillus species are used for the production of yogurt, cheese, sauerkraut, pickles, beer, wine, cider, kimchi, chocolate and other fermented foods, as well as animal feeds such as silage. In recent years, much interest has been shown in the use of lactobacilli as probiotic organisms and their potential for disease prevention in humans and animals.Bifidobacteria are considered as important probiotics, and are used in the food industry to relieve and treat many intestinal disorders.

Bifidobacteria exert a range of beneficial health effects, including the regulation of intestinal microbial homeostasis, the inhibition of pathogens and harmful bacteria that colonize and/or infect the gut mucosa, the modulation of local and systemic immune responses, the repression of procarcinogenic enzymatic activities within the microbiota, the production of vitamins, and the bioconversion of a number of dietary compounds into bioactive molecules.

Microbial Biopolymers

A variety of biopolymers, such as polysaccharides, polyesters and polyamides, are naturally produced by microorganisms. Several microbially-produced polymers are used in the food industry.

*n*Plant-pathogenic bacteria of the genus Xanthomonas are able to produce the acidic exopolysaccharide xanthan gum. Because of its physical properties, it is widely used as a viscosifer, thickener, emulsifier or stabilizer in the food industry. Xanthan consists of pentasaccharide repeat units composed of D-glucosyl, D-mannosyl, and D-glucuronyl acid residues in a molar ratio of 2:2:1 and variable proportions of O-acetyl and pyruvyl residues.

Alginate

Alginate is the main representative of a family of polysaccharides that neither show branching nor repeating blocks or unit patterns and this property distinguishes it from to other polymers like xanthan or dextran. Alginates can be used as thickening agents. Although listed here under the category 'Microbial polysaccharides', commercial alginates are currently only produced by extraction from brown seaweeds such as Laminaria hyperborea or L. japonica.

Cellulose

Cellulose is a simple polysaccharide, in that it consists only of one type of sugar (glucose), and the units are linearly arranged and linked together by ?-1,4 linkages only. The mechanism of biosynthesis is, however, rather complex, partly because in native celluloses, the chains are organized as highly ordered water-insoluble fibres. Currently, the key genes involved in cellulose biosynthesis and regulation are known in a number of bacteria, but many details of the biochemistry of its biosynthesis are still not clear. In spite of the enormous abundance of cellulose in plants, bacterial celluloses are being investigated for industrial exploitations.

Poly-g-glutamic Acid

Poly-γ-glutamic acid (ã-PGA) produced by various strains of Bacillus has potential applications as a thickener in the food industry.

Levan

Levan, a homopolysaccharide composed of D-fructofuranosyl residues joined by 2,6 with multiple branches by 2,1 linkages, has great potential as a functional biopolymer in foods, feeds, cosmetics, and the pharmaceutical and chemical industries. Levan can be used as food or a feed additive with prebiotic and hypocholesterolemic effects.

Exopolysaccharides

Microorganisms synthesize a wide spectrum of multifunctional polysaccharides, including intracellular polysaccharides, structural polysaccharides and extracellular polysaccharides or exopolysaccharides (EPSs). EPSs generally consist of monosaccharides and some noncarbohydrate substituents (such as acetate, pyruvate, succinate, and phosphate). Owing to the wide diversity in composition, they have found multifarious applications in various food and pharmaceutical industries.

Foodborne Pathogens

Foodborne pathogens are the leading causes of illness and death in less developed countries, killing approximately 1.8 million people annually. In developed countries, foodborne pathogens are responsible for millions of cases of infectious gastrointestinal diseases each year, costing billions of dollars in medical care and lost productivity. New foodborne pathogens and foodborne diseases are likely to emerge, driven by factors such as pathogen evolution, changes in agricultural and food manufacturing practices, and changes to the human host status. There are growing concerns that terrorists could use pathogens to contaminate food and water supplies in attempts to incapacitate thousands of people and disrupt economic growth.

Enteric Viruses

Food and waterborne viruses contribute to a substantial number of illnesses throughout the world. Among those most commonly known are hepatitis A virus, rotavirus, astrovirus, enteric adenovirus, hepatitis E virus, and the human caliciviruses consisting of the noroviruses and the Sapporo viruses. This diverse group is transmitted by the fecal-oral route, often by ingestion of contaminated food and water.

Protozoan Parasites

Protozoan parasites associated with food and water can cause illness in humans. Although parasites are more commonly found in developing countries, developed countries have also experienced several foodborne outbreaks. Contaminants may be inadvertently introduced to the foods by inadequate handling practices, either on the farm or during processing of foods. Protozoan parasites can be found worldwide, either infecting wild animals or in water and contaminating crops grown for human consumption. The disease can be much more severe and prolonged in immunocompromissed individuals.

Mycotoxins

Molds produce mycotoxins, which are secondary metabolites that can cause acute or chronic diseases in humans when ingested from contaminated foods. Potential diseases include cancers and tumors in different organs (heart, liver, kidney, nerves), gastrointestinal disturbances, alteration of the immune system, and reproductive problems.

Species of Aspergillus, Fusarium, Penicillium, and Claviceps grow in agricultural commodities or foods and produce the mycotoxins such as aflatoxins, deoxynivalenol, ochratoxin A, fumonisins, ergot alkaloids, T-2 toxin, and zearalenone and other minor mycotoxins such as cyclopiazonic acid and patulin. Mycotoxins occur mainly in cereal grains (barley, maize, rye, wheat), coffee, dairy products, fruits, nuts and spices.

Control of mycotoxins in foods has focused on minimizing mycotoxin production in the field, during storage or destruction once produced. Monitoring foods for mycotoxins is important to manage strategies such as regulations and guidelines, which are used by 77 countries, and for developing exposure assessments essential for accurate risk characterization. Aflatoxins are still recognized as the most important mycotoxins.

They are synthesized by only a few Aspergillus species, of which A. flavus and A. parasiticus are the most problematic. The expression of aflatoxin-related diseases is influenced by factors such as age, nutrition, sex, species and the possibility of concurrent exposure to other toxins. The main target organ in mammals is the liver, so aflatoxicosis is primarily a hepatic disease. Conditions increasing the likelihood of aflatoxicosis in humans include limited availability of food, environmental conditions that favour mold growth on foodstuffs, and lack of regulatory systems for aflatoxin monitoring and control.

Yersinia Enterocolitica

Yersinia enterocolitica includes pathogens and environmental strains that are ubiquitous in terrestrial and fresh water ecosystems. Evidence from large outbreaks of yersiniosis and from epidemiological studies of sporadic cases has shown that Y. enterocolitica is a foodborne pathogen. Pork is often implicated as the source of infection. The pig is the only animal consumed by man that regularly harbors pathogenic Y. enterocolitica.

An important property of the bacterium is its ability to multiply at temperatures near 0°C, and therefore in many chilled foods. The pathogenic serovars (mainly O:3, O:5, 27, O:8 and O:9) show different geographical distribution. However, the appearance of strains of serovars O:3 and O:9 in Europe, Japan in the 1970s, and in North America by the end of the 1980s, is an example of a global pandemic. There is a possible risk of reactive arthritis following infection with Y. enterocolitica.

Vibrio

Vibrio species are prevalent in estuarine and marine environments, and seven species can cause foodborne infections associated with seafood. Vibrio cholerae O1 and O139 serovtypes produce cholera toxin and are agents of cholera. However, fecal-oral route infections in the terrestrial environment are responsible for epidemic cholera. V. cholerae non-O1/O139 strains may cause gastroenteritis through production of known toxins or unknown mechanism.

Vibrio parahaemolytitcus strains capable of producing thermostable direct hemolysin (TDH) and/or TDH-related hemolysin are most important causes of gastroenteritis associated with seafood consumption. Vibrio vulnificus is responsible for seafoodborne primary septicemia, and its infectivity depends primarily on the risk factors of the host.

V. vulnificus infection has the highest case fatality rate (50%) of any foodborne pathogen. Four other species (V. mimicus, V. hollisae, V. fluvialis, and V. furnissii) can cause gastroenteritis. Some strains of these species produce known toxins, but the pathogenic mechanism is largely not understood. The ecology of and detection and control methods for all seafoodborne Vibrio pathogens are essentially similar.

Staphylococcus Aureus

Staphylococcus aureus is a common cause of bacterial foodborne disease worldwide. Symptoms include vomiting and diarrhea that occur shortly after ingestion of S. aureus toxin-contaminated food. The symptoms arise from ingestion of preformed enterotoxin, which accounts for the short incubation time. Staphylococcal enterotoxins are superantigens and, as such, have adverse effects on the immune system.

The enterotoxin genes are accessory genetic elements in S. aureus, meaning not all strains of this organism are enterotoxin-producing. The enterotoxin genes are found on prophages, plasmids, and pathogenicity islands in different strains of S. aureus. Expression of the enterotoxin genes is often under the control of global virulence gene regulatory systems.

Campylobacter

Campylobacter spp., primarily C. jejuni subsp. jejuni is one of the major causes of bacterial gastroenteritis in the U.S. and worldwide. Campylobacter infection is primarily a foodborne illness, usually without complications; however, serious sequelae, such as Guillain-Barre Syndrome, occur in a small subset of infected patients. Detection of C. jejuni in clinical samples is readily accomplished by culture and nonculture methods.

Listeria Monocytogenes

Listeria monocytogenes is Gram-positive foodborne bacterial pathogen and the causative agent of human listeriosis. Listeria infections are acquired primarily through the consumption of contaminated foods, including soft cheese, raw milk, deli salads, and ready-to-eat foods such as luncheon meats and frankfurters.

Although L. monocytogenes infection is usually limited to individuals that are immunocompromised, the high mortality rate associated with human listeriosis makes it the leading cause of death among foodborne bacterial pathogens. As a result, tremendous effort has been made to develop methods for the isolation, detection and control of L. monocytogenes in foods.

Salmonella

Salmonella serotypes continue to be a prominent threat to food safety worldwide. Infections are commonly acquired by animal to human transmission though consumption of undercooked food products derived from livestock or domestic fowl. The second half of the 20th century saw the emergence of Salmonella serotypes that became associated with new food sources (*i.e.* chicken eggs) and the emergence of Salmonella serotypes with resistance against multiple antibiotics.

Shigella

Shigella species are members of the family Enterobacteriaceae and are Gram negative, nonmotile rods. Four subgroups exist based on O-antigen structure and biochemical properties: S. dysenteriae (subgroup A), S. flexneri (subgroup B), S. boydii (subgroup C) and S. sonnei (subgroup D). Symptoms include mild to severe diarrhea with or without blood, fever, tenesmus and abdominal pain. Further complications of the disease may be seizures, toxic megacolon, reactive arthritis and hemolytic uremic syndrome. Transmission of the pathogen is by the fecal-oral route, commonly through food and water.

The infectious dose ranges from 10-100 organisms. Shigella spp. have a sophisticated pathogenic mechanism to invade colonic epithelial cells of the host, man and higher primates, and the ability to multiply intracellularly and spread from cell to adjacent cell via actin polymerization. Shigella spp. are one of the leading causes of bacterial foodborne illnesses and can spread quickly within a population.

Escherichia Coli

More information is available concerning Escherichia coli than any other organism, thus making E. coli the most thoroughly studied species in the microbial world. For many years, E. coli was considered a commensal of human and animal intestinal tracts with low virulence potential. It is now known that many strains of E. coli act as pathogens, inducing serious gastrointestinal diseases and even death in humans.

There are six major categories of E. coli strains that cause enteric diseases in humans, including the:

- Enterohemorrhagic E. coli, which cause hemorrhagic colitis and hemolytic uremic syndrome,
- Enterotoxigenic E. coli, which induce traveller's diarrhea,
- Enteropathogenic E. coli, which cause a persistent diarrhea in children living in developing countries,
- Enteroaggregative E. coli, which provokes diarrhea in children,
- Enteroinvasive E. coli that are biochemically and genetically related to Shigella species and can induce diarrhea,
- Diffusely adherent E. coli, which cause diarrhea and are distinguished by a characteristic type of adherence to mammalian cells.

Clostridium Botulinum and Clostridium Perfringens

Clostridium botulinum produces extremely potent neurotoxins that result in the severe neuroparalytic disease, botulism. The enterotoxin produced by C. perfringens during sporulation of vegetative cells in the host intestine results in debilitating acute diarrhea and abdominal pain. Sales of refrigerated, processed foods of extended durability including sous-vide foods, chilled ready-to-eat meals, and cook-chill foods have increased over recent years. Anaerobic spore-formers have been identified as the primary microbiological concerns in these foods. Heightened awareness over intentional food source tampering with botulinum neurotoxin has arisen with respect to genes encoding the toxins that are capable of transfer to nontoxigenic clostridia.

Bacillus Cereus

The Bacillus cereus group comprises six members: B. anthracis, B. cereus, B. mycoides, B. pseudomycoides, B. thuringiensis and B. weihenstephanensis. These species are closely related and should be placed within one species, except for B. anthracis that possesses specific large virulence plasmids. B. cereus is a normal soil inhabitant, and is frequently isolated from a variety of foods, including vegetables, dairy products and meat. It causes a vomiting or diarrhea illness that is becoming increasingly important in the industrialized world. Some patients may experience both types of illness simultaneously. The diarrheal type of illness is most prevalent in the western hemisphere, whereas the emetic type is most prevalent in Japan. Desserts, meat dishes, and dairy products are the foods most frequently associated with diarrheal illness, whereas rice and pasta are the most common vehicles of emetic illness.

The emetic toxin (cereulide) has been isolated and characterized; it is a small ring peptide synthesised nonribosomally by a peptide synthetase. Three types of B. cereus enterotoxins involved in foodborne outbreaks have been identified. Two of these enterotoxins are three-component proteins and are related, while the last is a one-component protein (CytK). Deaths have been recorded both by strains that produce the emetic toxin and by a strain producing only CytK.

Some strains of the B. cereus group are able to grow at refrigeration temperatures. These variants raise concern about the safety of cooked, refrigerated foods with an extended shelf life. B. cereus spores adhere to many surfaces and survive normal washing and disinfection (except for hypochlorite and UVC) procedures. B. cereus food borne illness is likely under-reported because of its relatively mild symptoms, which are of short duration.