HANDBOOK OF MICROBIOLOGICAL MEDIA FOR THE EXAMINATION OF FOOD

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Introduction to Food Microbiology

Food products serve not only as sources of nutrition for humans and other animals but also as substrates for the growth of microorganisms. The uncontrolled growth of microorganisms in food causes spoilage, a serious problem accounting for sizable losses of food products. Some disease-causing microorganisms also contaminate foods and potable waters. Foodborne and waterborne pathogens remain a major source for the spread of disease worldwide. A major concern in food microbiology is the preservation of food products and the prevention of foodborne and waterborne spread of disease. Careful quality control in the food industry is essential for preventing outbreaks of foodborne disease and for controlling food spoilage. Many foods are routinely examined for the presence of disease-causing and food spoilage microorganisms. Many of these quality control procedures involve the cultivation of microorganisms using a variety of media.

Food- and Waterborne Pathogens

The ingestion of foods containing toxins and human pathogens can cause a variety of diseases. It is essential to prevent contamination of food products with human pathogens and to control the potential proliferation of toxin-producing microorganisms, which can result in food poisoning and the transmission of foodborne pathogens. In some cases, the growth of pathogenic microorganisms in a food is accompanied by obvious signs of spoilage, such as the production of gas or foul-smelling compounds, which give a clear indication that the product should not be eaten. In other cases, there are no obvious signs of spoilage to indicate the presence of pathogens or toxins. It is therefore necessary to carry out inspection programs aimed at detecting the possible contamination of food products with pathogenic microorganisms. As a consequence of the high quality control procedures employed in the food industry, few occurrences of foodborne disease are traced back to the food processor. Rather, most outbreaks of food poisoning are caused by improper post process handling of the food, often as a result of improper preparation and/or handling at home or in a food service setting.

Quality control laboratories in the food industry routinely perform tests to detect pathogens in food products and to ensure that the numbers and types of microorganisms associated with a food are not likely to cause serious food spoilage and/or health problems. In some cases, quality control procedures are aimed at detecting the presence of specific microorganisms, but in most cases the tests consist of examining the food for indicator organisms. For example, coliform counts are routinely performed on representative samples of many food products as an indication of possible fecal contamination, since food contaminated with fecal material has a relatively high probability of containing human pathogens. Foods such as hamburger often have 100,000 total bacteria per gram, but as long as they do not contain any *Salmonella* or other pathogens, they are considered safe for human consumption. In the United States, agencies involved in the surveillance and regulation of foods are the Department of Agriculture (USDA), the Food and Drug Administration (FDA), and state boards of health. Standards based on the presence or absence of pathogens, such as those set by Congress and regulated by the USDA and FDA, do provide the safeguards needed for assurance of the safety of food products.

Microorganisms routinely enter the gastrointestinal tract in association with ingested food and water. The large resident microbiota that develops in the human intestinal tract after birth is important for the maintenance of good health and is usually not involved in disease processes. This microbiota is normally noninvasive and is associated with the surface tissues and ingested food material. Some pathogenic microorganisms, however, possess toxigenic or invasive properties that permit them to cause disease when they enter the gastrointestinal tract.

There are two distinct processes that can initiate disease through the gastrointestinal tract. In the first type, microorganisms growing in food or water can produce toxins, and their ingestion initiates a disease process. Such diseases are classified as food poisoning or intoxication because the etiological agents of the disease need not grow within the body; that is, there is no true infectious process. Toxins absorbed through the gastrointestinal tract can cause neural damage and death in some cases, as well as localized inflammation and gastrointestinal upset in others. In the second type of disease-causing process, invasive pathogens establish an initial infection through the gastrointestinal tract and cause localized gastrointestinal upset or systemic disease symptomatology. Generally, the establishment of infection through the gastrointestinal tract requires a relatively large infectious dose; that is, a relatively large number of pathogenic microorganisms is required to successfully overcome the inherent defense mechanisms of the gastrointestinal tract. Quite different measures are required to prevent and treat infectious gastrointestinal diseases compared to those for specific microorganisms responsible for food poisoning.

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Many outbreaks of gastroenteritis involve the foodborne or waterborne transmission of viruses that can replicate within the human gastrointestinal tract and cause an inflammation of the lining of the gastrointestinal tract. Contamination of food with fecal matter is an important route of transmission of viral gastroenteritis. Several different viruses cause viral gastroenteritis, including adenoviruses, coxsackieviruses, polioviruses, and members of the ECHO virus group. The Norwalk agent, a small DNA virus identified as being responsible for an outbreak of "winter vomiting disease" that occurred in Norwalk, Ohio, in 1968, appears to be an important etiological agent of various viral gastroenteritis outbreaks. Rotavirus, a large RNA virus, also appears to be a very common etiological agent of diarrhea in infants, particularly in socioeconomically depressed regions of the world. It is difficult to detect the diversity of viruses causing this disease and foods and waters are only rarely examined for their presence.

Hepatitis is a systemic viral infection that primarily affects the liver, caused by several types of hepatitis viruses, designated types A, B, C, D, and non-A, non-B. Type A hepatitis virus is normally associated with foodborne and waterborne cases of hepatitis. Type A hepatitis virus normally enters the body via the gastrointestinal tract and causes infectious hepatitis. Although there are some documented cases of foodborne hepatitis B, types B, D and non-A, non-B hepatitis viruses, these viruses normally enter the body through skin punctures and cause serum hepatitis. Hepatitis type A virus is usually transmitted by the fecal-oral route and is prevalent in areas with inadequate sewage treatment. Several outbreaks of viral hepatitis have been associated with contaminated shellfish, such as oysters, that have concentrated viruses from sewage effluents. There have also been outbreaks from vegetables contaminated with infected fecal matter used as fertilizer. Contaminated potable water supplies are probably the most common source of hepatitis A viral infections.

Polioviruses may enter the body through either the gastrointestinal or respiratory tracts. Transmission through the ingestion of food and water containing polioviruses is considered very important. Polio is effectively controlled through the use of vaccines. This has been responsible for the decline of this disease, particularly in developed nations. Global vaccination programs are aimed at eliminating this disease worldwide.

Several *Clostridium* species cause foodborne disease. Canned foods are often the sources of clostridia causing human disease because *Clostridium* species are endospore forming anaerobes. Hence they are resistant to elevated temperatures, sometimes surviving the canning process high temperature killing, and can grow in the can because of the absence of air. Botulism, the most serious form of bacterial food poisoning, is caused by neurotoxins produced by *Clostridium botulinum*. The toxins are absorbed from the intestinal tract and transported via the circulatory system to motor nerve synapses, where their action blocks normal neural transmissions. Various strains of *C. botulinum* elaborate different toxins. Types A, B, and E toxins cause food poisoning of humans. Type E toxins are associated with the growth of *C. botulinum* in fish or fish products, and most outbreaks of botulism in Japan are caused by type E toxins because large amounts of fish are consumed there. Type A is the predominant toxin in cases of botulism in the United States, and type B toxin is most prevalent in Europe. *C. botulinum* cultures form three distinct physiological groups that can be detected by culture methods. *C. botulinum* types C and D are nonproteolytic and do not digest coagulated egg white or meat. All type A and some type B and F strains of *C. botulinum* are proteolytic whereas all type E strains and the remaining type B strains are nonproteolytic. Type G strains of *C. botulinum* show slow proteolysis. Optimum temperature for toxin production by proteolytic strains is approximately 35°C and for nonproteolytic strains is about 27°C. Nonproteolytic strains of *C. botulinum* types B, E, and F produce toxin at 4°C.

Over 90 % of the cases of botulism involve improperly home-canned food. Of 236 outbreaks of this disease in the United States between 1899 and 1974, 57 % were caused by contaminated vegetables, 15 % by contaminated fish, and 12 % by contaminated fruit. Certain canned foods provide an optimal anaerobic environment for the growth of *C. botulinum* that results in the release of toxin into the food. *C. botulinum,* though, cannot grow and produce toxin at low pH and thus is not a problem in acidic food products. Another clostridial species, *Clostridium perfringens*, is a major cause of a less severe form of food poisoning. *C. perfringens* generally accounts for over 10 % of the outbreaks of foodborne disease in the United States. The ingestion of food containing toxin produced by *C. perfringens* and the adsorption of the toxin into the cells lining the gastrointestinal tract initiate this disease. Toxin type A of *C. perfringens* is associated with most cases of clostridial food poisoning, particularly with cooked meats if a gravy is prepared with the meat. The spores of *C. perfringens* type A can survive the temperatures used in cooking many meats, and if incubated in a warm gravy, there is sufficient time for the spores to germinate and the growing bacteria to produce enough toxin to cause this disease.

Bacillus cereus is responsible for a relatively mild form of gastroenteritis, and recovery normally occurs in less than a day. The occurrence of gastroenteritis due to *B. cereus* requires the ingestion of a large number of spores. The symptoms include abdominal pain, profuse diarrhea, and nausea. *B. cereus* foodborne disease most commonly is associated with custards, cereal products, and meat gravies.

Strains of *Staphylococcus aureus* produce a toxin that causes an inflammation of the lining of the gastrointestinal tract. *S. aureus* can reproduce within many different types of food products. Enterotoxin-producing strains of *S. aureus* often enter foods from the skin surfaces of people who handle food. Custard-filled bakery goods, dairy products, processed meats, potato salad, and various canned foods are frequently found to be the source of the toxin. The prevention of staphylococcal food poisoning depends on proper handling and preservation of food products to prevent contamination and subsequent growth of enterotoxin-producing strains of *Staphylococcus.*

Listeria monocytogenes infects cattle, sheep, and chickens. It is transmitted to humans via contaminated milk and poultry and fecally-contaminated water and vegetables. Unpasteurized milk often is the source of human infection and cheeses have been a major source of *Listeria* infections in the United States. Identification of *Listeria monocytogenes* is based upon observation of Gram-positive rods that exhibit tumbling motility, utilization of lactose, catalase positive and oxidase negative reactions, exculin and sodium hippurate hydrolysis, Methyl Red positive reaction, ammonia production from arginine, and negative reactions for hydrogen sulfide production, nitrate reduction, gelatin liquefaction, starch hydrolysis, and urea hydrolysis.

Various *Salmonella* species, including *S. choleraesuis* and especially the numerous serotypes of *S. enteritidis,* are commonly the etiological agents of salmonellosis. Like many enteropathogenic bacteria, *Salmonella* species have pili that enable them to adhere to the lining of the gastrointestinal tract. Although *Salmonella* species are able to reproduce within the intestines, causing inflammation, they do not normally penetrate the mucosal lining and enter the bloodstream; in some cases, however, *Salmonella* species can gain access to the circulatory system, causing bacteremia. For example, paratyphoid fever, which is caused by strains of *S. paratyphi* and *S. typhimurium,* is characterized by gastroenteritis and a relatively high rate of bacteremia. *Salmonella* species causing gastroenteritis are normally transmitted by ingestion of contaminated food. Birds and domestic fowl, especially ducks, turkeys, and chickens, including their eggs, are commonly identified as the sources of *Salmonella* infections. Inadequate cooking of large turkeys and the ingestion of raw eggs cause a significant number of cases of salmonellosis. Outbreaks of typhoid fever, a systemic infection caused by *Salmonella typhi,* are associated with contaminated water supplies and the handling of food products by individuals infected with this bacterium. A relatively low infectious dose is required for *S. typhi* to establish an infection. Some individuals develop a carrier state; such individuals do not develop typhoid fever but act as a source of *S. typhi* and spread the disease as in the infamous case of a cook in the early twentieth century who became known as typhoid Mary.

Shigellosis, or bacterial dysentery, is an acute inflammation of the intestinal tract caused by species of the Gram-negative genus *Shigella,* including *S. flexneri, S. sonnei,* and *S. dysenteriae.* Water and food supplies are involved in some outbreaks of bacterial dysentery. The severe dehydration associated with this disease can cause shock and lead to death in children, in whom the incidence of bacterial dysentery is highest.

Campylobacter fetus var. *jejuni* has been found to be the causative agent of many cases of gastroenteritis in infants. In fact, *C. fetus* may be more important in juvenile gastroenteritis than *Salmonella* species. The transmission of *C. fetus* appears to be via contaminated food or water. *C. fetus* is a Gram-negative, motile, spiral-shaped bacterium, formerly known as *Vibrio fetus,* which also causes fetal abortion in cattle and sheep.

Several *Vibrio* species, including *V. cholerae, V. parahaemolyticus, V. mimicus,* and *V. vulnificus* cause foodborne and waterborne diseases. *Vibrio* species are Gram negative straight or curved rods (single curve) and ferment glucose without producing gas. *V. vulnificus* is most often associated with seafoods as a source of foodborne infections. *V. parahaemolyticus* is responsible for many cases of gastroenteritis in Japan and perhaps in the United States. It occurs in marine environments, and the ingestion of contaminated seafood, particularly the eating of raw fish, is the main route of transmission. Gastroenteritis caused by *V. parahaemolyticus* requires the establishment of an infection within the gastrointestinal tract, rather than simple ingestion of an enterotoxin. The symptoms generally appear 12 hours after ingestion of contaminated food and include abdominal pain, diarrhea, nausea, and vomiting. Recovery from this form of gastroenteritis normally occurs in 2 to 5 days, and the mortality rate is very low.

In contrast, cholera, which is caused by *Vibrio cholerae*, serotypes *cholerae* and *El Tor*, often is fatal. Although we typically associate cholera with Asia, sometimes referring to the disease as *Asiatic cholera*, it also occurs in the United States, primarily in the Gulf Coast region, where cases have been traced to contaminated shellfish. Cholera is now endemic to South America where eating of contaminated raw fish is a major source of infection. Contaminated water supplies are also responsible for the geographic spread of cholera from the initial focus in Peru throughout Latin America. Cholera is a particular problem in socioeconomically depressed countries, where there is poor sanitation and inadequate sewage treatment and where medical facilities have only a limited capacity to deal with outbreaks. This disease is endemic in the Ganges delta, and there are annual epidemic outbreaks of cholera in India and Bangladesh. In these endemic areas of Asia the death rate is normally 5 to 15 %. Seasonal outbreaks of cholera often occur in Southeast Asia when monsoon rains wash sewage material into drinking water supplies. During sudden epidemics, the mortality rate may reach 75 %.

Yersinia enterocolitica and related species, such as *Y. pseudotuberculosis,* produce a severe form of enterocolitis. Outbreaks of yersiniosis are most common in Western Europe but have also been confirmed in the United States. *Y. pseudotuberculosis* is found in pork, raw milk, and various other foods. *Y. enterocolitica* is widely distributed and has been found in water, milk, fruits, vegetables, and seafoods. This organism is psychrotrophic and thus is able to reproduce within refrigerated foods, where it can multiply and reach an infectious dose. In fact, *Y. enterocolitica* grows better at 25°C than at 37°C.

Coliform bacteria, principally *Escherichia coli*, are widely used as indicators of human fecal contamination of food and water. When used in this manner *E. coli* is not screened as a pathogen but rather because it is found in high numbers in human feces and is relatively short lived in the environment. Finding *E. coli* in food or potable water indicates contamination with human fecal matter that may also carry pathogens such as *Salmonella* and *Shigella*. Some *E. coli* strains, however, are human pathogens. These are designated enterotoxigenic or enteropathogenic *E. coli* if they produce toxins or can invade the body through the gastrointestinal tract.

Enterotoxin-producing strains of *Escherichia coli* are also capable of causing both mild and severe forms of gastroenterocolitis. In most cases, enterotoxin-producing strains of *E. coli* do not invade the body through the gastrointestinal tract; rather, toxin released by cells growing on the surface lining of the gastrointestinal tract causes diarrhea. Aside from diarrhea, abdominal cramps are normally the only other clinical symptom of this disease. Travelers from the United States to Mexico often suffer severe diarrhea as a result of ingestion of strains of *E. coli* foreign to their own microbiota and therefore generally avoid drinking the water. Many cases of severe diarrhea in children are caused by noninvasive, enterotoxin-producing strains of *E. coli*. In some cases, enteropathogenic strains of *E. coli* invade the body through the mucosa of the large intestine to cause a serious form of dysentery. Invasive strains of *E. coli* are primarily associated with contaminated food and water in Southeast Asia and South America. The ability to invade the mucosa of the large intestine depends on the presence of a specific K antigen in enteropathogenic serotypes of *E. coli*. The enterotoxins produced by *E. coli* cause a loss of fluids from intestinal tissues. With proper replacement of body fluids and maintenance of the essential electrolyte balance, infections with enterotoxic *E. coli* normally are not fatal.

E. coli O157:H7 has emerged as an important foodborne and waterborne pathogen. This strain of *E. coli* causes hemorrhagic colitis and serious kidney disease. It frequently is fatal. Outbreaks of disease caused by infections with *E. coli* O157:H7 have most often been traced to contaminated undercooked hamburger meat. The U.S. Food and Drug Administration recommends thorough cooking of hamburger meat. Improved surveillance of meats for bacterial contamination is being implemented by the FDA.

Arizona hinshawii is associated with poultry, and products containing eggs such as custards. It causes gastroenteritis that generally lasts a few days. *Aeromonas hydrophila* has been the source of some foodborne disease outbreaks in the United States. It is ubiquitous in water and can infect various animals, including frogs. *Plesiomonas shigelloides* has been implicated in several foodborne disease outbreaks involving fish and shellfish. It can grow on minimal media containing ammonium salts as the nitrogen source and glucose as the carbon source. Infections cause gastrointestinal upsets.

Tuberculosis, which is caused by *Mycobacterium tuberculosis* and related mycobacterial species, can be transmitted by the ingestion of contaminated food. Before the extensive use of pasteurization, milk contaminated with *M. tuberculosis* was associated with outbreaks of this disease. The dairy industry routinely screens milk for the presence of mycobacteria, such as *M. bovis* that commonly infects cattle and can also cause human disease.

Legionella species from potable waters can cause human infections when aerosols are inhaled. Most outbreaks of Legionnaire's disease have been traced to air-conditioning cooling systems. *Legionella* bacteria multiply in the cooling system waters, which are rapidly evaporated to provide cooling, and inadvertently become airborne and circulate through the air-conditioning system. In other cases potable waters, including aerosols from dental instruments and shower heads, are the source of *Legionella* infections. There have also been outbreaks of Legionnaire's disease due to contaminated aerosols used to moisten produce and keep fruits and vegetables fresh.

Mycotoxins produced by some fungi are responsible for serious cases of food poisoning. Many mycotoxins are potent neurotoxins. Various species of mushrooms contain toxins that can be absorbed through the gastrointestinal tract, and the ingestion of poisonous mushrooms, such as *Amanita phalloides*, is normally fatal. The amatoxins and phallotoxins produced by *A. phalloides* and other species of *Amanita* cause symptoms of food poisoning 8 to 24 hours after their ingestion. Initial symptoms include vomiting and diarrhea; later, degenerative changes occur in liver and kidney cells, and death may ensure within a few days of ingesting as little as 5 to 10 mg of toxin.

Some filamentous fungi, other than mushrooms, also produce toxins that can cause human disease. *Aspergillus* species growing on peanuts and grains produce aflatoxins, which are potent carcinogens, as well as toxic. They are known to cause death in sheep and cattle and may be involved in some human disease conditions. Aflatoxins are the only known carcinogens for which the United States government has set permissible levels; all other products with carcinogenic activity are banned outright.

Ergotism, another disease caused by fungi, results from ingesting grain containing ergot alkaloids produced by *Claviceps purpurea*. The toxins of *C. purpurea* cause degeneration of the capillary blood vessels, and this type of food poisoning has a relatively high mortality rate. Symptoms of ergotism may include vomiting, diarrhea, thirst, hallucinations, convulsions, and lesions of the extremities. Various outbreaks of mass hallucinations have been traced to contamination of food with ergot alkaloids, and there are even theories that the Salem witch hunts in colonial Massachusetts were related to grain contamination and widespread ergotism.

Algae are rarely considered as the etiological agents of disease, but paralytic shellfish poisoning is caused by toxins produced by dinoflagellate *Gonyaulax*. Blooms of *Gonyaulax* cause red tides in coastal marine environments. During such algal blooms, the algae and the toxins they produce can be concentrated in bivalve shellfish, such as clams and oysters. The ingestion of shellfish containing algal toxins can lead to symptoms that resemble those of botulism. Shellfishing is banned in areas of *Gonyaulax* blooms to prevent this form of food poisoning.

Amebic dysentery or amebiasis is caused by the protozoan *Entamoeba histolytica*. Infections with *E. histolytica* may be asymptomatic or may involve mild or severe diarrhea and abdominal pain. Amebic dysentery occurs as a result of inadequate sewage treatment and contamination of water with *E. histolytica*, whose cysts are not killed by the chlorination methods normally used to treat municipal drinking water. Infection is acquired by ingesting contaminated food or water containing cysts of *E. histolytica*.

Giardia lamblia, a flagellated protozoan, is responsible for most cases of diarrhea infection caused by protozoa. The cysts of *G. lamblia* can enter the gastrointestinal tract through contaminated water. A high incidence of giardiasis occurred among groups touring Leningrad during the 1970s as a result of contaminated water supplies. In 1973 a major outbreak of giardiasis occurred in upstate New York, with an estimated 4,800 individuals developing symptoms of the disease. The following year, giardiasis was the most common waterborne disease in the United States. *G. lamblia* can live saprophytically within the small intestine without causing any symptoms of giardiasis, and in the United States almost 4 % of the population appears to be infected by this organism. Excessive growth of the organism, however, can cause disease symptoms that include diarrhea, dehydration, mucus secretion, and flatulence.

Cryptosporidium is a newly emerging pathogen that has caused major outbreaks of waterborne disease in the United States. A 1993 outbreak of cryptosporidiosis in Milwaukee infected over 400,000 individuals. Excessive growth of this protozoan in municipal water supplies is the source of infection. New surveillance requirements are being implemented to prevent future major outbreaks of *Cryptosporidium* caused disease.

Food Spoilage

The growth of microorganisms in foods can alter the quality of the product, causing sizable economic losses to the food industry due to food spoilage. Food spoilage is a change in a food that renders it undesirable or unsafe for human consumption, and the growth of microorganisms in a food represents only one process that may cause food to spoil. The growth of pathogenic microorganisms in a food is certainly undesirable, as it can make that food unsafe to eat, but other microbially induced changes in food, such as decreased nutritional content and altered taste, odor, color, and texture, can also make a food undesirable for human consumption. Such food spoilage is difficult to define because it depends on the culture of the consumer; for example, Eskimos bury fish to produce a stinky mess and in Britain meat should have the strong aroma of aging, but these same foods would likely be rejected as spoiled in most parts of the United States.

Foods are classified according to their susceptibility to microbial spoilage as nonperishable, semiperishable, or perishable. Perishable foods, such as meats, fish, poultry, most fruits and vegetables, eggs, and milk, readily spoil because of microbial activities and generally have short shelf lives unless steps are taken to remove, kill, or prevent the growth of associated microorganisms. Semiperishable foods, such as potatoes and apples, generally remain unspoiled for prolonged periods of time unless improperly handled. Nonperishable foods, such as sugar, flour, and numerous dry products, normally do not spoil, but even these foods can spoil under appropriate conditions. For example, if cereals, grains, and flours are stored under conditions of high moisture, various fungal and bacterial populations are able to grow and spoil the products. We have all observed the growth of fungi on bread that has been stored too long.

The changes that occur in a food during microbial spoilage depend on the particular microbial populations involved, their enzymatic activities, environmental conditions, and the nature of the food. The microorganisms involved in the spoilage process generally do not originate within the food, the inner tissues of most plants and animals being sterile, but rather come from the surface tissues or are a result of contamination during processing. Several factors controlling food spoilage are intrinsic to the food, but others are extrinsic environmental parameters. The most important extrinsic factors influencing food spoilage are (1) temperature of storage; (2) relative humidity; and (3) oxygen concentration. By controlling these parameters, it is possible to alter the rate of food spoilage. It is somewhat more difficult to control the inherent properties of animal and plant tissues that influence microbial spoilage of foods. The intrinsic moisture content, pH, and physical and chemical nature of the food, in part, control the numbers and types of microorganisms involved in the spoilage process. Fruits, vegetables, meats, poultry, seafoods, milk and dairy products, and various other food products differ in their biochemical composition and therefore are subject to spoilage by differing microbial populations.

Fruits and vegetables are subject to rot because of the microbial degradation of pectin, the biochemical responsible for maintaining the firmness and texture of fruits and vegetables. Microbially produced pectinesterases and polygalacturonases hydrolyze pectins, resulting in the formation of soft spots in fruits and vegetables. About 20 % of the harvested crops of fruits and vegetables are lost to spoilage primarily because of the activities of bacteria and fungi. Carbohydrates, which are present in high concentrations in fruits, vegetables, and other foods, are readily degraded by numerous microorganisms, resulting in the production of various degradation products, such as low molecular weight acids and alcohols. The accumulation of such products of microbial metabolism can alter the taste of a food, generally causing it to sour. The taste of sour milk, for example, is associated with the accumulation of lactic acid from the microbial transformation of carbohydrates. The accumulation of low molecular weight acids may also alter the smell of the product; sour milk often can be recognized just by smelling it. In canned foods, production of acid and no gas is referred to as flat-sour spoilage because the food becomes sour, but the can shows no evidence of food spoilage because no gas is produced; that is, the can remains flat. When gas is produced during spoilage of canned foods, the can swells, as can be seen by examining the lids, which are designed to push outward if pressure builds within the can because of microbial action. Thermoduric microorganisms, that is, those that can withstand exposure to elevated temperatures, and especially endospore formers, are important in the spoilage of canned foods.

Meats and other proteinaceous products can be decomposed by anaerobic bacteria, resulting in putrefaction. Putrefaction of meat is the result of the breakdown of proteins by proteinases. The subsequent degradation of amino acids produces foul-smelling, low molecular weight sulfur- and nitrogen-containing compounds, such as mercaptans, hydrogen sulfide, ammonia, and amines. The evolution of noxious, odoriferous compounds from putrefying proteins renders food unacceptable for human consumption. The characteristic odor of hydrogen sulfide, for example, renders rotting eggs inedible, and the development of off odors and slime on poultry and beef reflects the presence of increased microbial populations and spoilage of the meat. In canned foods such spoilage is referred to as sulfide stinkers. Under aerobic conditions, the decomposition of proteins generally does not result in the production of compounds with obnoxious odors. The spoilage of meat under aerobic conditions, though, can result in the accumulation of surface slime, generally because of the growth of *Pseudomonas, Achromobacter, Streptococcus, Leuconostoc, Bacillus, Micrococcus,* and *Lactobacillus* species. The physical state of the meat influences which microbial species may be involved in spoilage. The spoilage of fresh whole meats is normally associated with lactic acid bacteria, particularly *Lactobacillus, Leuconostoc,* and *Streptococcus* species, whereas the spoilage of ground beef is primarily due to the growth of *Pseudomonas, Achromobacter,* and *Micrococcus.*

Spoilage by microbial degradation of fats and oils produces rancidity, caused by oxidation or hydrolysis of lipids. Spoiled butter, for example, becomes rancid because of the hydrolysis of butterfat, with the production of free fatty acids and glycerol and the accompanying development of undesirable flavors. Fish with high lipid levels, such as salmon and mackerel, may also become rancid because of the hydrolysis of fats and oils.

The growth of some microorganisms can alter the color of the food. For example, the growth of *Serratia marcescens* can produce bright red bread; the combined growth of *Pseudomonas* species and *Streptococcus lactis* produces blue milk; the growth of fungi on meat surfaces can produce off colors, such as black spot caused by *Cladosporium herbarum,* white spot caused by *Sporotrichium carnis,* and green patches produced by *Penicillium* species; and the growth of heterolactic fermenters on cured meats, such as frankfurters, causes greening because of the action of peroxidases on the meat pigments. The development of unnatural colors in a food reduces its acceptability, regardless of whether there is any associated development of an off taste or smell. Microbial growth can also alter the texture of a food. For example, the growth of *Bacillus* species in products such as milk and dough can produce ropiness, a textural change resulting from the growth of encapsulated *Bacillus* species together with the hydrolysis of starch and proteins.

Microbiological Production of Food

Although microbial growth is a problem when it results in food spoilage, microorganisms are used beneficially in the food industry for food production. Many of the foods and beverages we commonly enjoy, such as wine and cheese, are the products of microbial enzymatic activity. For the most part, it is the fermentative metabolism of microorganisms that is exploited in the production of food products. The accumulation of fermentation products, such as ethanol and lactic acid, is desirable because of their characteristic flavors and other properties. Only a few processes, such as the production of vinegar, make use of microbial oxidative metabolism. The microbial production of foods can be viewed as an exercise in harnessing microbial biochemistry to produce desired, rather than adverse, changes in food products.

The production of fermented foods requires the proper substrates, microbial populations, and environmental conditions to obtain the desired end product. Quality control is essential in food fermentation to ensure that the product is of high quality. A fermented food may require additional preservation to prevent spoilage because further uncontrolled microbial growth could render it inedible. For example, once wine is produced, it must be maintained under anaerobic conditions in order to prevent its oxidation to vinegar.

The microbial processes used in food production traditionally employ microbial enzymatic activities to transform one food into another, with the microbially produced food product having properties vastly different from those of the starting material. In addition to the use of microorganisms to produce fermented food products, microbial biomass is now considered a potential source of protein for meeting the food needs of an expanding world population. Some microorganisms, such as mushrooms, have been used as food products for centuries. The growth of bacteria, algae, and fungi as proteinaceous food, however, is a relatively new concept. Microbial biomass can be used as an animal feed supplement or may be developed as a direct source of protein for human consumption.

Numerous products are made by the microbial fermentation of milk, including buttermilk, yogurt, and many cheeses. The fermentation of milk is primarily carried out by lactic acid bacteria. The lactic acid fermentation pathway and the accumulation of lactic acid from the metabolism of the milk sugar lactose are common to the production of fermented dairy products. The accumulated lactic acid in these products acts as a natural preservative. The differences in the flavor and aroma of the various fermented dairy products are due to additional fermentation products that may be present in only relatively low concentrations.

Different fermented dairy products are produced by using different strains of lactic acid bacteria as starter cultures and different fractions of whole milk as the starting substrate. Sour cream, for example, uses *Streptococcus cremoris* or *S. lactis* for the production of lactic acid, and *Leuconostoc cremoris* or *S. lactis diacetilactis* for the production of the characteristic flavor compounds. Cream is the starting

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