Food Microbiology

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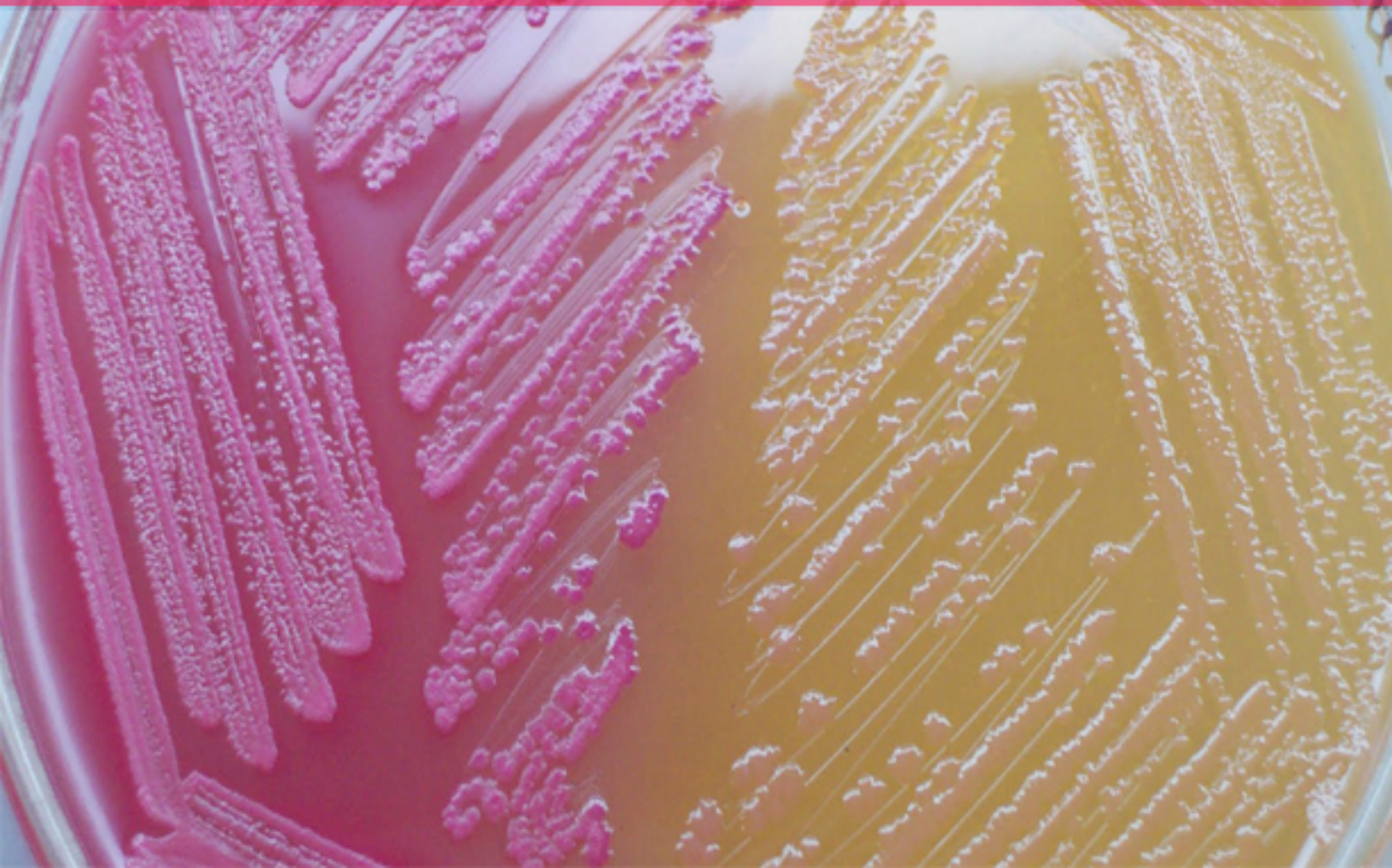

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Chapter 1

Food Microbiology

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.

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 flavors.

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.

Xanthan

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 β-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 fibers. 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-γ-glutamic acid

Poly-γ-glutamic acid (γ-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 immunocompromised 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 favor 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 (1) enterohemorrhagic *E. coli*, which cause hemorrhagic colitis and hemolytic uremic syndrome, (2) enterotoxigenic *E. coli*, which induce traveler's diarrhea, (3) enteropathogenic *E. coli*, which cause a persistent diarrhea in children living in developing countries, (4) enteroaggregative *E. coli*, which provokes diarrhea in children, (5) enteroinvasive *E. coli* that are biochemically and genetically related to *Shigella* species and can induce diarrhea, and (6) 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.

Food authenticity

It is important to be able to detect microorganisms in food, in particular pathogenic microorganisms or genetically modified microorganisms. Real-time PCR is an accepted analytical tool within the food industry. Its principal role has been one of assisting the legislative authorities, major manufacturers and retailers to confirm the authenticity of foods. The most obvious role is the detection of genetically modified organisms, but real-time PCR makes a significant contribution to other areas of the food industry, including food safety.

Chapter 2

Probiotic

Probiotics are live microorganisms thought to be beneficial to the host organism. According to the currently adopted definition by FAO/WHO, probiotics are: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host". Lactic acid bacteria (LAB) and bifidobacteria are the most common types of microbes used as probiotics; but certain yeasts and bacilli may also be helpful. Probiotics are commonly consumed as part of fermented foods with specially added active live cultures; such as in yogurt, soy yogurt, or as dietary supplements.

Etymologically, the term appears to be a composite of the Latin preposition *pro* ("for") and the Greek adjective *βιωτικός* (biotic), the latter deriving from the noun *βίος* (bios, "life").

At the start of the 20th century, probiotics were thought to beneficially affect the host by improving its intestinal microbial balance, thus inhibiting pathogens and toxin producing bacteria. Today, specific health effects are being investigated and documented including alleviation of chronic intestinal inflammatory diseases, prevention and treatment of pathogen-induced diarrhea, urogenital infections, and atopic diseases.

History

The original observation of the positive role played by certain bacteria was first introduced by Russian scientist and Nobel laureate Eli Metchnikoff, who in the beginning of the 20th century suggested that it would be possible to modify the gut flora and to replace harmful microbes with useful microbes. Metchnikoff, at that time a professor at the Pasteur Institute in Paris, produced the notion that the aging process results from the activity of putrefactive (proteolytic) microbes producing toxic substances in the large bowel. Proteolytic bacteria such as clostridia, which are part of the normal gut flora, produce toxic substances including phenols, indols and ammonia from the digestion of

proteins. According to Metchnikoff these compounds were responsible for what he called "intestinal auto-intoxication", which caused the physical changes associated with old age.

It was at that time known that milk fermented with lactic-acid bacteria inhibits the growth of proteolytic bacteria because of the low pH produced by the fermentation of lactose. Metchnikoff had also observed that certain rural populations in Europe, for example in Bulgaria and the Russian steppes who lived largely on milk fermented by lactic-acid bacteria were exceptionally long lived. Based on these facts, Metchnikoff proposed that consumption of fermented milk would "seed" the intestine with harmless lactic-acid bacteria and decrease the intestinal pH and that this would suppress the growth of proteolytic bacteria. Metchnikoff himself introduced in his diet sour milk fermented with the bacteria he called "Bulgarian Bacillus" and found his health benefited. Friends in Paris soon followed his example and physicians began prescribing the sour milk diet for their patients.

Bifidobacteria were first isolated from a breast-fed infant by Henry Tissier who also worked at the Pasteur Institute. The isolated bacterium named Bacillus bifidus communis was later renamed to the genus *Bifidobacterium*. Tissier found that bifidobacteria are dominant in the gut flora of breast-fed babies and he observed clinical benefits from treating diarrhea in infants with bifidobacteria. The claimed effect was bifidobacterial displacement of proteolytic bacteria causing the disease.

During an outbreak of shigellosis in 1917, German professor Alfred Nissle isolated a strain of *Escherichia coli* from the feces of a soldier who was not affected by the disease. Methods of treating infectious diseases were needed at that time when antibiotics were not yet available, and Nissle used the *Escherichia coli* Nissle 1917 strain in acute gastrointestinal infectious salmonellosis and shigellosis.

In 1920, Rettger demonstrated that Metchnikoff's "Bulgarian Bacillus", later called *Lactobacillus delbrueckii subsp. bulgaricus*, could not live in the human intestine, and the fermented food phenomena petered out. Metchnikoff's theory was disputable (at this stage), and people doubted his theory of longevity.

After Metchnikoff's death in 1916, the centre of activity moved to the United States. It was reasoned that bacteria originating from the gut were more likely to produce the desired effect in the gut, and in 1935 certain strains of *Lactobacillus acidophilus* were found to be very active when implanted in the human digestive tract. Trials were carried out using this organism, and encouraging results were obtained especially in the relief of chronic constipation.

The term "probiotics" was first introduced in 1953 by Kollath. Contrasting antibiotics, probiotics were defined as microbially derived factors that stimulate the growth of other microorganisms. In 1989 Roy Fuller suggested a definition of probiotics which has been widely used: "*A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance*". Fuller's definition emphasizes the

requirement of viability for probiotics and introduces the aspect of a beneficial effect on the host.

In the following decades intestinal lactic acid bacterial species with alleged health beneficial properties have been introduced as probiotics, including *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Lactobacillus johnsonii*.

Benefits

Experiments into the benefits of probiotic therapies suggest a range of potentially beneficial medicinal uses for probiotics. For many of the potential benefits, research is limited and only preliminary results are available. It should be noted that the effects described are *not* general effects of probiotics. Recent research on the molecular biology and genomics of *Lactobacillus* has focused on the interaction with the immune system, anti-cancer potential, and potential as a biotherapeutic agent in cases of antibiotic-associated diarrhoea, travellers' diarrhoea, pediatric diarrhoea, inflammatory bowel disease and irritable bowel syndrome.

All effects can only be attributed to the individual strain(s) tested. Testing of a supplement does not indicate benefit from any other strain of the same species, and testing does not indicate benefit from the whole group of LAB (or other probiotics).

Managing lactose intolerance

As lactic acid bacteria actively convert lactose into lactic acid, ingestion of certain active strains may help lactose intolerant individuals tolerate more lactose than they would have otherwise.

Preventing colon cancer

In laboratory investigations, some strains of LAB (*Lactobacillus bulgaricus*) have demonstrated anti-mutagenic effects thought to be due to their ability to bind with heterocyclic amines, which are carcinogenic substances formed in cooked meat. Animal studies have demonstrated that some LAB can protect against colon cancer in rodents, though human data is limited and conflicting. Most human trials have found that the strains tested may exert anti-carcinogenic effects by decreasing the activity of an enzyme called β-glucuronidase (which can generate carcinogens in the digestive system). Lower rates of colon cancer among higher consumers of fermented dairy products have been observed in one population study.

Lowering cholesterol

Animal studies have demonstrated the efficacy of a range of LAB to be able to lower serum cholesterol levels, presumably by breaking down bile in the gut, thus inhibiting its reabsorption (which enters the blood as cholesterol). Some, but not all human trials have shown that dairy foods fermented with specific LAB can produce modest reductions in

total and LDL cholesterol levels in those with normal levels to begin with, however trials in hyperlipidemic subjects are needed.

Lowering blood pressure

Several small clinical trials have indicated that consumption of milk fermented with various strains of LAB may result in modest reductions in blood pressure. It is thought that this is due to the ACE inhibitor-like peptides produced during fermentation.

Improving immune function and preventing infections

LAB are thought to have several presumably beneficial effects on immune function. They may protect against pathogens by means of competitive inhibition (i.e., by competing for growth) and there is evidence to suggest that they may improve immune function by increasing the number of IgA-producing plasma cells, increasing or improving phagocytosis as well as increasing the proportion of T lymphocytes and Natural Killer cells. Clinical trials have demonstrated that probiotics may decrease the incidence of respiratory tract infections and dental caries in children. LAB foods and supplements have been shown to aid in the treatment and prevention of acute diarrhea, and in decreasing the severity and duration of rotavirus infections in children and travelers' diarrhea in adults.

A 2010 study suggested that the anecdotal benefits of probiotic therapies as beneficial for preventing secondary infections, a common complication of antibiotic therapy, may be because keeping the immune system primed by eating foods enhanced with "good" bacteria may help counteract the negative effects of sickness and antibiotics. It was thought that antibiotics may turn the immune system "off" while probiotics turns it back on "idle", and more able to quickly react to new infections.

Helicobacter pylori

LAB are also thought to aid in the treatment of *Helicobacter pylori* infections (which cause peptic ulcers) in adults when used in combination with standard medical treatments. However more studies are required into this area.

Antibiotic-associated diarrhea

Antibiotic-associated diarrhea (AAD) results from an imbalance in the colonic microbiota caused by antibiotic therapy. Microbiota alteration changes carbohydrate metabolism with decreased short-chain fatty acid absorption and an osmotic diarrhea as a result. Another consequence of antibiotic therapy leading to diarrhea is overgrowth of potentially pathogenic organisms such as *Clostridium difficile*.

Probiotic treatment can reduce the incidence and severity of AAD as indicated in several meta-analyses. However, further documentation of these findings through randomized, double blind, placebo-controlled trials are warranted.

Efficacy of probiotic AAD prevention is dependent on the probiotic strain(s) used and on the dosage. Up to a 50% reduction of AAD occurrence has been found. No side-effects have been reported in any of these studies. Caution should, however, be exercised when administering probiotic supplements to immunocompromised individuals or patients who have a compromised intestinal barrier.

Reducing inflammation

LAB and supplements have been found to modulate inflammatory and hypersensitivity responses, an observation thought to be at least in part due to the regulation of cytokine function. Clinical studies suggest that they can prevent reoccurrences of inflammatory bowel disease in adults, as well as improve milk allergies. They are not effective for treating eczema, a persistent skin inflammation. How probiotics counteract immune system overactivity remains unclear, but a potential mechanism is desensitization of T lymphocytes, an important component of the immune system, towards pro-inflammatory stimuli.

Improving mineral absorption

It is hypothesized that probiotic lactobacilli may help correct malabsorption of trace minerals, found particularly in those with diets high in phytate content from whole grains, nuts, and legumes.

Preventing harmful bacterial growth under stress

In a study done to see the effects of stress on intestinal flora, rats that were fed probiotics had little occurrence of harmful bacteria latched onto their intestines compared to rats that were fed sterile water.

Treating irritable bowel syndrome and colitis

B. infantis 35624, sold as Align, was found to improve some symptoms of irritable bowel syndrome in women in a recent study. Another probiotic bacterium, *Lactobacillus plantarum* 299v, was also found to be effective in reducing IBS symptoms. Additionally, a probiotic formulation, VSL#3, was found to be safe in treating ulcerative colitis, though efficacy in the study was uncertain. *Bifidobacterium animalis* DN-173 010 may help. For maintenance of remission of ulcerative colitis, Mutaflor (*E.coli* Nissle 1917) there are 3 controlled, randomized, double blind clinical studies which have proven equivalence of Mutaflor and mesalazine (5-ASAs).

Managing urogenital health

Several in vitro studies have revealed probiotics' potential in relieving urinary tract infections and bacterial vaginosis. Results have been varied on these studies, and in vivo studies are still required in this area to determine efficacy.

Other

A study in 2004 testing the immune system of students given either milk or Actimel over a 6 week exam period (3 weeks of studying, 3 weeks of exams) tested 19 different biomarkers. Of these 19 biomarkers only 2 were shown to be different between the two groups, increased production of lymphocytes and increased production of CD56 cells. The tests were not blind and show that certain probiotic strains may have no overall effect on the immune system or on its ability.

A 2007 study at University College Cork in Ireland showed that a diet including milk fermented with *Lactobacillus* bacteria prevented *Salmonella* infection in pigs.

A 2007 clinical study at Imperial College London showed that preventive consumption of a commercially available probiotic drink containing *L casei DN-114001*, *L bulgaricus*, and *S thermophilus* can reduce the incidence of antibiotic-associated diarrhea and *C difficile*-associated diarrhea.

The efficacy and safety of a daily dose of *Lactobacillus acidophilus* CL1285 in the prevention of AAD was demonstrated by Montreal's Maisonneuve-Rosemont Hospital, in a clinical study of hospitalized patients.

Current research is focusing on the molecular biology and genomics of *Lactobacillus* and bifidobacteria. The application of modern whole genome approaches is providing insights into bifidobacterial evolution, while also revealing genetic functions that explain their presence in the particular ecological environment of the gastrointestinal tract.

Probiotics are used in industry to improve yields of pork and chicken production.

Disadvantages

In some situations, such as where the person consuming probiotics is critically ill, probiotics could be harmful. In a therapeutic clinical trial conducted by the Dutch Pancreatitis Study Group, the consumption of a mixture of six probiotic bacteria, increased the death rate of patients with predicted severe acute pancreatitis.

In a clinical trial conducted at the University of Western Australia, aimed at showing the effectiveness of probiotics in reducing childhood allergies, Dr Susan Prescott and her colleagues gave 178 children either a probiotic or a placebo for the first six months of their life. Those given the good bacteria were more likely to develop a sensitivity to allergens.

Some hospitals have reported treating lactobacillus septicaemia, which is a potentially fatal disease caused by the consumption of probiotics by people with lowered immune systems or who are already very ill.

There is no published evidence that probiotic supplements are able to replace the body's natural flora when these have been killed off; indeed bacterial levels in feces disappear within days when supplementation ceases.

Probiotics taken orally can be destroyed by the acidic conditions of the stomach. A number of micro-encapsulation techniques are being developed to address this problem.

Recent studies indicate that probiotic products such as yogurts could be a cause for obesity trends. However, this is contested as the link to obesity and other health related issue with yogurt may link to its dairy attributes.

Some experts are skeptical on the efficacy of many strains and believe not all subjects will benefit from the use of probiotics. A criticism of probiotic supplements is the cost and value of probiotics products.

Strains

Live probiotic cultures are available in fermented dairy products and probiotic fortified foods. However, tablets, capsules, powders and sachets containing the bacteria in freeze dried form are also available.

Probiotic Research and Producer Information

Strain	Brandname	Producer	Potential effect in humans
Bacillus coagulans GBI-30, 6086	**GanedenBC^{30}**	**Ganeden Biotech**	Improves abdominal pain and bloating in IBS patients. Increases immune response to viral challenge.
Bifidobacterium LAFTI® B94 Bifidobacterium sp	LAFTI B94	Institut Rosell-Lallemand	Protects against Salmonella typhimurium in mice. Uses prebiotics for improved colonization. Facilitates apoptotic response when used in combination with resistant starch in a colon cancer model. Reduces inflammation and incidence of

			diarrhea in an IBS model. Reduces allergic responses in an allergy model. Reduces the severity of H.pylori infection of the stomach mucosa. Inhibits pathogenic bacteria, including H. pylori, monocytogenes, E. coli, and salmonella typhimurium. Survives in the conditions of the gastro-intestinal tract. Adheres to human intestinal cells. Synthesizes folate from yogurts.
Lactobacillus acidophilus LAFTI® L10 Lactobacillus acidophilus	LAFTI L10	Institut Rosell-Lallemand	Enhances clearance of Candida albicans by induction of an immune response. Reduces allergic responses in an allergy model. Protects against Listeria monocytogenes in the gastro- intestinal tract of mice. Reduces the incidence of tumor formation and the size of intestinal tumors in rats. Uses prebiotics for improved colonization. Reduces inflammation in an IBS model. Inhibits pathogenic bacteria, including H. pylori,

			monocytogenes, E. colim, and Salmonella typhimurium. Superior survival in the conditions of the gastro-intestinal tract compared to other probiotics. Adheres to human intestinal cells. Produces anti-microbial substances like H202.
Lactobacillus casei LAFTI® L26 Lactobacillus casei	LAFTI L26	Institut Rosell-Lallemand	Protects against Salmonella typhimurium in mice. Uses prebiotics for improved colonization. Reduces inflammation in an IBS model. Reduces allergic responses in an allergy model. Reduces the severity of H.pylori infection of the stomach mucosa. Inhibits pathogenic bacteria, including H. pylori, monocytogenes, E. coli, and Salmonella typhimurium. Survives in the conditions of the gastro-intestinal tract. Adheres to human intestinal cells.
Bifidobacterium animalis subsp. *lactis* BB-12	Probio-Tec® Bifidobacterium BB-12®	Chr. Hansen	Alleviates symptoms of atopic eczema. Has an

			immune modulating effect.
Bifidobacterium breve Yakult	**Bifiene**	Yakult	
Bifidobacterium infantis 35624	**Align**	Procter & Gamble	Showed significant improvement for abdominal pain/discomfort, bloating/distention, and bowel movement difficulty.
Bifidobacterium animalis subsp. *lactis* HN019 (DR10)	**Howaru Bifido**	Danisco	
Bifidobacterium longum BB536		Morinaga Milk Industry	
Escherichia coli M-17	**ProBactrix**	BioBalance	
Escherichia coli Nissle 1917	**Mutaflor**	Ardeypharm	
Lactobacillus acidophilus DDS-1		Nebraska Cultures	
Lactobacillus acidophilus LA-5		Chr. Hansen	
Lactobacillus acidophilus NCFM		Danisco	Shown to reduce the side effects of antibiotic therapy.
Lactobacillus casei DN114-001 (*Lactobacillus casei* Immunitas(s)/Defensis)	**Actimel/DanActive**	Danone	
Lactobacillus casei 431		Chr. Hansen	
Lactobacillus casei F19	**Cultura**	Arla Foods	
Lactobacillus casei Shirota	**Yakult**	Yakult	
Lactobacillus paracasei St11 (or NCC2461)	Lactobacillus fortis	Nestlé	
Lactobacillus johnsonii La1 (= Lactobacillus LC1, *Lactobacillus johnsonii* NCC533)		Nestlé	Reduces incidences of H pylori-caused gastritis and reduces inflammation
Lactococcus lactis L1A		Norrmejerier	Immune

			stimulation, improves digestive health, reduces antibiotic-associated diarrhoea
Lactobacillus plantarum 299v	**GoodBelly / ProViva/ TuZen/ Bion Transit / ProbiMage**	Probi	Shown to improve symptoms of IBS.
Lactobacillus reuteri ATTC 55730 (*Lactobacillus reuteri* SD2112)		BioGaia Biologics	Diarrhea prevention and mitigation in children, eradication of H. pylori infection, amelioration of gingivitis, general illness prevention in children and adults.
Lactobacillus rhamnosus ATCC 53013 (Also strain number GG, discovered by Gorbach & Goldin)	**LGG, Gefilus, Vifit and others**	Valio	
Lactobacillus rhamnosus LB21	**Verum**	Norrmejerier	Immune stimulation, improves digestive health, reduces antibiotic-associated diarrhoea
Saccharomyces boulardii	**DiarSafe and others**	**Wren Laboratories and others**	Protects against antibiotic-associated diarrhoea and infections of Clostridium difficile and other clostridial species; helps treat acute diarrhoea in adults & children.
tested as mixture: *Lactobacillus rhamnosus* GR-1 & *Lactobacillus reuteri* RC-14	**Bion Flore Intime Jarrow Fem-Dophilus**	Chr. Hansen	Oral ingestion results in vaginal colonisation and prevention of vaginitis.
tested as mixture: *Lactobacillus*	**Florajen3**	American Lifeline, Inc	Reduction of C. difficile–associated

acidophilus NCFM & *Bifidobacterium bifidum* BB-12			disease (CDAD).
			Improves digestive health. Prevents Antiobic Associated Diarrhea (AAD) and Clostridium difficile (C. difficile).
tested as mixture: *Lactobacillus acidophilus* CL1285 & *Lactobacillus casei* LBC80R	**Bio-K+ CL1285**	Bio-K+ International	In vitro inhibition of *Listeria monocytogenes* and *L. innocua*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium*. Reduction of symptoms of lactose intolerance and immune stimulation.
Lactobacillus plantarum HEAL 9 & *Lactobacillus paracasei* 8700:2	**Bravo Friscus/ ProbiFrisk**	Probi	Reduces the risk of acquiring common cold infections.
Lactobacillus helveticus R0052 & *Lactobacillus rhamnosus* R0011	**A'Biotica and others**	Institut Rosell	
Lactobacillus casei var. rhamnosus MG001 & *Lactobacillus acidophilus* MG002 & *Lactobacillus plantarum* MG003 & *Enterococcus faecium* MG004	**Symprove Probiotic**	Symprove Ltd.	

Some additional forms of yogurt bacteria include:

- *Lactobacillus bulgaricus*
- *Streptococcus thermophilus*
- *Lactobacillus bifidus* - became new genus *Bifidobacterium*

Some fermented products containing similar lactic acid bacteria include:

- Pickled vegetables
- Fermented bean paste such as tempeh, miso and doenjang
- Kefir
- Buttermilk or Karnemelk
- Kimchi
- Pao cai
- Sauerkraut
- Soy sauce
- Zha cai

Multi-probiotic

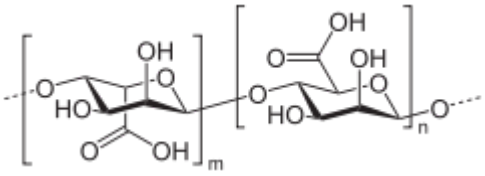
Research is emerging on the potential health benefits of multiple probiotic strains as a health supplement as opposed to a single strain. The human gut is home to some 400-500 types of microbes. It is thought that this diverse environment may benefit from multiple probiotic strains; different strains populate different areas of the digestive tract, and studies are beginning to link different probiotic strains to specific health benefits.

Incomplete list of supplement products that contain more than one probiotic strain.

Company	Product	Probiotic Strains	Strain Qty
EMD Canada Inc.	Multibionta	*Lactobacillus gasseri PA16/8, Bifidobacterium bifidum MF20/5, Bifidobacterium longum SP07/3*	3
OptiBac Probiotics	For daily wellbeing	*Bifidobacterium longum Rosell-175, Lactococcus lactis Rosell-1058, Bifidobacterium breve Rosell-70, Lactobacillus rhamnosus Rosell-11, Lactobacillus acidophilus Rosell-52, Bifidobacterium bifidum rosell-71*	6
Symprove Ltd. UK	Symprove Probiotic	*Lactobacillus casei var. rhamnosus MG001, Lactobacillus acidophilus MG002, Lactobacillus plantarum MG003, Enterococcus faecium MG004*	

Chapter 3

Alginic Acid

Alginic acid	
	
Other names	
E400	
Identifiers	
CAS number	9005-32-7 ✓
UNII	8C3Z4148WZ ✗
EC number	232-680-1
ATC code	A02BX13
Properties	
Molecular formula	$(C_6H_8O_6)_n$
Molar mass	10,000 - 600,000
Appearance	white to yellow, fibrous powder
Density	1.601 g/cm^3
Acidity (pK_a)	1.5-3.5

Alginic acid, also called **algin** or **alginate**, is an anionic polysaccharide distributed widely in the cell walls of brown algae, where it, through binding water, forms a viscous gum. In extracted form it absorbs water quickly; it is capable of absorbing 200-300 times its own weight in water. Its colour ranges from white to yellowish-brown. It is sold in filamentous, granular or powdered forms.

Structure

It is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks.

The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks), alternating M and G-residues (MG-blocks), or randomly organized blocks.

Forms

Commercial varieties of alginate are extracted from seaweed, including the giant kelp *Macrocystis pyrifera*, *Ascophyllum nodosum*, and various types of *Laminaria*. It is also produced by two bacterial genera *Pseudomonas* and *Azotobacter*, which played a major role in the unravelling of its biosynthesis pathway. Bacterial alginates are useful for the production of micro- or nanostructures suitable for medical applications.

Uses

Alginate absorbs water quickly, which makes it useful as an additive in dehydrated products such as slimming aids, and in the manufacture of paper and textiles. It is also used for waterproofing and fireproofing fabrics, as a gelling agent, and for thickening drinks, ice cream and cosmetics.

Alginate is used in various pharmaceutical preparations such as Gaviscon, Bisodol, and Asilone. Alginate is used extensively as an impression-making material in dentistry, prosthetics, lifecasting and occasionally for creating positives for small-scale casting. It is also used in the food industry, for thickening soups and jellies.

Calcium alginate is used in different types of medical products, including burn dressings that promote healing and can be removed with less pain than conventional dressings.

Also, due to alginate's biocompatibility and simple gelation with divalent cations such as Ca^{2+}, it is widely used for cell immobilization and encapsulation.

Alginic acid (alginato) is also used in culinary arts, most notably in the "Esferificación" (Sphereification) techniques of Ferran Adrià of El Bulli in Roses, Girona, where natural juices of fruits and vegetables are encapsulated in bubbles that "explode" on the tongue when consumed. One of the most famous examples of this use of alginic acid was when Ferran Adrià used alginic acid to make apple caviar.

Due to its ability to absorb water quickly, alginate can be changed through a lyophilization process to a new structure that has the ability to expand. It is used in the weight loss industry as an appetite suppressant.

In March, 2010 researchers at Newcastle University announced that dietary alginates can reduce human fat uptake by more than 75%.

Sodium alginate

The chemical compound **sodium alginate** is the sodium salt of alginic acid. Its empirical formula is $NaC_6H_7O_6$. Sodium alginate is a gum, extracted from the cell walls of brown algae.

Uses

Its form as a flavorless gum, is used by the foods industry to increase viscosity and as an emulsifier. It is also used in indigestion tablets and the preparation of dental impressions.

A major application for sodium alginate is in reactive dye printing, as as thickener for reactive dyestuffs (such as the Procion cotton-reactive dyes) in textile screen-printing and carpet jet-printing. Alginates do not react with these dyes and wash out easily, unlike starch-based thickeners.

Sodium alginate is a good chelator for pulling radioactive toxins from the body, such as iodine-131 and strontium-90 that have taken the place of their non-radioactive counterparts. It is also used in immobilizing enzymes by inclusion.

As a food additive, sodium alginate is used especially in the production of gel-like foods. For example, bakers' "Chellies" are often gelled alginate "jam." Also, the pimento stuffing in prepared cocktail olives is usually injected as a slurry at the same time that the stone is ejected; the slurry is subsequently set by immersing the olive in a solution of a calcium salt, which causes rapid gelation by electrostatic cross-linking. A similar process can be used to make "chunks" of everything from cat food through "reformed" ham or fish to "fruit" pieces for pies. It has the E-number 401.

Nowadays, it is also used in the biological experiments for the immobilization of cells to obtain important products like alcohols, organic acids, etc.

In recent years, sodium alginate has been used in molecular gastronomy at some of the best restaurants in the world. Ferran Adria pioneered the technique, and it has since been used by chefs such as Grant Achatz and Heston Blumenthal. Sodium alginate is combined with calcium lactate or similar compound to create spheres of liquid surrounded by a thin jelly membrane.

Potassium alginate

Potassium alginate is a chemical compound that is the potassium salt of alginic acid. It is an extract of seaweed. Its empirical chemical formula is $KC_6H_7O_6$.

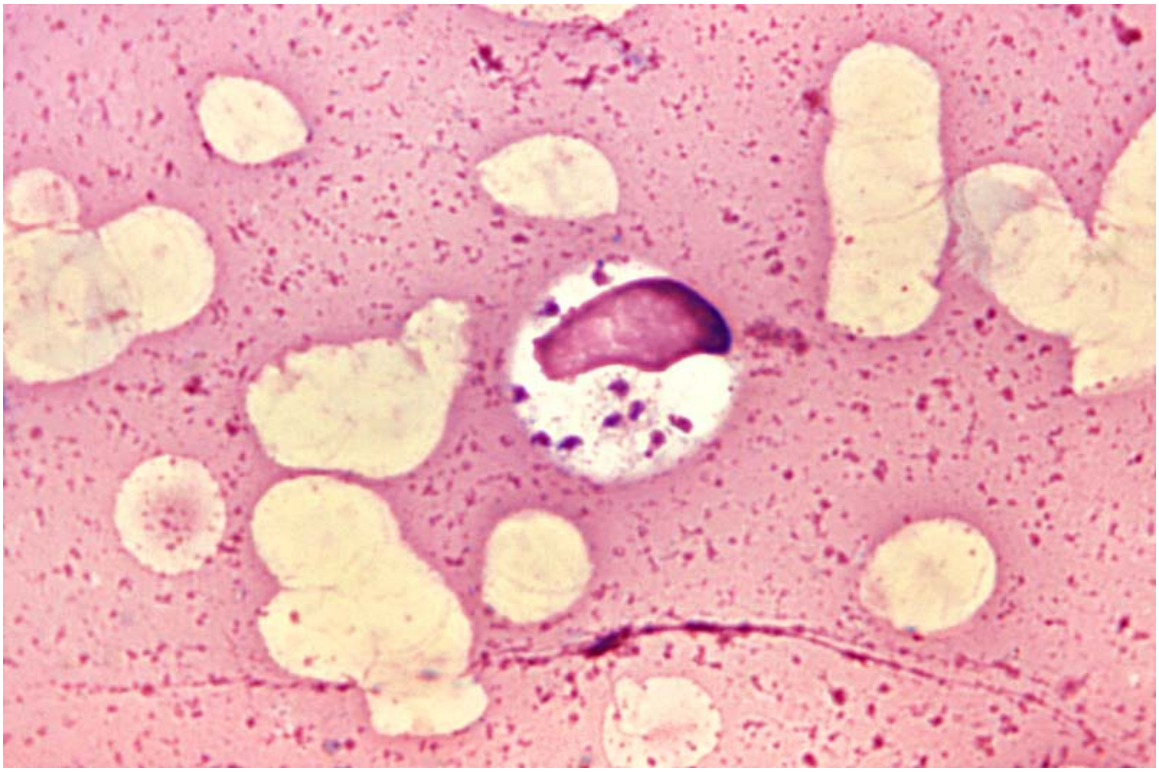
Uses

Potassium alginate is widely used in foods as a stabilizer, thickener, and emulsifier.

Its use as a pharmaceutical excipient is currently limited to experimental hydrogel systems. The viscosity, adhesiveness, elasticity, stiffness, and cohesiveness of potassium alginate hydrogels have been determined and compared with values from a range of other hydrogel-forming materials. The effect of calcium ions on the rheological properties of procyanidin hydrogels containing potassium alginate and intended for oral administration has also been investigated.

Chapter 4

Protozoa



Leishmania donovani, (a species of protozoa) in a bone marrow cell

Protozoa (from the Greek words *proto*, meaning "first", and *zoa*, meaning "animals") are a diverse group of single cell eukaryotic organisms, many of which are motile. Historically protozoa were defined as single cell protists with animal-like behaviour, e.g. movement. Protozoa were regarded as the partner group of protists to protophyta which have plant-like behaviour, e.g. photosynthesis. The term protozoa is generally not used in modern scientific contexts.

Terminology

The word *protozoan* was originally an adjective but can also be used as a noun.

Protozoans are referred to generally as animal-like protists because of movement (motile). However, both protozoa and protists are paraphyletic groups (not including all genetic relatives of the group). For example, *Entamoeba* is more closely related to humans than to *Euglena*. "Protozoa" is considered an outdated classification in more formal contexts. However, the term is still used in children's education.

While there is no exact definition for the term *protozoan*, it often refers to a unicellular heterotrophic protist, such as the amoeba and ciliates. The term *algae* is used for microorganisms that photosynthesize. However, the distinction between protozoa and algae is often vague. For example, the algae *Dinobryon* has chloroplasts for photosynthesis, but it can also feed on organic matter and is motile.

- Protozoa are their own "kingdom" by the Integrated Taxonomic Information System 2009 classification.
- It is sometimes considered a subkingdom.
- It was traditionally considered a phylum under Animalia.

Characteristics

The most important protozoans range usually from 10 to 52 micrometers, but can grow as large as 1 mm, and are seen easily by microscope.

They were considered formerly to be part of the protista family. Protozoa exist throughout aqueous environments and soil, occupying a range of trophic levels.

Motility and digestion

Tulodens are one of the slow-moving form of protozoans. They move around with whip-like tails called flagella, hair-like structures called cilia, or foot-like structures called pseudopodia. Others do not move at all.

Protozoa may absorb food via their cell membranes, some, e.g. amoebas, surround food and engulf it, and yet others have openings or "mouth pores" into which they sweep food. All protozoa digest their food in stomach-like compartments called vacuoles.

Ecological role

As components of the micro- and meiofauna, protozoa are an important food source for microinvertebrates. Thus, the ecological role of protozoa in the transfer of bacterial and algal production to successive trophic levels is important. As predators, they prey upon

unicellular or filamentous algae, bacteria, and microfungi. Protozoa are both herbivores and consumers in the decomposer link of the food chain. They also control bacteria populations and biomass to some extent. Protozoa such as the malaria parasites (*Plasmodium* spp.), trypanosomes and leishmania are also important as parasites and symbionts of multicellular animals.

Life cycle

Some protozoa have life stages alternating between proliferative stages (e.g. trophozoites) and dormant cysts. As cysts, protozoa can survive harsh conditions, such as exposure to extreme temperatures or harmful chemicals, or long periods without access to nutrients, water, or oxygen for a period of time. Being a cyst enables parasitic species to survive outside of a host, and allows their transmission from one host to another. When protozoa are in the form of trophozoites (Greek, *tropho* = to nourish), they actively feed. The conversion of a trophozoite to cyst form is known as encystation, while the process of transforming back into a trophozoite is known as excystation.

Protozoa can reproduce by binary fission or multiple fission. Some protozoa reproduce sexually, some asexually, while some use a combination, (e.g. Coccidia). An individual protozoon is hermaphroditic.

Classification

Protozoa were previously often grouped in the kingdom of Protista, together with the plant-like algae and fungus-like slime molds. As a result of 21st-century systematics, protozoa, along with ciliates, mastigophorans, and apicomplexans, are arranged as animal-like protists. With the possible exception of Myxozoa, protozoa are not categorized as Metazoa. Protozoans are unicellular organisms and are often called the animal-like protists because they subsist entirely on other organisms for food. Most protozoans can move about on their own. Amoebas, Paramecia, and Trypanosomes are all examples of animal-like Protists.

Sub-groups

Protozoa have been divided traditionally on the basis of their means of locomotion, although this character is no longer believed to represent genuine relationships:

- Flagellates (e.g. *Giardia lamblia*)
- Amoeboids (e.g. *Entamoeba histolytica*)
- Sporozoans (e.g. *Plasmodium knowlesi*)
    - Apicomplexa
    - Myxozoa
    - Microsporidia
- Ciliates (e.g. *Balantidium coli*)

Human disease

Some protozoa are human parasites, causing diseases.

Examples of human diseases caused by protozoa:

- Malaria
- Amoebiasis
- Giardiasis
- Toxoplasmosis
- Cryptosporidiosis
- Trichomoniasis
- Leishmaniasis
- Sleeping Sickness
- Dysentery

Chapter 5

Mycotoxin

A **mycotoxin** (from Greek μύκης (mykes, mukos) "fungus" and Latin (toxicum) "poison") is a toxic secondary metabolite produced by organisms of the fungus kingdom, commonly known as molds. The term 'mycotoxin' is usually reserved for the toxic chemical products produced by fungi that readily colonize crops. Most fungi are aerobic (use oxygen) and are found almost everywhere in extremely small quantities due to the minute size of their spores. They consume organic matter wherever humidity and temperature are sufficient. One mold species may produce many different mycotoxins and/or the same mycotoxin as another species.

Where conditions are right, fungi proliferate into colonies and mycotoxin levels become high. The reason for the production of mycotoxins is not yet known; they are neither necessary for growth nor the development of the fungi. Because mycotoxins weaken the receiving host, the fungus may use them as a strategy to better the environment for further fungal proliferation. The production of toxins depends on the surrounding intrinsic and extrinsic environments and the toxins vary greatly in their severity, depending on the organism infected and its susceptibility, metabolism, and defense mechanisms. Some of the health effects found in animals and humans include death, identifiable diseases or health problems, weakened immune systems without specificity to a toxin, and as allergens or irritants. Some mycotoxins are harmful to other micro-organisms such as other fungi or even bacteria; penicillin is one example.

Mycotoxins can appear in the food chain as a result of fungal infection of crops, either by being eaten directly by humans, or by being used as livestock feed. Mycotoxins greatly resist decomposition or being broken down in digestion, so they remain in the food chain in meat and dairy products. Even temperature treatments, such as cooking and freezing, do not destroy mycotoxins.

Although various wild mushrooms contain an assortment of poisons that are definitely fungal metabolites causing noteworthy health problems for humans, they are rather

arbitrarily excluded from discussions of mycotoxicology. In such cases the distinction is based on the size of the producing fungus and human intention. Mycotoxin exposure is almost always accidental whereas with mushrooms improper identification and ingestion causing mushroom poisoning is commonly the case. Ingestion of misidentified mushrooms containing mycotoxins may result in hallucinations. The cyclopeptide-produced *Amanita phalloide* is well known for its toxic potential and is responsible for approximately 90% of all mushroom fatalities. The other primary mycotoxin groups found in mushrooms include: orellanine, monomethylhydrazine, disulfiram-like, hallucinogenic indoles, muscarinic, isoxazole, and gastrointestinal (GI)-specific irritants.

Many international agencies are trying to achieve universal standardization of regulatory limits for mycotoxins. Currently, over 100 countries have regulations regarding mycotoxins in the feed industry, in which 13 mycotoxins or groups of mycotoxins are of concern. The process of assessing a need for mycotoxin regulation includes a wide array of in-laboratory testing which includes extracting, clean-up and separation techniques. Most official regulations and control methods are based on high-performance liquid techniques (e.g., HPLC) through international bodies. It is implied that any regulations regarding these toxins will be in co-ordinance with any other countries with which a trade agreement exists. Many of the standards for the method performance analysis for mycotoxins is set by the European Committee for Standardization (CEN). However, one must take note that scientific risk assessment is commonly influenced by culture and politics which, in turn, will affect trade regulations of mycotoxins.

Food-based mycotoxins were studied extensively worldwide throughout the 20th century. In Europe, statutory levels of a range of mycotoxins permitted in food and animal feed are set by a range of European directives and Commission regulations. The U.S. Food and Drug Administration has regulated and enforced limits on concentrations of mycotoxins in foods and feed industries since 1985. It is through various compliance programs that the FDA monitors these industries to guarantee that mycotoxins are kept at a practical level. These compliance programs sample food products including peanuts and peanut products, tree nuts, corn and corn products, cottonseed, and milk. There is still a lack of sufficient surveillance data on some mycotoxins that occur in the U.S. which is largely due to the lack of reliable analytical methods.

Major groups

Aflatoxins are a type of mycotoxin produced by *Aspergillus* species of fungi, such as *A. flavus* and *A. parasiticus*. The umbrella term aflatoxin refers to four different types of mycotoxins produced, which are B1, B2, G1, and G2. Aflatoxin B_1, the most toxic, is a potent carcinogen and has been directly correlated to adverse health effects, such as liver cancer, in many animal species. Aflatoxins are largely associated with commodities produced in the tropics and subtropics, such as cotton, peanuts, spices, pistachios and maize.

Ochratoxin is a mycotoxin that comes in three secondary metabolite forms, A, B, and C. All are produced by *Penicillium* and *Aspergillus* species. The three forms differ in that

Ochratoxin B (OTB) is a nonchlorinated form of Ochratoxin A (OTA) and that Ochratoxin C (OTC) is an ethyl ester form Ochatoxin A. *Aspergillus ochraceus* is found as a contaminant of a wide range of commodities including beverages such as beer and wine. *Aspergillus carbonarius* is the main species found on vine fruit, which releases its toxin during the juice making process. OTA has been labeled as a carcinogen and a nephrotoxin, and has been linked to tumors in the human urinary tract, although research in humans is limited by confounding factors.

Citrinin is a toxin that was first isolated from *Penicillium citrinum*, but has been identified in over a dozen species of *Penicillium* and several species of *Aspergillus*. Some of these species are used to produce human foodstuffs such as cheese (*Penicillium camemberti*), sake, miso, and soy sauce (*Aspergillus oryzae*). Citrinin is associated with yellow rice disease in Japan and acts as a nephrotoxin in all animal species tested. Although it is associated with many human foods (wheat, rice, corn, barley, oats, rye, and food colored with Monascus pigment) its full significance for human health is unknown. Citrinin can also act synergistically with Ochratoxin A to depress RNA synthesis in murine kidneys.

Ergot Alkaloids are compounds produced as a toxic mixture of alkaloids in the sclerotia of species of *Claviceps*, which are common pathogens of various grass species. The ingestion of ergot sclerotia from infected cereals, commonly in the form of bread produced from contaminated flour, cause ergotism the human disease historically known as St. Anthony's Fire. There are two forms of ergotism gangrenous affecting blood supply to extremities and convulsive which affects the central nervous system. Modern methods of grain cleaning have significantly reduced ergotism as a human disease, however it is still an important veterinarian problem. Ergot alkaloids have been used pharmaceutically.

Patulin is a toxin produced by the *P. expansum*, *Aspergillus*, *Penicillium*, and *Paecilomyces* fungal species. *P. expansum* is especially associated with a range of moldy fruits and vegetables, in particular rotting apples and figs. It is destroyed by the fermentation process and so is not found in apple beverages, such as cider. Although patulin has not been shown to be carcinogenic, it has been reported to damage the immune system in animals. In 2004, the European Community set limits to the concentrations of patulin in food products. They currently stand at 50 μg/kg in all fruit juice concentrations, at 25 μg/kg in solid apple products used for direct consumption, and at 10 μg/kg for children's apple products, including apple juice.

Fusarium toxins are produced by over 50 species of *Fusarium* and have a history of infecting the grain of developing cereals such as wheat and maize. They include a range of mycotoxins, such as: the **fumonisins**, which affect the nervous systems of horses and may cause cancer in rodents; the **trichothecenes**, which are most strongly associated with chronic and fatal toxic effects in animals and humans; and **zearalenone**, which is not correlated to any fatal toxic effects in animals or humans. Some of the other major types of *Fusarium* toxins include: beauvercin and enniatins, butenolide, equisetin, and fusarins.

Binding agents and deactivators

In the feed and food industry it has become common practice to add mycotoxin binding agents such as Montmorillonite or bentonite clay in order to affectively adsorb the mycotoxins. To reverse the adverse effects of mycotoxins, the following criteria are used to evaluate the functionality of any binding additive:

- Efficacy of active component verified by scientific data
- A low effective inclusion rate
- Stability over a wide pH range
- High capacity to adsorb high concentrations of mycotoxins
- High affinity to adsorb low concentrations of mycotoxins
- Affirmation of chemical interaction between mycotoxin and adsorbent
- Proven *in vivo* data with all major mycotoxins
- Non-toxic, environmentally friendly component

Since not all mycotoxins can be bound to such agents, the latest approach to mycotoxin control is mycotoxin deactivation. By means of enzymes (esterase, epoxidase), yeast (*Trichosporon mycotoxinvorans*) or bacterial strains (Eubacterium BBSH 797), mycotoxins can be reduced during pre-harvesting contamination. Other removal methods include physical separation, washing, milling, heat-treatment, radiation, extraction with solvents, and the use of chemical or biological agents. Irradiation methods have proven to be effective treatment against mold growth and toxin production.

In the indoor environment

Buildings are another source of mycotoxins and people living or working in areas with mold increase their chances of adverse health effects. Molds growing in buildings can be divided into three groups — Primary, Secondary, and Tertiary colonizers. Each group is categorized by the ability to grow at a certain water activity requirement. It has become difficult to identify mycotoxins production by indoor molds for many variables, such as (i) they may be masked as derivatives (ii) they are poorly documented and (iii) the fact that they are likely to produce different metabolites on building materials. Some of the mycotoxins in the indoor environment are produced by Alternaria, Aspergillus (multiple forms), Penicillium, and Stachybotrys. Stachybotrys chartarum contains a higher number of mycotoxins than other molds grown in the indoor environment and has been associated with allergies and respiratory inflammation. The infestation of S. chartarum in buildings containing gypsum board, as well as on ceiling tiles, is very common and has recently become a more recognized problem. When gypsum board has been repeatedly introduced to moisture S. chartarum grows readily on its cellulose face. This stresses the importance of moisture controls and ventilation within residential homes and other buildings. The negative health effects of mycotoxins are a function of the concentration, the duration of exposure and the subject's sensitivities. The concentrations experienced in a normal home, office or school are often too low to trigger a health response in occupants.

In the 1990s, public concern over mycotoxins increased following multi-million dollar toxic mold settlements. The lawsuits took place after the Center for Disease Control (CDC) did a study in Cleveland Ohio and claimed that there was an association between mycotoxins from *Stachybotrys* spores and pulmonary hemorrhage in infants. However in 2000, based on internal and external reviews of their data, the CDC concluded that because of flaws in their methods the association was not proven. *Stachybotrys* spores in animal studies have been shown to cause lung hemorrhaging but only at very high concentrations.

One study by the Center of Integrative Toxicology at Michigan State University investigated the causes of Damp Building Related Illness (DBRI). They found that *Stachybotrys* is possibly an important contributing factor to DBRI. So far animal models indicate that airway exposure to *S. chartarum* can evoke allergic sensitization, inflammation, and cytotoxicity in the upper and lower respiratory tracts. Trichothecene toxicity appears to be an underlying cause of many these adverse effects. Recent findings indicate that lower doses (studies usually involve high doses) can cause these symptoms.

Some toxicologists have used the Concentration of No Toxicological Concern (CoNTC) measure to represent the airborne concentration of mycotoxins that are expected to cause no hazard to humans (exposed continuously throughout a 70–yr lifetime). The resulting data of several studies have thus far demonstrated that common exposures to airborne mycotoxins in the built indoor environment are below the CoNTC, however agricultural environments have potential to produce levels greater than the CoNTC.

Human health effects

Mycotoxicoses is the term used for poisoning associated with exposures to mycotoxins. The symptoms of a mycotoxicosis depend on the type of mycotoxin; the concentration and length of exposure; as well as age, health, and sex of the exposed individual. The synergistic effects associated with several other factors such as genetics, diet, and interactions with other toxics have been poorly studied. Therefore it is possible that vitamin deficiency, caloric deprivation, alcohol abuse, and infectious disease status can all have compounded effects with mycotoxins. In turn, mycotoxins have the potential for both acute and chronic health effects via ingestion, skin contact, and inhalation. These toxins can enter the blood stream and lymphatic system, they inhibit protein synthesis, damage macrophage systems, inhibit particle clearance of the lung, and increase sensitivity to bacterial endotoxin.

Notably Severe Cases of Aflatoxin Ingestion: In 2004 in Kenya 125 people died and nearly 200 others were treated after eating aflatoxin contaminated maize. The deaths were mainly associated with homegrown maize that had not been treated with fungicides or properly dried before storage. Due to food shortages at the time, farmers may have been harvesting maize earlier than normal to prevent thefts from their fields, so that the grain had not fully matured and was more susceptible to infection.

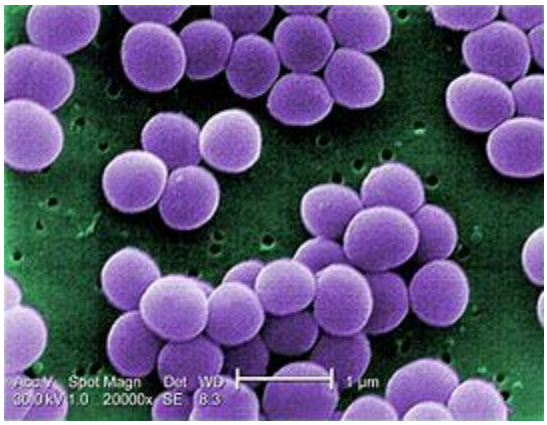
In pet food

There have been outbreaks of pet food containing mycotoxins in North America.

Chapter 6

Staphylococcus Aureus

Scanning electron micrograph of S. aureus, 20,000x, false color added.



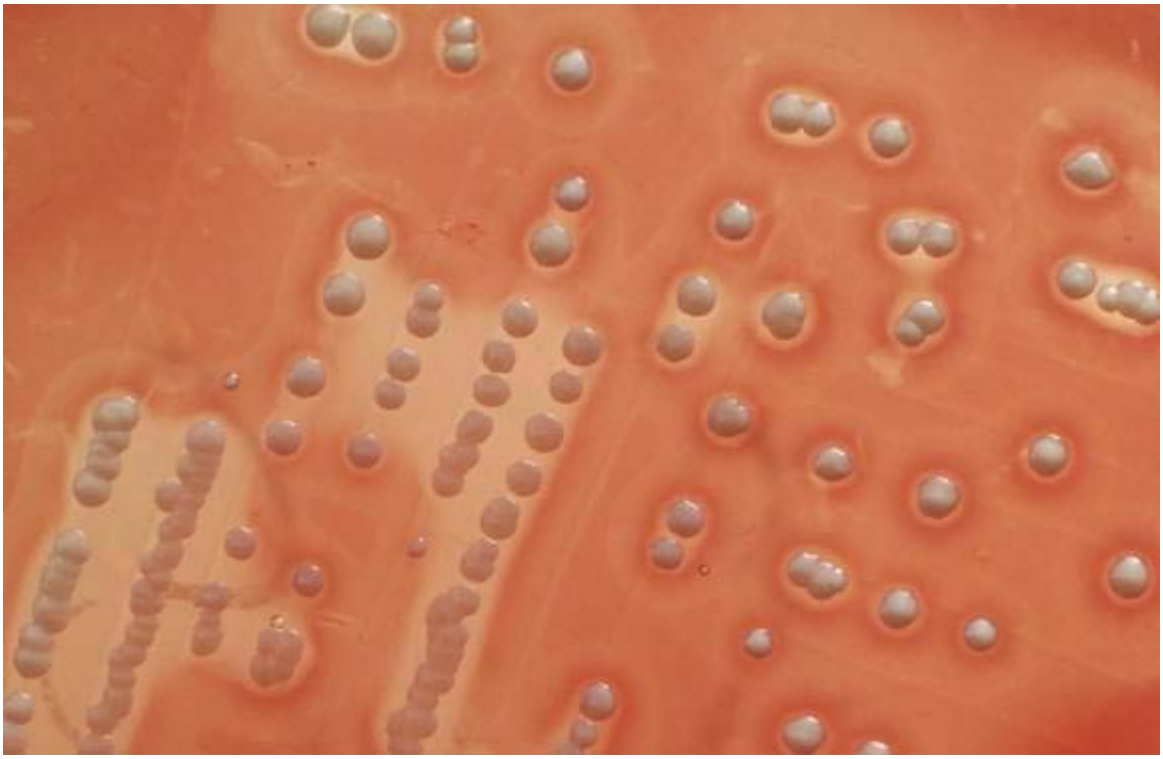
Scientific classification	
Domain:	Bacteria
Kingdom:	Eubacteria
Phylum:	Firmicutes
Class:	Bacilli
Order:	Bacillales
Family:	Staphylococcaceae
Genus:	*Staphylococcus*
Species:	***aureus***

Binomial name

Staphylococcus aureus
Rosenbach 1884

Staphylococcus Aureus

ICD-9 041.11



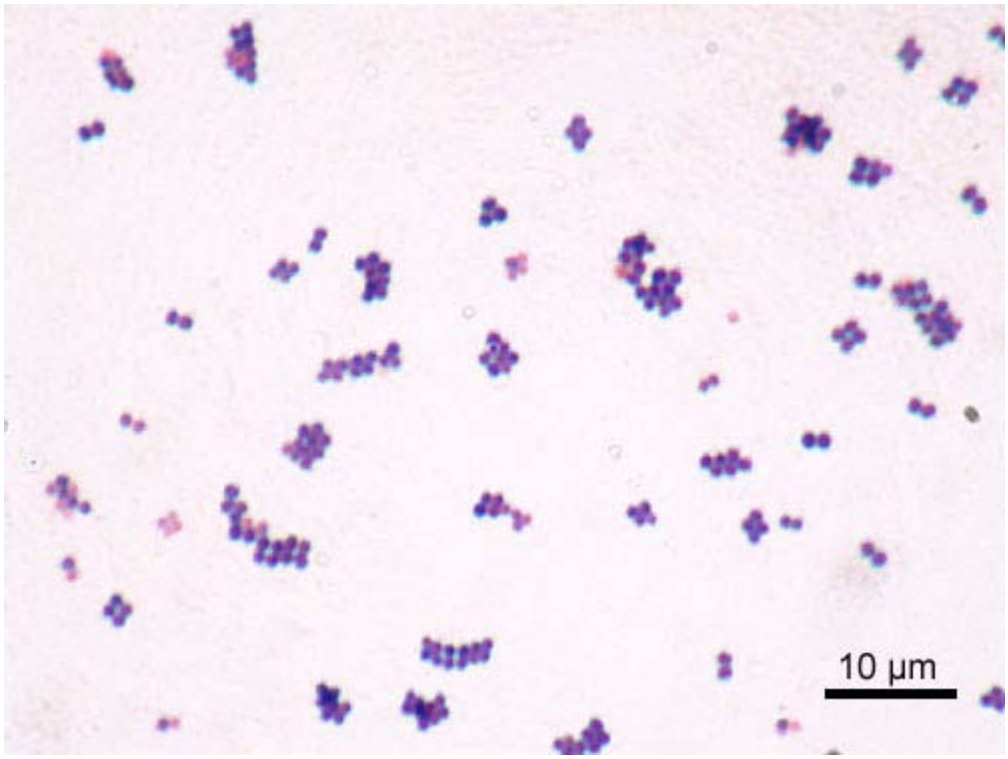
Yellow colonies of *S. aureus* on a blood agar plate. Note regions of clearing around colonies, caused by lysis of red cells in the agar (beta hemolysis)

Staphylococcus aureus is a facultative anaerobic, Gram-positive coccus and is the most common cause of staph infections. It is frequently part of the skin flora found in the nose and on skin. About 20% of the human population are long-term carriers of *S. aureus*. The carotenoid pigment staphyloxanthin is responsible for S. aureus' characteristic golden colour, which may be seen in colonies of the organism. This pigment acts as a virulence factor with an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system. Staph organisms which lack the pigment are more easily killed by host defenses.

S. aureus can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections. Abbreviated to *S. aureus* or *Staph aureus* in medical literature, *S. aureus* should not be confused with the similarly named and similarly dangerous (and also medically relevant) species of the genus *Streptococcus*.

S. aureus was discovered in Aberdeen, Scotland in 1880 by the surgeon Sir Alexander Ogston in pus from surgical abscesses. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection.

Microbiology

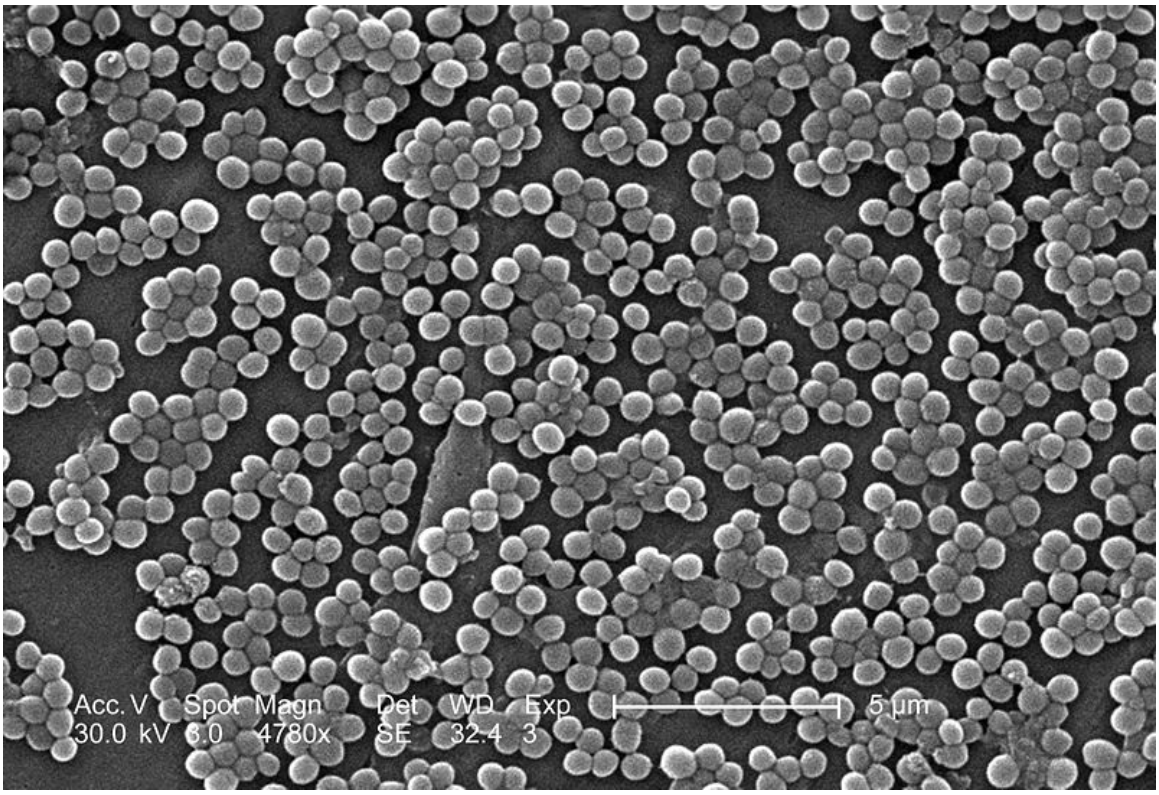


Gram stain of *S. aureus*. Staph aureus typically occur more in clusters than chains, and the cells take up Gram stain well

S. aureus is a facultatively anaerobic, Gram-positive coccus, which appears as grape-like clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. The golden appearance is the etymological root of the bacteria's name; *aureus* means "golden" in Latin.

S. aureus is catalase-positive (meaning that it can produce the enzyme "catalase") and able to convert hydrogen peroxide (H_2O_2) to water and oxygen, which makes the catalase test useful to distinguish staphylococci from enterococci and streptococci. A small percentage of *S. aureus* can be differentiated from most other staphylococci by the coagulase test: *S. aureus* is primarily coagulase-positive (meaning that it can produce the enzyme "coagulase") that causes clot formation, whereas most other *Staphylococcus* species are coagulase-negative. However, while the majority of *S. aureus* are coagulase-positive, some may be atypical in that they do not produce coagulase (the most common organism in patients with nosocomial bacteremia is coagulase-negative staphylococcus). Incorrect identification of an isolate can impact implementation of effective treatment and/or control measures.

Role in disease



SEM micrograph of Methicillin-resistant Staphylococcus aureus

Strains are responsible for food poisoning through the production of an enterotoxin and pathogenicity is also associated with coagulase positivity.

S. aureus may occur as a commensal on skin; it also occurs in the nose frequently (in about a third of the population) and throat less commonly. The occurrence of *S. aureus* under these circumstances does not always indicate infection and, therefore, does not always require treatment (indeed, treatment may be ineffective and re-colonisation may occur). It can survive on domesticated animals such as dogs, cats, and horses, and can cause bumblefoot in chickens. It can survive for hours to days, weeks, or even months on dry environmental surfaces depending on strain. It can host phages, such as Panton-Valentine leukocidin, that increase its virulence.

S. aureus can infect other tissues when barriers have been breached (e.g., skin or mucosal lining). This leads to furuncles and carbuncles (a collection of furuncles). In infants *S. aureus* infection can cause a severe disease staphylococcal scalded skin syndrome (SSSS).

S. aureus infections can be spread through contact with pus from an infected wound, skin-to-skin contact with an infected person by producing hyaluronidase that destroys tissues, and contact with objects such as towels, sheets, clothing, or athletic equipment used by an infected person. Deeply penetrating *S. aureus* infections can be severe.

Prosthetic joints put a person at particular risk for septic arthritis, and staphylococcal endocarditis (infection of the heart valves) and pneumonia, which may be rapidly spread.

Atopic dermatitis

S. aureus is extremely prevalent in atopic dermatitis patients who are less resistant to it than other people. It often causes complications, and this disease is mostly found in fertile active places including, the armpits, hair, and scalp. The large pimples that appear in those areas may cause the worst of the infection if popped. This can lead to scalded skin syndrome. A severe form of this is Ritter's disease seen in neonates.

Toxic shock syndrome and *S. aureus* food poisoning

Some strains of *S. aureus*, which produce the exotoxin TSST-1, are the causative agents of toxic shock syndrome. Some strains of *S. aureus* also produce an enterotoxin that is the causative agent of *S. aureus* gastroenteritis. The gastroenteritis is self-limiting, with the person recovering in 8–24 hours. Symptoms include nausea, vomiting, diarrhea, and major abdominal pain. Lack of antibody to TSST-1 plays a part in the pathogenesis of toxic shock syndrome.

Mastitis in cows

S. aureus is one of the causal agents of mastitis in dairy cows. Its large polysaccharide capsule protects the organism from recognition by the cow's immune defenses.

Reproduction

S. aureus reproduces asexually. It starts this process by reproducing its DNA. The membrane stretches out and separates the DNA molecules. The cells form a hollow space that eventually divides out into two new cells. The new cell wall does not fully separate from the existing cell wall, which is why the cells are observed in clusters. This cell will eventually reproduce and cells will attach onto it.

Virulence factors

Toxins

Depending on the strain, *S. aureus* is capable of secreting several exotoxins, which can be categorized into three groups. Many of these toxins are associated with specific diseases.

Superantigens
: (PTSAgs) have superantigen activities that induce toxic shock syndrome (TSS). This group includes the toxin TSST-1, which causes TSS associated with tampon use. This is characterized by fever, erythematous rash, hypotension, shock, multi organ failure, and skin desquamation. The staphylococcal enterotoxins, which

cause a form of food poisoning, characterized by vomiting and diarrhea 1-6 hours after ingested S aureus food, are included in this group.

Exfoliative toxins

EF toxins are implicated in the disease staphylococcal scalded-skin syndrome (SSSS), which occurs most commonly in infants and young children. It also may occur as epidemics in hospital nurseries. The protease activity of the exfoliative toxins causes peeling of the skin observed with SSSS.

Other toxins

Staphylococcal toxins that act on cell membranes include alpha toxin, beta toxin, delta toxin, and several bicomponent toxins. The bicomponent toxin Panton-Valentine leukocidin (PVL) is associated with severe necrotizing pneumonia in children. The genes encoding the components of PVL are encoded on a bacteriophage found in community-associated methicillin-resistant *S. aureus* (MRSA) strains.

Protein A

Protein A is a protein that is anchored to staphylococcal peptidoglycan pentaglycine bridges (chains of five glycine residues) by the transpeptidase Sortase A. Protein A is an IgG-binding protein that binds to the Fc region of an antibody. In fact, studies involving mutation of genes coding for Protein A resulted in a lowered virulence of *S. aureus* as measured by survival in blood, which has led to speculation that Protein A contributed virulence requires binding of antibody Fc regions. Protein A in various recombinant forms has been used for decades to bind and purify a wide range of antibodies by immunoaffinity chromatography. Transpeptidases such as the sortases that are responsible for anchoring factors like Protein A to the staphylococcal peptidoglycan are being studied in hopes of developing new antibiotics to target MRSA infections.

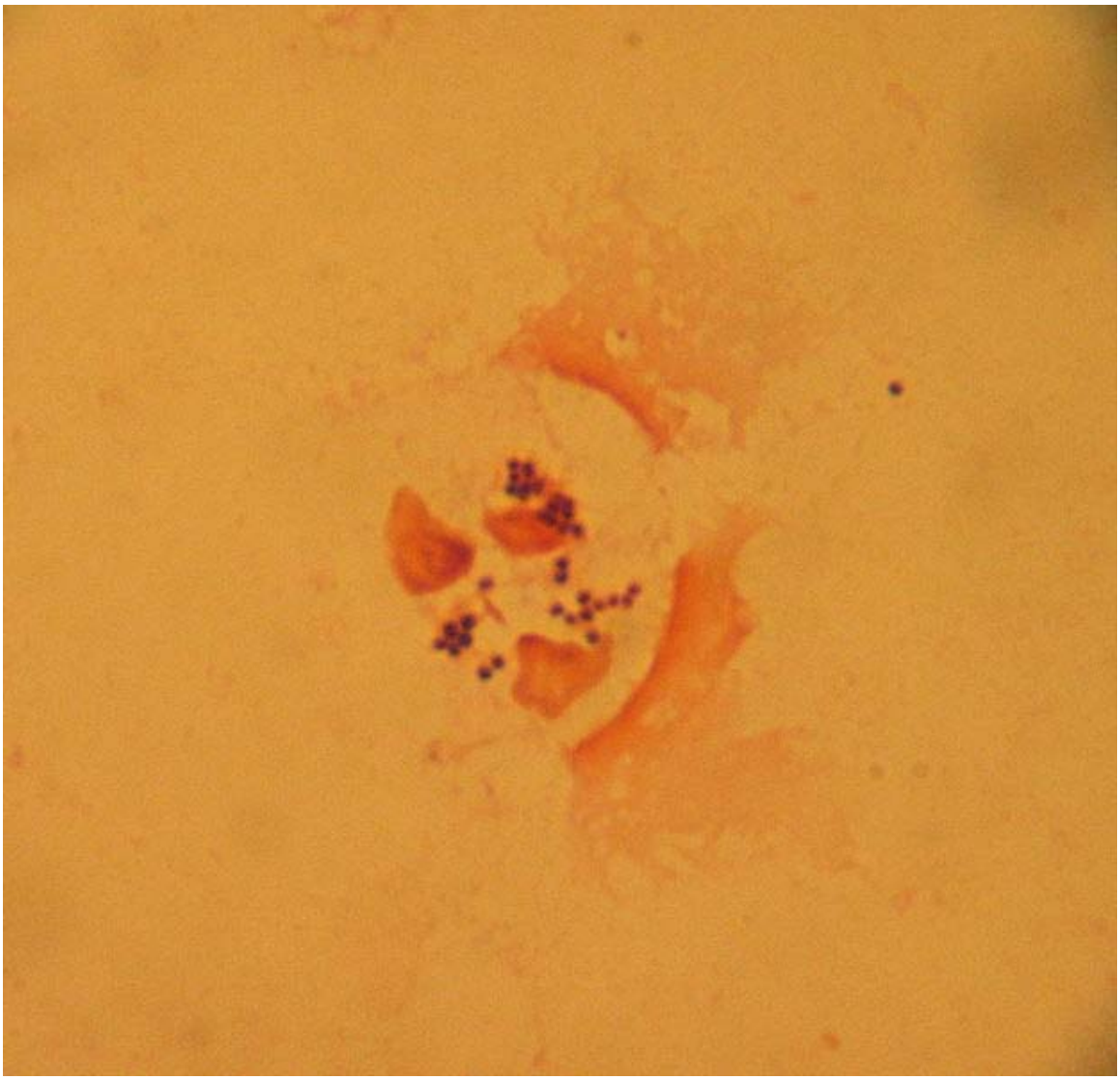
Role of pigment in virulence

Some strains of *S. aureus* are capable of producing *staphyloxanthin* - a carotenoid pigment that acts as a virulence factor. It has an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system. Staphyloxanthin is responsible for S. aureus' characteristic golden colour. When comparing a normal strain of *S. aureus* with a strain modified to lack staphyloxanthin, the wildtype pigmented strain was more likely to survive incubation with an oxidizing chemical such as hydrogen peroxide than the mutant strain was. Colonies of the two strains were also exposed to human neutrophils. The mutant colonies quickly succumbed while many of the pigmented colonies survived. Wounds on mice were inoculated with the two strains. The pigmented strains created lingering abscesses. Wounds with the unpigmented strains healed quickly.

These tests suggest that the staphyloxanthin may be key to the ability of *S. aureus* to survive immune system attacks. Drugs designed to inhibit the bacterium's production of the staphyloxanthin may weaken it and renew its susceptibility to antibiotics. In fact, because of similarities in the pathways for biosynthesis of staphyloxanthin and human

cholesterol, a drug developed in the context of cholesterol-lowering therapy was shown to block *S. aureus* pigmentation and disease progression in a mouse infection model.

Classical diagnosis



Typical gram-positive cocci, in clusters, from a sputum sample Gram stain.

Depending upon the type of infection present, an appropriate specimen is obtained accordingly and sent to the laboratory for definitive identification by using biochemical or enzyme-based tests. A Gram stain is first performed to guide the way, which should show typical gram-positive bacteria, cocci, in clusters. Second, the isolate is cultured on mannitol salt agar, which is a selective medium with 7–9% NaCl that allows *S. aureus* to grow, producing yellow-colored colonies as a result of mannitol fermentation and subsequent drop in the medium's pH. Furthermore, for differentiation on the species level, catalase (positive for all *Staphylococcus* species), coagulase (fibrin clot formation, positive for *S. aureus*), DNAse (zone of clearance on nutrient agar), lipase (a yellow

color and rancid odor smell), and phosphatase (a pink color) tests are all done. For staphylococcal food poisoning, phage typing can be performed to determine if the staphylococci recovered from the food to determine the source of infection.

Rapid diagnosis and typing

Diagnostic microbiology laboratories and reference laboratories are key for identifying outbreaks and new strains of *S. aureus*. Recent genetic advances have enabled reliable and rapid techniques for the identification and characterization of clinical isolates of *S. aureus* in real-time. These tools support infection control strategies to limit bacterial spread and ensure the appropriate use of antibiotics. Real-time PCR is being increasingly employed in clinical laboratories as a technique to identifying outbreaks.

Treatment and antibiotic resistance

The treatment of choice for *S. aureus* infection is penicillin; but, in most countries, penicillin-resistance is extremely common and first-line therapy is most commonly a penicillinase-resistant β-lactam antibiotic (for example, oxacillin or flucloxacillin). Combination therapy with gentamicin may be used to treat serious infections like endocarditis, but its use is controversial because of the high risk of damage to the kidneys. The duration of treatment depends on the site of infection and on severity.

Antibiotic resistance in *S. aureus* was uncommon when penicillin was first introduced in 1943. Indeed, the original petri dish on which Alexander Fleming of Imperial College London observed the antibacterial activity of the *penicillium* fungus was growing a culture of *S. aureus*. By 1950, 40% of hospital *S. aureus* isolates were penicillin-resistant; and, by 1960, this had risen to 80%.

Researchers from Italy have identified a bacteriophage active against *Staphylococcus aureus*, including methicillin-resistant strains (MRSA), in mice and possibly humans.

Mechanisms of antibiotic resistance

Staphylococcal resistance to penicillin is mediated by penicillinase (a form of β-lactamase) production: an enzyme that cleaves the β-lactam ring of the penicillin molecule, rendering the antibiotic ineffective. Penicillinase-resistant β-lactam antibiotics such as methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, and flucloxacillin are able to resist degradation by staphylococcal penicillinase.

Resistance to methicillin is mediated via the *mec* operon, part of the staphylococcal cassette chromosome mec (SCC*mec*). Resistance is conferred by the *mecA* gene, which codes for an altered penicillin-binding protein (PBP2a or PBP2') that has a lower affinity for binding β-lactams (penicillins, cephalosporins, and carbapenems). This allows for resistance to all β-lactam antibiotics and obviates their clinical use during MRSA infections. As such, the glycopeptide vancomycin is often deployed against MRSA.

Aminoglycoside antibiotics such as kanamycin, gentamicin, streptomycin, etc. were once effective against Staphylococcal infections until strains evolved mechanisms to inhibit the aminoglycosides action, which occurs via protonated amine and/or hydroxyl interactions with the ribosomal RNA of the bacterial 30S ribosomal subunit There are three main mechanisms of aminoglycoside resistance mechanisms which are currently and widely accepted: Aminoglycoside modifying enzymes, Ribosomal mutations, and active efflux of the drug out of the bacteria.

Aminoglycoside-modifying enzymes inactivate the aminoglycoside by covalently attaching either a phosphate, nucleotide, or acetyl moiety to either the amine and/or the alcohol key functional group of the antibiotic. This changes the charge or sterically hinders the antibiotic, decreasing its ribosomal binding affinity. In *S. aureus*, the best-characterized aminoglycoside modifying enzyme is ANT(4')IA *Aminoglycoside adenylyltransferase 4′ IA*. This enzyme has been solved by x-ray crystallography. The enzyme is able to attach an adenyl moiety to the 4' hydroxyl group of many aminoglycosides including kamamycin and gentamicin.

Glycopeptide resistance is mediated by acquisition of the *vanA* gene. The *vanA* gene originates from the *enterococci* and codes for an enzyme that produces an alternative peptidoglycan to which vancomycin will not bind.

Today, *S. aureus* has become resistant to many commonly used antibiotics. In the UK, only 2% of all *S. aureus* isolates are sensitive to penicillin with a similar picture in the rest of the world. The β-lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin, and flucloxacillin) were developed to treat penicillin-resistant *S. aureus* and are still used as first-line treatment. Methicillin was the first antibiotic in this class to be used (it was introduced in 1959), but, only two years later, the first case of methicillin-resistant *S. aureus* (MRSA) was reported in England.

Despite this, MRSA generally remained an uncommon finding even in hospital settings until the 1990s when there was an explosion in MRSA prevalence in hospitals where it is now endemic.

MRSA infections in both the hospital and community setting are commonly treated with non-β-lactam antibiotics such as clindamycin (a lincosamine) and co-trimoxazole (also commonly known as trimethoprim/sulfamethoxazole). Resistance to these antibiotics has also led to the use of new, broad-spectrum anti-Gram-positive antibiotics such as linezolid because of its availability as an oral drug. First-line treatment for serious invasive infections due to MRSA is currently glycopeptide antibiotics (vancomycin and teicoplanin). There are number of problems with these antibiotics, such as the need for intravenous administration (there is no oral preparation available), toxicity, and the need to monitor drug levels regularly by blood tests. There are also concerns that glycopeptide antibiotics do not penetrate very well into infected tissues (this is a particular concern with infections of the brain and meninges and in endocarditis). Glycopeptides must not be used to treat methicillin-sensitive *S. aureus* (MSSA) as outcomes are inferior.

Because of the high level of resistance to penicillins and because of the potential for MRSA to develop resistance to vancomycin, the Centers for Disease Control and Prevention have published guidelines for the appropriate use of vancomycin. In situations where the incidence of MRSA infections is known to be high, the attending physician may choose to use a glycopeptide antibiotic until the identity of the infecting organism is known. After the infection is confirmed to be due to a methicillin-susceptible strain of *S. aureus*, treatment can be changed to flucloxacillin or even penicillin as appropriate.

Vancomycin-resistant *S. aureus* (VRSA) is a strain of *S. aureus* that has become resistant to the glycopeptides. The first case of vancomycin-intermediate *S. aureus* (VISA) was reported in Japan in 1996; but the first case of *S. aureus* truly resistant to glycopeptide antibiotics was only reported in 2002. Three cases of VRSA infection have been reported in the United States as of 2005.

Infection control

Spread of *S. aureus* (including MRSA) is through human-to-human contact, although recently some veterinarians have discovered that the infection can be spread through pets , with environmental contamination thought to play a relatively unimportant part. Emphasis on basic hand washing techniques are, therefore, effective in preventing the transmission of *S. aureus*. The use of disposable aprons and gloves by staff reduces skin-to-skin contact and, therefore, further reduces the risk of transmission.

Recently, there have been myriad reported cases of *S. aureus* in hospitals across America. The pathogen has had facilitated transportation in medical facilities mainly because of insufficient healthcare worker hygiene. *S. aureus* is an incredibly hardy bacterium, as was shown in a study where it survived on polyester for just under three months, polyester being the main material used in hospital privacy curtains.

The bacterium is transported on the hands of healthcare workers who may pick up the bacteria from a seemingly healthy patient carrying a "benign" or commensal strain of *S. aureus* and then pass it on to the next patient being cared for. Introduction of the bacterium into the bloodstream can lead to various complications including, but not limited to, endocarditis, meningitis, and, if it is widespread, sepsis.

Ethanol has proven to be an effective topical sanitizer against MRSA. Quaternary ammonium can be used in conjunction with ethanol to increase the duration of the sanitizing action. The prevention of nosocomial infections involves routine and terminal cleaning. Nonflammable alcohol vapor in CO_2 NAV-CO2 systems have an advantage as they do not attack metals or plastics used in medical environments, and do not contribute to antibacterial resistance.

An important and previously unrecognized means of community-associated MRSA colonization and transmission is during sexual contact.

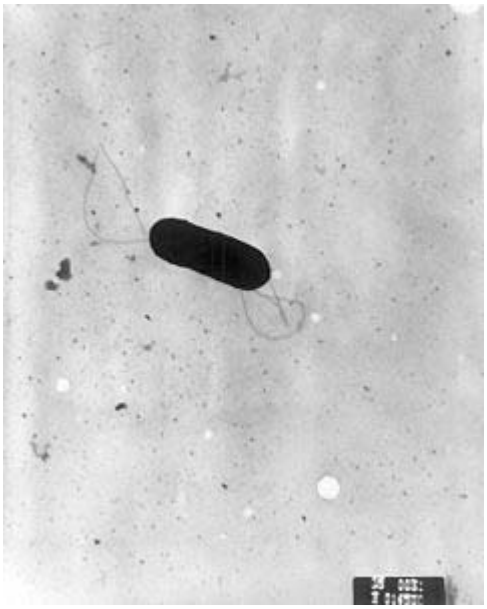
Staff or patients who are found to carry resistant strains of *S. aureus* may be required to undergo "eradication therapy," which may include antiseptic washes and shampoos (such as chlorhexidine) and application of topical antibiotic ointments (such as mupirocin or neomycin) to the anterior nares of the nose.

The nonprotein amino acid L-homoarginine is a growth inhibitor of Staphylococcus aureus as well as *Candida albicans*. It is assumed to be an antimetabolite of arginine.

Chapter 7

Listeria Monocytogenes

Listeria monocytogenes



Scanning electron micrograph of *Listeria monocytogenes*.

Scientific classification	
Kingdom:	Bacteria
Division:	Firmicutes
Class:	Bacilli
Order:	Bacillales
Family:	Listeriaceae
Genus:	*Listeria*
Species:	***L. monocytogenes***

Binomial name
Listeria monocytogenes

(Murray et al. 1926) Pirie 1940

Listeria monocytogenes is a facultative anaerobe, intracellular bacterium that is the causative agent of Listeriosis. It is one of the most virulent foodborne pathogens with 20 to 30 percent of clinical infections resulting in death. Responsible for approximately 2,500 illnesses and 500 deaths in the United States (U.S.) annually, Listeriosis is the leading cause of death among foodborne bacterial pathogens with fatality rates exceeding even Salmonella and Clostridium botulinum.

L. monocytogenes is a Gram-positive bacterium, in the division Firmicutes, named for Joseph Lister. Motile via flagella at 30 °C and below but usually not at 37 °C, *L. monocytogenes* can instead move within eukaryotic cells by explosive polymerization of actin filaments (known as *comet tails* or *actin rockets*).

Studies suggest that up to 10% of human gastrointestinal tracts may be colonized by *L. monocytogenes*.

Nevertheless, clinical diseases due to *L. monocytogenes* are more frequently recognized by veterinarians, especially as meningo-encephalitis in ruminants. See: listeriosis in animals.

Due to its frequent pathogenicity causing meningitis in newborns (acquired transvaginally), pregnant mothers are often advised not to eat soft cheeses such as Brie, Camembert, feta and queso blanco fresco, which may be contaminated with and permit growth of *L. monocytogenes*. It is the third most common cause of meningitis in newborns.

More recently, *L. monocytogenes* has been used as the model organism to illustrate the Patho-biotechnology concept.

Classification

L. monocytogenes is a gram-positive, non-spore forming, motile, facultatively anaerobic, rod-shaped bacterium. It is catalase-positive, oxidase-negative, and expresses a Beta hemolysin which causes destruction of red blood cells. This bacterium exhibits characteristic tumbling motility when viewed with light microscopy. Although *L. monocytogenes* is actively motile by means of peritrichous flagella at room temperature (20−25 °C), the organism does not synthesize flagella at body temperatures (37 °C).

The genus *Listeria* belongs to the *Clostridium* sub-branch, together with *Staphylococcus*, *Streptococcus*, *Lactobacillus* and *Brochothrix*. The genus *Listeria* includes 6 different species (*L. monocytogenes*, *L. ivanovii*, *L. innocua*, *L. welshimeri*, *L. seeligeri*, and *L. grayi*). Both *L. ivanovii* and *L. monocytogenes* are pathogenic in mice, but only *L. monocytogenes* is consistently associated with human illness. There are 13 serotypes of *L. monocytogenes* which can cause disease, but more than 90 percent of human isolates belong to only three serotypes: 1/2a, 1/2b, and 4b. *L. monocytogenes* serotype 4b strains

are responsible for 33 to 50 percent of sporadic human cases worldwide and for all major foodborne outbreaks in Europe and North America since the 1980s.

History

L. monocytogenes was first described by E.G.D.Murray in 1926 based on six cases of sudden death in young rabbits. Murray referred to the organism as *Bacterium monocytogenes* before J.H. Harvey Pirie changed the genus name to Listeria in 1940. Although clinical descriptions of *L. monocytogenes* infection in both animals and humans were published in the 1920s, not until 1952 in East Germany was it recognized as a significant cause of neonatal sepsis and meningitis. Listeriosis in adults would later be associated with patients living with compromised immune systems, such as individuals taking immunosuppressant drugs and corticosteroids for malignancies or organ transplants, and those with HIV infection.

It wasn't until 1981, however, that *L. monocytogenes* was identified as a cause of foodborne illness. An outbreak of listeriosis in Halifax, Nova Scotia involving 41 cases and 18 deaths, mostly in pregnant women and neonates, was epidemiologically linked to the consumption of coleslaw containing cabbage that had been treated with *L. monocytogenes* contaminated raw sheep manure. Since then a number of cases of foodborne listeriosis have been reported, and *L. monocytogenes* is now widely recognized as an important hazard in the food industry.

Pathogenesis

Infection by *L. monocytogenes* causes the disease listeriosis. The manifestations of listeriosis include septicemia, meningitis (or meningoencephalitis), encephalitis, corneal ulcer, pneumonia, and intrauterine or cervical infections in pregnant women, which may result in spontaneous abortion (2nd/3rd trimester) or stillbirth. Surviving neonates of Fetomaternal Listeriosis may suffer granulomatosis infantiseptica - pyogenic granulomas distributed over the whole body, and may suffer from physical retardation. Influenza-like symptoms, including persistent fever, usually precede the onset of the aforementioned disorders. Gastrointestinal symptoms such as nausea, vomiting, and diarrhea may precede more serious forms of listeriosis or may be the only symptoms expressed. Gastrointestinal symptoms were epidemiologically associated with use of antacids or cimetidine. The onset time to serious forms of listeriosis is unknown but may range from a few days to three weeks. The onset time to gastrointestinal symptoms is unknown but probably exceeds 12 hours. An early study suggested that *L. monocytogenes* was unique among Gram-positive bacteria in that it possessed lipopolysaccharide, which served as an endotoxin. Later it was found to not be a true endotoxin, Listeria cell walls consistently contain lipoteichoic acids, in which a glycolipid moiety, such as a galactosyl-glucosyl-diglyceride, is covalently linked to the terminal phosphomonoester of the teichoic acid. This lipid region anchors the polymer chain to the cytoplasmic membrane. These lipoteichoic acids resemble the lipopolysaccharides of gram-negative bacteria in both structure and function, being the only amphipathic polymers at the cell surface.

The infective dose of *L. monocytogenes* varies with the strain and with the susceptibility of the victim. From cases contracted through raw or supposedly pasteurized milk, one may safely assume that in susceptible persons, fewer than 1,000 total organisms may cause disease. *L. monocytogenes* may invade the gastrointestinal epithelium. Once the bacterium enters the host's monocytes, macrophages, or polymorphonuclear leukocytes, it becomes blood-borne (septicemic) and can grow. Its presence intracellularly in phagocytic cells also permits access to the brain and probably transplacental migration to the fetus in pregnant women. The pathogenesis of *L. monocytogenes* centers on its ability to survive and multiply in phagocytic host cells.

Regulation of pathogenesis

L. monocytogenes can act as a saprophyte or a pathogen depending on its environment. When this bacteria is present within a host organism quorum sensing causes the up regulation of several virulence genes. Depending on the location of the bacteria within the host organism different activators up regulate the virulence genes. SigB, an alternative sigma factor, up regulates Vir genes in the intestines whereas PrfA up regulates gene expression when the bacteria is present in blood. Little is known about the mechanism in how this bacteria switches between acting as a saprophyte and a pathogen however, it is thought that several non-coding RNAs are required to induce this change.

Pathogenicity of Lineages

L. monocytogenes has three distinct lineages with differing evolutionary histories and pathogenic potentials. Lineage I strains contain the majority of human clinical isolates and all human epidemic clones, but are underrepresented in animal clinical isolates. Lineage II strains are overrepresented in animal cases and underrepresented in human clinical cases as well as more prevalent in environmental and food samples. Lineage III isolates are very rare but significantly more common in animal isolates than human.

Treatment

When listeric meningitis occurs, the overall mortality may reach 70%; from septicemia 50%, from perinatal/neonatal infections greater than 80%. In infections during pregnancy, the mother usually survives. Reports of successful treatment with parenteral penicillin or ampicillin exist. Trimethoprim-sulfamethoxazole has been shown effective in patients allergic to penicillin.

Bacteriophage treatments have been developed by several companies. EBI Food Safety and Intralytix both have products suitable for treatment of the bacteria. The Food and Drug Administration of the United States approved a cocktail of six bacteriophages from Intralytix, and a one type phage product from EBI Food Safety designed to kill the bacteria *L. monocytogenes*. Uses would potentially include spraying it on fruits and ready-to-eat meat such as sliced ham and turkey.

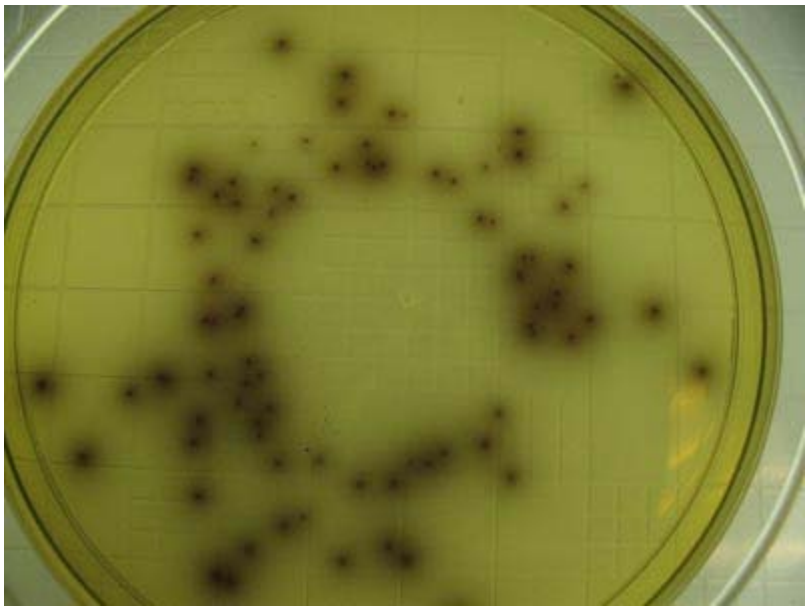
Use as a transfection vector

Because *L. monocytogenes* is an intracellular parasite, some studies have used this bacterium as a vector to deliver genes *in vitro*. Current transfection efficiency remains poor. One example of the successful use of *L. monocytogenes* in *in vitro* transfer technologies is in the delivery of gene therapies for cystic fibrosis cases.

Cancer vaccine

A live attenuated *L. monocytogenes* cancer vaccine named ADXS11-001 is under development as a possible treatment for cervical carcinoma.

Detection



Colonies of typical *Listeria monocytogenes* as they appear when grown on Listeria selective agar

The methods for analysis of food are complex and time-consuming. The present U.S. Food and Drug Administration (FDA) method, revised in September, 1990, requires 24 and 48 hours of enrichment, followed by a variety of other tests. Total time to identification takes from 5 to 7 days, but the announcement of specific nonradiolabled DNA probes should soon allow a simpler and faster confirmation of suspect isolates.

Recombinant DNA technology may even permit 2-to-3 day positive analysis in the future. Currently, the FDA is collaborating in adapting its methodology to quantitate very low numbers of the organisms in foods.

Epidemiology

Researchers have found *L. monocytogenes* in at least 37 mammalian species, both domesticated and feral, as well as in at least 17 species of birds and possibly in some species of fish and shellfish. Laboratories can isolate *L. monocytogenes* from soil, silage, and other environmental sources. *L. monocytogenes* is quite hardy and resists the deleterious effects of freezing, drying, and heat remarkably well for a bacterium that does not form spores. Most *L. monocytogenes* are pathogenic to some degree.

Routes of infection

L. monocytogenes has been associated with such foods as raw milk, pasteurized fluid milk, cheeses (particularly soft-ripened varieties), ice cream, raw vegetables, fermented raw-meat sausages, raw and cooked poultry, raw meats (of all types), and raw and smoked fish. Its ability to grow at temperatures as low as 0°C permits multiplication in refrigerated foods. In refrigeration temperature such as 4°C the amount of ferric iron promotes the growth of *L. monocytogenes*.

Infectious cycle

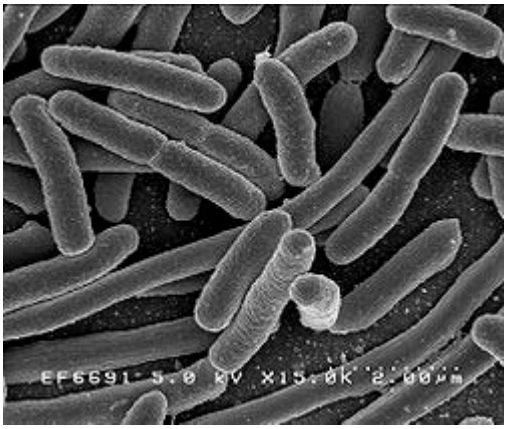
The primary site of infection is the intestinal epithelium where the bacteria invade non-phagocytic cells via the "zipper" mechanism. Uptake is stimulated by the binding of listerial internalins (Inl) to host cell adhesion factors such as E-cadherin or Met. This binding activates certain Rho-GTPases which subsequently bind and stabilize Wiskott Aldrich Syndrome Protein (WASp). WASp can then bind the Arp2/3 complex and serve as an actin nucleation point. Subsequent actin polymerization extends the cell membrane around the bacterium, eventually engulfing it. The net effect of internalin binding is to exploit the junction forming-apparatus of the host into internalizing the bacterium. Note that *L. monocytogenes* can also invade phagocytic cells (e.g. macrophages) but only requires internalins for invasion of non-phagocytic cells.

Following internalisation, the bacterium must escape from the vacuole/phagosome before fusion with a lysosome can occur. Three main virulence factors which allow the bacterium to escape are listeriolysin O (LLO - encoded by *hly*) phospholipase A (encoded by *plcA*) and phospholipase B (*plcB*). Secretion of LLO and PlcB disrupts the vacuolar membrane and allows the bacterium to escape into the cytoplasm where it may proliferate.

Once in the cytoplasm, *L. monocytogenes* exploits host actin for the second time. ActA proteins associated with the old bacterial cell pole (being a bacillus, *L. monocytogenes* septates in the middle of the cell and thus has one new pole and one old pole) are capable of binding the Arp2/3 complex and thus induce actin nucleation at a specific area of the bacterial cell surface. Actin polymerization then propels the bacterium unidirectionally into the host cell membrane. The protrusion which is formed may then be internalised by a neighbouring cell, forming a double-membrane vacuole from which the bacterium must escape using LLO and PlcB.

Chapter 8

Escherichia Coli

Escherichia coli

Scientific classification
Domain: Bacteria
Phylum: Proteobacteria
Class: Gammaproteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Escherichia*
Species: ***E. coli***
Binomial name
Escherichia coli (Migula 1895) Castellani and Chalmers 1919
Synonyms
Bacillus coli communis Escherich 1885

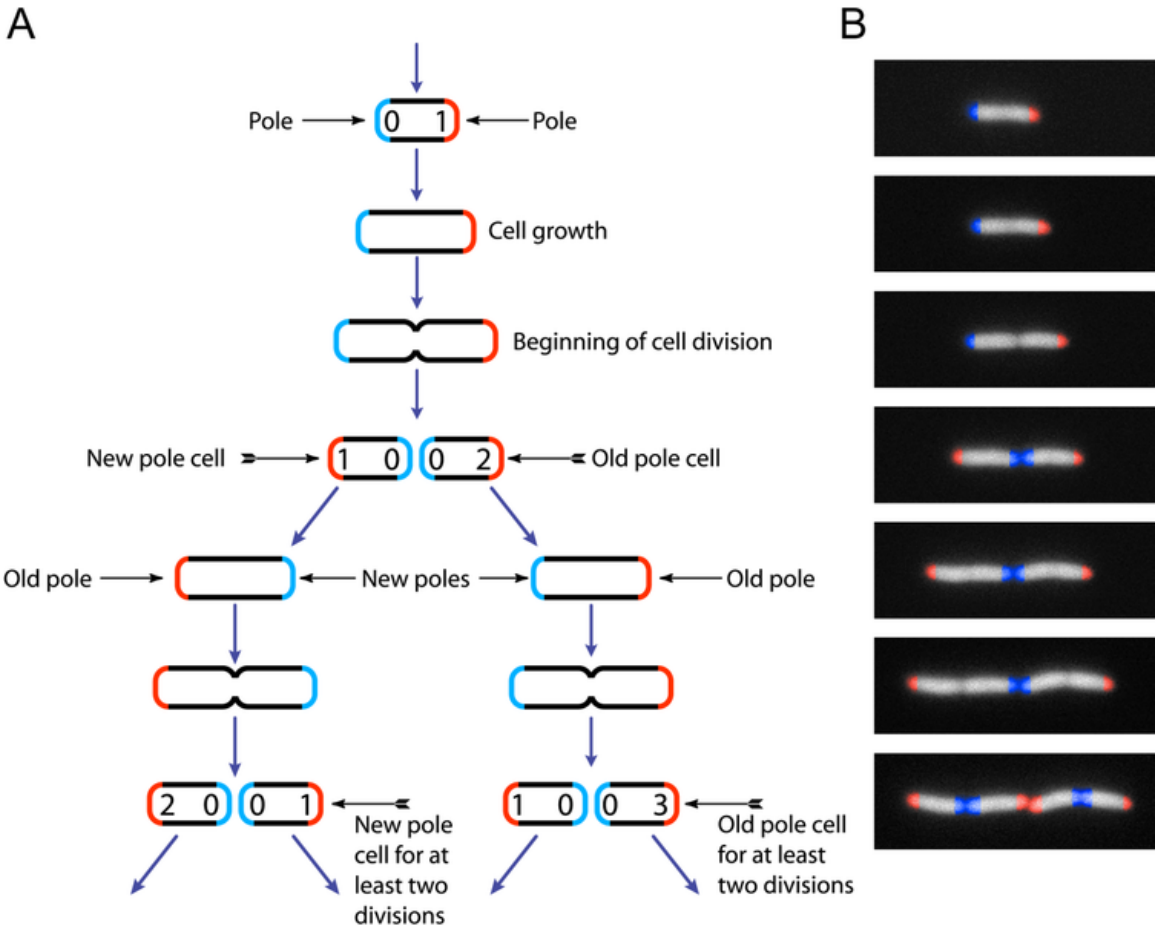
Escherichia coli is a Gram-negative rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms (endotherms). Most *E. coli* strains are

harmless, but some, such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for product recalls. The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, and by preventing the establishment of pathogenic bacteria within the intestine.

E. coli are not always confined to the intestine, and their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination. The bacteria can also be grown easily and its genetics are comparatively simple and easily manipulated or duplicated through a process of metagenics, making it one of the best-studied prokaryotic model organisms, and an important species in biotechnology and microbiology.

E. coli was discovered by German paediatrician and bacteriologist Theodor Escherich in 1885, and is now classified as part of the Enterobacteriaceae family of gamma-proteobacteria.

Biology and biochemistry



Model of successive binary fission in *E. coli*

E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped and are about 2 micrometres (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 - 0.7 $(\mu m)^3$. It can live on a wide variety of substrates. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E. coli* lives together with hydrogen-consuming organisms such as methanogens or sulphate-reducing bacteria.

Optimal growth of *E. coli* occurs at 37°C (98.6°F) but some laboratory strains can multiply at temperatures of up to 49°C (120.2°F). Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and trimethylamine N-oxide.

Strains that possess flagella can swim and are motile. The flagella have a peritrichous arrangement.

E. coli and related bacteria possess the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population. This process led to the spread of the gene encoding shiga toxin from *Shigella* to E. coli O157:H7, carried by a bacteriophage.

Diversity

As more is known about certain organisms, such as genetic information, the taxonomic classification of species is changed to reflect the advance in knowledge, however in the case of Escherichia coli due to its medical importance, this has not occurred (namely split into several genera/species) and remains one of the most diverse bacterial species: only 20% of the genome is common to all strains. In fact, from the evolutionary point of view, the members of genus Shigella (*dysenteriae*, *flexneri*, *boydii*, *sonnei*) are actually E. coli strains "in disguise" (i.e. E.coli is paraphyletic to the genus).

A strain of *E. coli* is a sub-group within the species that has unique characteristics that distinguish it from other *E. coli* strains. These differences are often detectable only at the molecular level; however, they may result in changes to the physiology or lifecycle of the bacterium. For example, a strain may gain pathogenic capacity, the ability to use a unique carbon source, the ability to take upon a particular ecological niche or the ability to resist antimicrobial agents. Different strains of *E. coli* are often host-specific, making it possible to determine the source of faecal contamination in environmental samples. For example, knowing which *E. coli* strains are present in a water sample allows researchers to make assumptions about whether the contamination originated from a human, another mammal or a bird.

New strains of *E. coli* evolve through the natural biological process of mutation and through horizontal gene transfer. Some strains develop traits that can be harmful to a host

animal. These virulent strains typically cause a bout of diarrhoea that is unpleasant in healthy adults and is often lethal to children in the developing world. More virulent strains, such as O157:H7 cause serious illness or death in the elderly, the very young or the immunocompromised.

E. coli is the type species of the genus and the type strain is ATCC 11775.

A common subdivison system of E.coli, but not based on evolutionary relatedness, is by serotype, which is based on major surface antigens (O antigen: part of lipopolysaccharide layer; H: flagellin; K antigen: capsule), e.g. O157:H7) (NB: K-12, the common laboratory strain is not a serotype.)

Role as normal microbiota

E. coli normally colonizes an infant's gastrointestinal tract within 40 hours of birth, arriving with food or water or with the individuals handling the child. In the bowel, it adheres to the mucus of the large intestine. It is the primary facultative anaerobe of the human gastrointestinal tract. (Facultative anaerobes are organisms that can grow in either the presence or absence of oxygen.) As long as these bacteria do not acquire genetic elements encoding for virulence factors, they remain benign commensals.

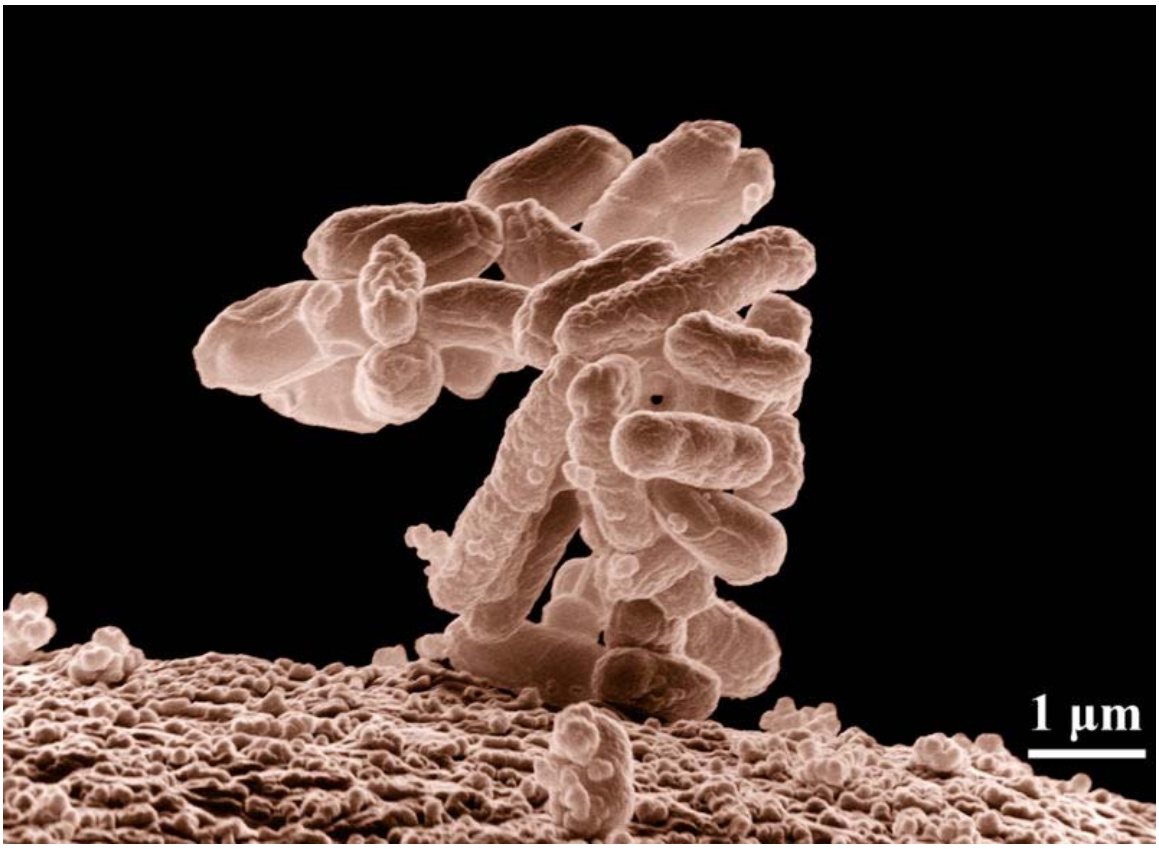
Therapeutic use of nonpathogenic *E. coli*

Nonpathogenic *Escherichia coli* strain Nissle 1917 also known as Mutaflor is used as a probiotic agent in medicine, mainly for the treatment of various gastroenterological diseases, including inflammatory bowel disease.

Role in disease

Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rarer cases, virulent strains are also responsible for haemolytic-uremic syndrome, peritonitis, mastitis, septicaemia and Gram-negative pneumonia.

Gastrointestinal infection



Low-temperature electron micrograph of a cluster of *E. coli* bacteria, magnified 10,000 times. Each individual bacterium is a rounded cylinder.

Certain strains of *E. coli*, such as O157:H7, O121 and O104:H21, produce potentially lethal toxins. Food poisoning caused by *E. coli* is usually caused by eating unwashed vegetables or undercooked meat. O157:H7 is also notorious for causing serious and even life-threatening complications such as haemolytic-uremic syndrome. This particular strain is linked to the 2006 United States *E. coli* outbreak due to fresh spinach. Severity of the illness varies considerably; it can be fatal, particularly to young children, the elderly or the immunocompromised, but is more often mild. Earlier, poor hygienic methods of preparing meat in Scotland killed seven people in 1996 due to *E. coli* poisoning, and left hundreds more infected. *E. coli* can harbour both heat-stable and heat-labile enterotoxins. The latter, termed LT, contains one A subunit and five B subunits arranged into one holotoxin, and is highly similar in structure and function to cholera toxins. The B subunits assist in adherence and entry of the toxin into host intestinal cells, while the A subunit is cleaved and prevents cells from absorbing water, causing diarrhea. LT is secreted by the Type 2 secretion pathway.

If *E. coli* bacteria escape the intestinal tract through a perforation (for example from an ulcer, a ruptured appendix, or due to a surgical error) and enter the abdomen, they usually cause peritonitis that can be fatal without prompt treatment. However, *E. coli* are

extremely sensitive to such antibiotics as streptomycin or gentamicin. This could change since, as noted below, *E. coli* quickly acquires drug resistance. Recent research suggests that treatment with antibiotics does not improve the outcome of the disease, and may in fact significantly increase the chance of developing haemolytic-uremic syndrome.

Intestinal mucosa-associated *E. coli* are observed in increased numbers in the inflammatory bowel diseases, Crohn's disease and ulcerative colitis. Invasive strains of *E. coli* exist in high numbers in the inflamed tissue, and the number of bacteria in the inflamed regions correlates to the severity of the bowel inflammation.

Virulence properties

Enteric *E. coli* (EC) are classified on the basis of serological characteristics and virulence properties. Virotypes include:

Name	Hosts	Description
Enterotoxigenic *E. coli* (ETEC)	causative agent of diarrhea (without fever) in humans, pigs, sheep, goats, cattle, dogs, and horses	ETEC uses fimbrial adhesins (projections from the bacterial cell surface) to bind enterocyte cells in the small intestine. ETEC can produce two proteinaceous enterotoxins: • the larger of the two proteins, **LT enterotoxin**, is similar to cholera toxin in structure and function. • the smaller protein, **ST enterotoxin** causes cGMP accumulation in the target cells and a subsequent secretion of fluid and electrolytes into the intestinal lumen. ETEC strains are non-invasive, and they do not leave the intestinal lumen. ETEC is the leading bacterial cause of diarrhoea in children in the developing world, as well as the most common cause of traveler's diarrhea. Each year, ETEC causes more than 200 million cases of diarrhoea and 380,000 deaths, mostly in children in developing countries.
Enteropathogenic *E. coli* (EPEC)	causative agent of diarrhea in humans, rabbits, dogs, cats and horses	Like ETEC, EPEC also causes diarrhoea, but the molecular mechanisms of colonization and aetiology are different. EPEC lack fimbriae, ST and LT toxins, but they utilize an adhesin known as intimin to bind host intestinal cells. This virotype has an array of virulence factors

		that are similar to those found in *Shigella*, and may possess a shiga toxin. Adherence to the intestinal mucosa causes a rearrangement of actin in the host cell, causing significant deformation. EPEC cells are moderately invasive (i.e. they enter host cells) and elicit an inflammatory response. Changes in intestinal cell ultrastructure due to "attachment and effacement" is likely the prime cause of diarrhoea in those afflicted with EPEC.
Enteroinvasive *E. coli* *(EIEC)*	found only in humans	EIEC infection causes a syndrome that is identical to Shigellosis, with profuse diarrhoea and high fever.
Enterohemorrhagic *E. coli* (EHEC)	found in humans, cattle, and goats	The most famous member of this virotype is strain O157:H7, which causes bloody diarrhoea and no fever. EHEC can cause hemolytic-uremic syndrome and sudden kidney failure. It uses bacterial fimbriae for attachment (E. coli common pilus, ECP), is moderately invasive and possesses a phage-encoded Shiga toxin that can elicit an intense inflammatory response.
Enteroaggregative *E. coli* (EAEC)	found only in humans	So named because they have fimbriae which aggregate tissue culture cells, EAEC bind to the intestinal mucosa to cause watery diarrhea without fever. EAEC are non-invasive. They produce a hemolysin and an ST enterotoxin similar to that of ETEC.

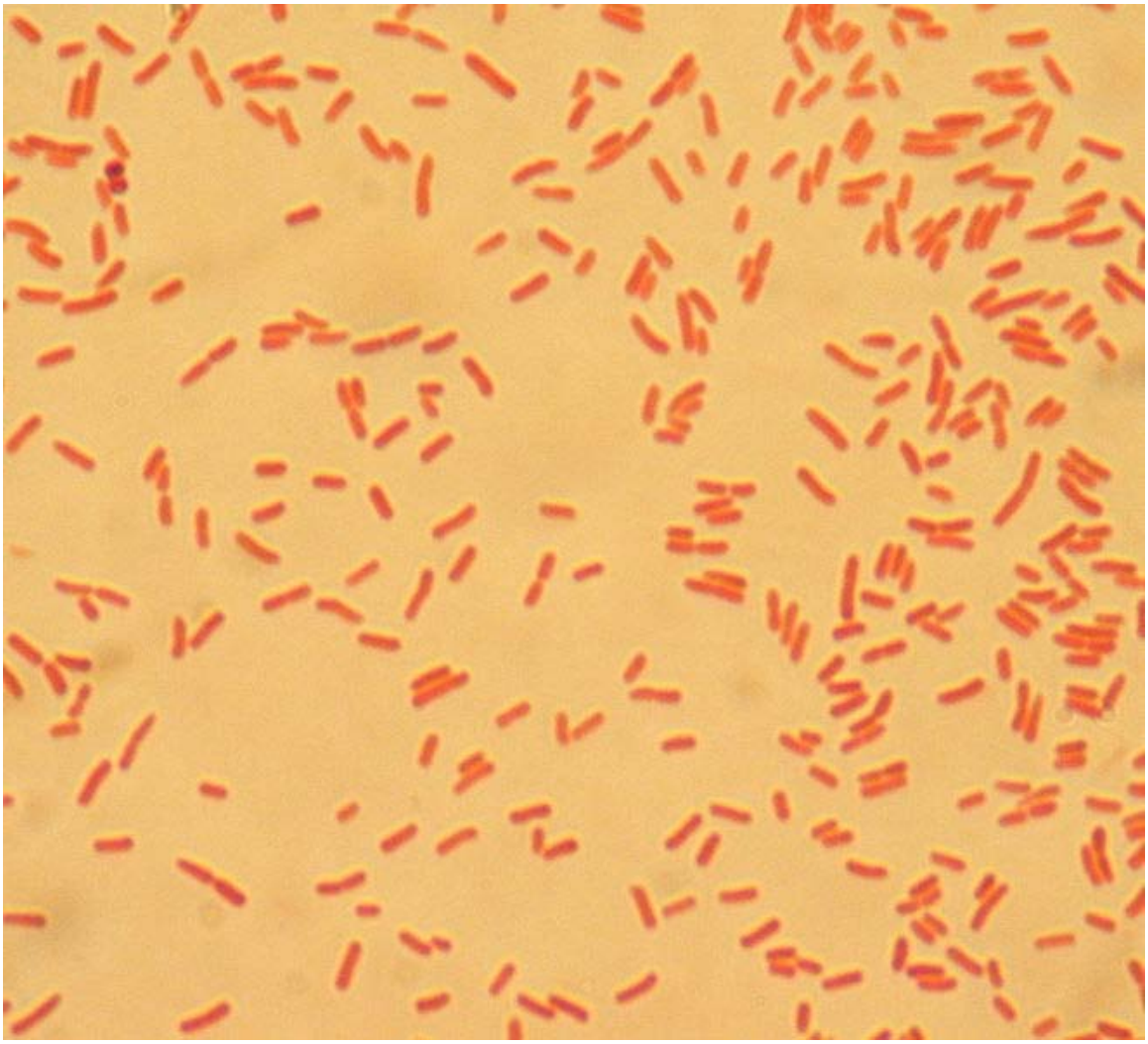
Epidemiology of gastrointestinal infection

Transmission of pathogenic *E. coli* often occurs via faecal-oral transmission. Common routes of transmission include: unhygienic food preparation, farm contamination due to manure fertilization, irrigation of crops with contaminated greywater or raw sewage, feral pigs on cropland, or direct consumption of sewage-contaminated water. Dairy and beef cattle are primary reservoirs of *E. coli* O157:H7, and they can carry it asymptomatically and shed it in their faeces. Food products associated with *E. coli* outbreaks include raw ground beef, raw seed sprouts or spinach, raw milk, unpasteurized juice, unpasteurized cheese and foods contaminated by infected food workers via faecal-oral route.

According to the U.S. Food and Drug Administration, the faecal-oral cycle of transmission can be disrupted by cooking food properly, preventing cross-contamination, instituting barriers such as gloves for food workers, instituting health care policies so food industry employees seek treatment when they are ill, pasteurization of juice or dairy products and proper hand washing requirements.

Shiga toxin-producing *E. coli* (STEC), specifically serotype O157:H7, have also been transmitted by flies, as well as direct contact with farm animals, petting zoo animals, and airborne particles found in animal-rearing environments.

Urinary tract infection



E. coli bacteria, the most prevalent gram-negative flora in the intestine.

Uropathogenic *E. coli* (UPEC) is responsible for approximately 90% of urinary tract infections (UTI) seen in individuals with ordinary anatomy. In *ascending infections*, fecal bacteria colonize the urethra and spread up the urinary tract to the bladder as well as to the kidneys (causing pyelonephritis), or the prostate in males. Because women have a shorter urethra than men, they are 14-times more likely to suffer from an ascending UTI.

Uropathogenic *E. coli* utilize P fimbriae (pyelonephritis-associated pili) to bind urinary tract endothelial cells and colonize the bladder. These adhesins specifically bind D-galactose-D-galactose moieties on the P blood-group antigen of erythrocytes and uroepithelial cells. Approximately 1% of the human population lacks this receptor, and its

presence or absence dictates an individual's susceptibility to *E. coli* urinary tract infections. Uropathogenic *E. coli* produce alpha- and beta-hemolysins, which cause lysis of urinary tract cells.

UPEC can evade the body's innate immune defences (e.g. the complement system) by invading superficial umbrella cells to form intracellular bacterial communities (IBCs). They also have the ability to form K antigen, capsular polysaccharides that contribute to biofilm formation. Biofilm-producing *E. coli* are recalcitrant to immune factors and antibiotic therapy and are often responsible for chronic urinary tract infections. K antigen-producing *E. coli* infections are commonly found in the upper urinary tract.

Descending infections, though relatively rare, occur when *E. coli* cells enter the upper urinary tract organs (kidneys, bladder or ureters) from the blood stream.

Neonatal meningitis

It is produced by a serotype of *Escherichia coli* that contains a capsular antigen called K1. The colonisation of the newborn's intestines with these stems, that are present in the mother's vagina, lead to bacteraemia, which leads to meningitis. And because of the absence of the IgM antibodies from the mother (these do not cross the placenta because FcRn only mediates the transfer of IgG), plus the fact that the body recognises as self the K1 antigen, as it resembles the cerebral glicopeptides, this leads to a severe meningitis in the neonates.

Laboratory diagnosis

In stool samples microscopy will show Gram negative rods, with no particular cell arrangement. Then, either MacConkey agar or EMB agar (or both) are inoculated with the stool. On MacConkey agar, deep red colonies are produced as the organism is lactose-positive, and fermentation of this sugar will cause the medium's pH to drop, leading to darkening of the medium. Growth on Levine EMB agar produces black colonies with greenish-black metallic sheen. This is diagnostic of *E. coli*. The organism is also lysine positive, and grows on TSI slant with a (A/A/g+/H_2S-) profile. Also, IMViC is {+ + - -} for *E. coli*; as it's indole-positive (red ring) and methyl red-positive (bright red), but VP-negative (no change-colourless) and citrate-negative (no change-green colour). Tests for toxin production can use mammalian cells in tissue culture, which are rapidly killed by shiga toxin. Although sensitive and very specific, this method is slow and expensive.

Typically diagnosis has been done by culturing on sorbitol-MacConkey medium and then using typing antiserum. However, current latex assays and some typing antisera have shown cross reactions with non-*E. coli* O157 colonies. Furthermore, not all *E. coli* O157 strains associated with HUS are nonsorbitol fermentors.

The Council of State and Territorial Epidemiologists recommend that clinical laboratories screen at least all bloody stools for this pathogen. The American Gastroenterological Association Foundation (AGAF) recommended in July 1994 that all stool specimens

should be routinely tested for *E. coli* O157:H7. It is recommended that the clinician check with their state health department or the Centers for Disease Control and Prevention to determine which specimens should be tested and whether the results are reportable.

Other methods for detecting *E. coli* O157 in stool include ELISA tests, colony immunoblots, direct immunofluorescence microscopy of filters, as well as immunocapture techniques using magnetic beads. These assays are designed as screening tool to allow rapid testing for the presence of *E. coli* O157 without prior culturing of the stool specimen.

Antibiotic therapy and resistance

Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of *E. coli* vary widely. As Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms. Antibiotics which may be used to treat *E. coli* infection include amoxicillin as well as other semi-synthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin and the aminoglycosides.

Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is probably due to the use of antibiotics as growth promoters in food of animals. A study published in the journal *Science* in August 2007 found that the rate of adaptative mutations in *E. coli* is "on the order of 10^{-5} per genome per generation, which is 1,000 times as high as previous estimates," a finding which may have significance for the study and management of bacterial antibiotic resistance.

Antibiotic-resistant *E. coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *Staphylococcus aureus*, through a process called horizontal gene transfer. *E. coli* often carry multidrug resistant plasmids and under stress readily transfer those plasmids to other species. Indeed, *E. coli* is a frequent member of biofilms where many species of bacteria exist in close proximity to each other. This mixing of species allows *E. coli* strains that are piliated to accept and transfer plasmids from and to other bacteria. Thus *E. coli* and the other enterobacteria are important reservoirs of transferable antibiotic resistance.

Beta-lactamase strains

Resistance to beta-lactam antibiotics has become a particular problem in recent decades, as strains of bacteria that produce extended-spectrum beta-lactamases have become more common. These beta-lactamase enzymes make many, if not all, of the penicillins and cephalosporins ineffective as therapy. Extended-spectrum beta-lactamase–producing *E. coli* are highly resistant to an array of antibiotics and infections by these strains are difficult to treat. In many instances, only two oral antibiotics and a very limited group of intravenous antibiotics remain effective. In 2009, a gene called New Delhi metallo-beta-lactamase (shortened NDM-1) that even gives resistance to intravenous antibiotic carbapenem, were discovered in India and Pakistan on *E. coli* bacteria.

Increased concern about the prevalence of this form of "superbug" in the United Kingdom has led to calls for further monitoring and a UK-wide strategy to deal with infections and the deaths. Susceptibility testing should guide treatment in all infections in which the organism can be isolated for culture.

Phage therapy

Phage therapy—viruses that specifically target pathogenic bacteria—has been developed over the last 80 years, primarily in the former Soviet Union, where it was used to prevent diarrhoea caused by *E. coli*. Presently, phage therapy for humans is available only at the Phage Therapy Center in the Republic of Georgia and in Poland. However, on January 2, 2007, the United States FDA gave Omnilytics approval to apply its *E. coli* O157:H7 killing phage in a mist, spray or wash on live animals that will be slaughtered for human consumption. The Bacteriophage T4 is a highly studied phage that targets E. coli for infection.

Vaccination

Researchers have actively been working to develop safe, effective vaccines to lower the worldwide incidence of *E. coli* infection. In March 2006, a vaccine eliciting an immune response against the *E. coli* O157:H7 O-specific polysaccharide conjugated to recombinant exotoxin A of *Pseudomonas aeruginosa* (O157-rEPA) was reported to be safe in children two to five years old. Previous work had already indicated that it was safe for adults. A phase III clinical trial to verify the large-scale efficacy of the treatment is planned.

In 2006 Fort Dodge Animal Health (Wyeth) introduced an effective live attenuated vaccine to control airsacculitis and peritonitis in chickens. The vaccine is a genetically modified avirulent vaccine that has demonstrated protection against O78 and untypeable strains.

In January 2007 the Canadian bio-pharmaceutical company Bioniche announced it has developed a cattle vaccine which reduces the number of O157:H7 shed in manure by a factor of 1000, to about 1000 pathogenic bacteria per gram of manure.

In April 2009 a Michigan State University researcher announced that he has developed a working vaccine for a strain of *E. coli*. Mahdi Saeed, professor of epidemiology and infectious disease in MSU's colleges of Veterinary Medicine and Human Medicine, has applied for a patent for his discovery and has made contact with pharmaceutical companies for commercial production.

Model organism in life science research

Role in biotechnology

Because of its long history of laboratory culture and ease of manipulation, *E. coli* also plays an important role in modern biological engineering and industrial microbiology. The work of Stanley Norman Cohen and Herbert Boyer in *E. coli*, using plasmids and restriction enzymes to create recombinant DNA, became a foundation of biotechnology.

Considered a very versatile host for the production of heterologous proteins, researchers can introduce genes into the microbes using plasmids, allowing for the mass production of proteins in industrial fermentation processes. Genetic systems have also been developed which allow the production of recombinant proteins using *E. coli*. One of the first useful applications of recombinant DNA technology was the manipulation of *E. coli* to produce human insulin. Modified *E. coli* have been used in vaccine development, bioremediation, and production of immobilised enzymes. *E. coli* cannot, however, be used to produce some of the more large, complex proteins which contain multiple disulfide bonds and, in particular, unpaired thiols, or proteins that also require post-translational modification for activity.

Studies are also being performed into programming *E. coli* to potentially solve complicated mathematics problems such as the Hamiltonian path problem.

Model organism

E. coli is frequently used as a model organism in microbiology studies. Cultivated strains (e.g. *E. coli* K12) are well-adapted to the laboratory environment, and, unlike wild type strains, have lost their ability to thrive in the intestine. Many lab strains lose their ability to form biofilms. These features protect wild type strains from antibodies and other chemical attacks, but require a large expenditure of energy and material resources.

In 1946, Joshua Lederberg and Edward Tatum first described the phenomenon known as bacterial conjugation using *E. coli* as a model bacterium, and it remains the primary model to study conjugation. *E. coli* was an integral part of the first experiments to understand phage genetics, and early researchers, such as Seymour Benzer, used *E. coli* and phage T4 to understand the topography of gene structure. Prior to Benzer's research, it was not known whether the gene was a linear structure, or if it had a branching pattern.

E. coli was one of the first organisms to have its genome sequenced; the complete genome of *E. coli* K12 was published by *Science* in 1997.

The long-term evolution experiments using *E. coli*, begun by Richard Lenski in 1988, have allowed direct observation of major evolutionary shifts in the laboratory. In this experiment, one population of *E. coli* unexpectedly evolved the ability to aerobically metabolize citrate. This capacity is extremely rare in *E. coli*. As the inability to grow aerobically is normally used as a diagnostic criterion with which to differentiate *E. coli*

from other, closely related bacteria such as *Salmonella*, this innovation may mark a speciation event observed in the lab.

By combining nanotechnologies with landscape ecology complex habitat landscapes can be generated with details at the nanoscale. On such synthetic ecosystems evolutionary experiments with *E. coli* have been performed in order to study the spatial biophysics of adaptation in an island biogeography on-chip.

Environmental quality

E. coli bacteria have been commonly found in recreational waters and their presence is used to indicate the presence of recent faecal contamination, but *E. coli* presence may not be indicative of human waste. *E. coli* are harboured in all warm-blooded animals: birds and mammals alike. *E. coli* bacteria have also been found in fish and turtles. Sand and soil also harbor *E. coli* bacteria and some strains of *E. coli* have become naturalized. Some geographic areas may support unique populations of *E. coli* and conversely, some *E. coli* strains are cosmopolitan.

Chapter 9

Clostridium Botulinum

Clostridium botulinum

Clostridium botulinum stained with gentian violet.

Scientific classification

Domain:	Bacteria
Class:	Clostridia
Order:	Clostridiales
Family:	Clostridiaceae
Genus:	*Clostridium*
Species:	***C. botulinum***

Binomial name

Clostridium botulinum
van Ermengem, 1896

Clostridium botulinum is a gram-positive, rod-shaped bacterium that produces neurotoxins known as botulinum neurotoxins types A-G, which causes flaccid muscular paralysis seen in botulism, and is also the main paralytic agent in botox. It is an anaerobic

spore-former, which produces oval, subterminal endospores and is commonly found in soil.

Microbiology

Clostridium botulinum is a rod-shaped microorganism. It is an obligate anaerobe, meaning that oxygen is poisonous to the cells. However, *C. botulinum* tolerates traces of oxygen due to the enzyme called superoxide dismutase (SOD) which is an important antioxidant defense in nearly all cells exposed to oxygen. *C. botulinum* is only able to produce the neurotoxin during sporulation, which can only happen in an anaerobic environment. Other bacterial species produce spores in an unfavorable growth environment to preserve the organism's viability and permit survival in a dormant state until the spores are exposed to favorable conditions.

In the laboratory *Clostridium botulinum* is usually isolated in tryptose sulfite cycloserine (TSC) growth media in an anaerobic environment with less than 2% of oxygen. This can be achieved by several commercial kits that use a chemical reaction to replace O_2 with CO_2 (E.J. GasPak System). *C. botulinum* is a lipase negative microorganism that grows between pH of 4.8 and 7 and it can't use lactose as a primary carbon source, characteristics important during a biochemical identification.

Taxonomy history

Clostridium botulinum was first recognized and isolated in 1895 by Emile van Ermengem from home cured ham implicated in a botulism outbreak. The isolate was originally named *Bacillus botulinus*. However, isolates from subsequent outbreaks were always found to be anaerobic spore formers, so Bengston proposed that the organism be placed into the genus *Clostridium* as the *Bacillus* genus was restricted to aerobic spore-forming rods.

Since 1959 all species producing the botulinum neurotoxins (types A-G) have been designated *C. botulinum*. Substantial phenotypic and genotypic evidence exists to demonstrate heterogeneity within the species. This has led to the reclassification of *C. botulinum* type G strains as a new species *Clostridium argentinense*.

Clostridium botulinum strains that do not produce a botulin toxin are referred to as *Clostridium sporogenes*.

The complete genome of *C. botulinum* has been sequenced Sanger.

Phenotypes

The current nomenclature for *C. botulinum* recognises four physiological groups (I-IV). The classification is based on the ability of the organism to digest complex proteins. Studies at the DNA and rRNA level support the subdivision of the species into groups I-IV. Most outbreaks of human botulism are caused by group I (proteolytic) or II (non-

proteolytic) *C. botulinum*. Group III organisms mainly cause diseases in animals. There has been no record of Group IV *C. botulinum* causing human or animal disease.

Pathology

Botulism poisoning can occur due to improperly preserved or home canned low-acid food that was not processed using correct preservation times and/or pressure.

Neurotoxin types

Neurotoxin production is the unifying feature of the species *C. botulinum*. Seven types of toxins have been identified and allocated a letter (A-G). Most strains produce one type of neurotoxin but strains producing multiple toxins have been described. *Clostridium botulinum* producing B and F toxin types have been isolated from human botulism cases in New Mexico and California. The toxin type has been designated Bf as the type B toxin was found in excess to the type F. Similarly, strains producing Ab and Af toxins have been reported. There is evidence that the neurotoxin genes have been the subject of horizontal gene transfer, possibly from a viral source. This theory is supported by the presence of integration sites flanking the toxin in some strains of *C. botulinum*. However, these integrations sites are degraded indicating that the *C. botulinum* acquired the toxin genes quite far into the evolutionary past.

Only types A, B, E, and F cause disease in humans while types C and D cause disease in cows, birds, and other animals but not in humans. The "gold standard" for determining toxin type is a mouse bioassay, but the genes for types A, B, E, and F can now be readily differentiated using Real-time polymerase chain reaction (PCR).

Organisms genetically as they identified as other *Clostridium* species have caused human botulism; *Clostridium butyricum* producing type E toxin and *Clostridium baratii* producing type F toxin. The ability of *C. botulinum* to naturally transfer neurotoxin genes to other clostridia is concerning, especially in the food industry where preservation systems are designed to destroy or inhibit only *C. botulinum* but not other *Clostridium* species.

Phenotypic groups of *Clostridium botulinum*				
Properties	**Group I**	**Group II**	**Group III**	**Group IV**
Toxin Types	A, B, F	B, E, F	C, D	G
Proteolysis	+	-	weak	-
Saccharolysis	-	+	-	-
Disease host	human	human	animal	-
Toxin gene	chromosome	chromosome	bacteriophage	plasmid
Close relatives	*C. sporogenes*, *C. putrificum*	*C. butyricum*, *C. beijerinickii*	*C. haemolyticum*, *C. novyi type A*	*C. subterminale*, *C. haemolyticum*

Clostridium botulinum *in different geographical locations*

A number of quantitative surveys for *C. botulinum* spores in the environment have suggested a prevalence of specific toxin types in given geographic areas, which remain unexplained.

North America

Type A *C. botulinum* predominates the soil samples from the western regions while type B is the major type found in eastern areas. The type B organisms were of the proteolytic type I. Sediments from the Great Lake regions were surveyed after outbreaks of botulism among commercially reared fish and only type E spores were detected. It has been noted in a survey that type A strains were isolated from soils that were neutral to alkaline (average pH 7.5) while type B strains were isolated from slightly acidic soils (average pH 6.25).

Europe

Clostridium botulinum type E is prevalent in aquatic sediments in Norway and Sweden, Denmark, the Netherlands, the Baltic coast of Poland and Russia. It was then suggested that the type E *C. botulinum* is a true aquatic organism, which was indicated by the correlation between the level of type E contamination and flooding of the land with seawater. As the land dried, the level of type E decreased and type B became dominant.

In soil and sediment from the United Kingdom, *C. botulinum* type B predominates. In general, the incidence is usually lower in soil than in sediment. In Italy, a survey was conducted in the vicinity of Rome, and a low level of contamination was found; all strains were proteolytic *C. botulinum* type A or B.

Australia

Clostridium botulinum type A was found to be present in soil samples from mountain areas of Victoria. Type B organisms were detected in marine mud from Tasmania. Type A *C. botulinum* have been found in Sydney suburbs and types A and B were isolated from urban areas. In a well defined area of the Darling-Downs region of Queensland, a study showed the prevalence and persistence of *C. botulinum* type B after many cases of botulism in horses.

Other

A "mouse protection" or "mouse bioassay" test determines the type of *C. botulinum* present using monoclonal antibodies. This can now also be accomplished using real-time PCR.

Clostridium botulinum is also used to prepare the medicaments Botox, Dysport, Xeomin, and Neurobloc used to selectively paralyze muscles to temporarily relieve muscle

function. It has other "off-label" medical purposes, such as treating severe facial pain, such as that caused by trigeminal neuralgia.

Botulin toxin produced by *C. botulinum* is often believed to be a potential bioweapon as it is so potent that it takes about 75 nanograms to kill a person (LD_{50} of 1 ng/kg, assuming an average person weighs ~75 kg); 500 grams of it would be enough to kill half of the entire human population.

Clostridium botulinum is a soil bacterium. The spores can survive in most environments and are very hard to kill. They can survive the temperature of boiling water at sea level, thus many foods are canned with a pressurized boil that achieves an even higher temperature, sufficient to kill the spores.

Growth of the bacterium can be prevented by high acidity, high ratio of dissolved sugar, high levels of oxygen, very low levels of moisture or storage at temperatures below 3°C (38°F) for type A. For example in a low acid, canned vegetable such as green beans that are not heated hot enough to kill the spores (i.e., a pressurized environment) may provide an oxygen free medium for the spores to grow and produce the toxin. On the other hand, pickles are sufficiently acidic to prevent growth; even if the spores are present, they pose no danger to the consumer. Honey, corn syrup, and other sweeteners may contain spores but the spores cannot grow in a highly concentrated sugar solution; however, when a sweetener is diluted in the low oxygen, low acid digestive system of an infant, the spores can grow and produce toxin. As soon as infants begin eating solid food, the digestive juices become too acidic for the bacterium to grow.

Chapter 10

Fermentation (Food)

Beer fermenting at a brewery

Fermentation in food processing typically is the conversion of carbohydrates to alcohols and carbon dioxide or organic acids using yeasts, bacteria, or a combination thereof, under anaerobic conditions. A more restricted definition of fermentation is the chemical conversion of sugars into ethanol. The science of fermentation is known as zymurgy.

Fermentation usually implies that the action of microorganisms is desirable, and the process is used to produce alcoholic beverages such as wine, beer, and cider. Fermentation is also employed in the leavening of bread, and for preservation techniques to create lactic acid in sour foods such as sauerkraut, dry sausages, kimchi and yogurt, or vinegar (acetic acid) for use in pickling foods.

History

Natural fermentation precedes human history. Since ancient times, however, humans have been controlling the fermentation process. The earliest evidence of winemaking dates from eight thousand years ago, in Georgia, in the Caucasus area. Seven-thousand-year-old jars containing the remains of wine have been excavated in the Zagros Mountains in Iran, which are now on display at the University of Pennsylvania. There is strong evidence that people were fermenting beverages in Babylon circa 5000 BC, ancient Egypt circa 3150 BC, pre-Hispanic Mexico circa 2000 BC, and Sudan circa 1500 BC. There is also evidence of leavened bread in ancient Egypt circa 1500 BC and of milk fermentation in Babylon circa 3000 BC.

French chemist Louis Pasteur was the first known *zymologist*, when in 1854 he connected yeast to fermentation. Pasteur originally defined fermentation as "respiration without air". Pasteur performed careful research and concluded;

"I am of the opinion that alcoholic fermentation never occurs without simultaneous organization, development and multiplication of cells.... If asked, in what consists the chemical act whereby the sugar is decomposed ... I am completely ignorant of it."

Contributions to biochemistry

When studying the fermentation of sugar to alcohol by yeast, Louis Pasteur concluded that the fermentation was catalyzed by a vital force, called "ferments," within the yeast cells. The "ferments" were thought to function only within living organisms. "Alcoholic fermentation is an act correlated with the life and organization of the yeast cells, not with the death or putrefaction of the cells," he wrote.

Nevertheless, it was known that yeast extracts ferment sugar even in the absence of living yeast cells. While studying this process in 1897, Eduard Buchner of Humboldt University of Berlin, Germany, found that sugar was fermented even when there were no living yeast cells in the mixture, by a yeast secretion that he termed *zymase*. In 1907 he received the Nobel Prize in Chemistry for his research and discovery of "cell-free fermentation."

One year prior, in 1906, ethanol fermentation studies led to the early discovery of NAD^+.

Uses

The primary benefit of fermentation is the conversion of sugars and other carbohydrates, e.g., converting juice into wine, grains into beer, carbohydrates into carbon dioxide to leaven bread, and sugars in vegetables into preservative organic acids.

Food fermentation has been said to serve five main purposes:

- Enrichment of the diet through development of a diversity of flavors, aromas, and textures in food substrates
- Preservation of substantial amounts of food through lactic acid, alcohol, acetic acid and alkaline fermentations
- Biological enrichment of food substrates with protein, essential amino acids, essential fatty acids, and vitamins
- Elimination of antinutrients
- A decrease in cooking times and fuel requirements

Some fermentation products (e.g., fusel alcohol) are deleterious.

Fermented foods by region

Nattō, a Japanese fermented soybean food

- **Worldwide:** alcohol, wine, vinegar, olives, yogurt, bread, cheese

- **Asia**
  - **East and Southeast Asia:** amazake, asinan, atsara, bai-ming, belacan, burong mangga, com ruou, dalok, doenjang (된장), douchi, jeruk, lambanog, kimchi (김치), kombucha, leppet-so, narezushi, miang, miso, nata de coco, nata de pina, natto, naw-mai-dong, oncom, pak-siam-dong, paw-tsaynob (雪裡蕻), prahok, ruou nep, sake, seokbakji, soy sauce, stinky tofu, szechwan cabbage (四川泡菜), tai-tan tsoi, chiraki, tape, tempeh, totkal kimchi, yen tsai (醃菜), zha cai (榨菜)
  - **Central Asia:** kumis (mare milk), kefir, shubat (camel milk)
  - **India:** achar, appam, dosa, dhokla, dahi (yogurt), idli, mixed pickle, u-to-nga (Northeast fermented fish)
  - **Nepal:** achar, dahi (yogurt), gundruk, *jaand* (rice beer), sinki, tongba, Panir
- **Africa:** fermented millet porridge, garri, hibiscus seed, hot pepper sauce, injera, lamoun makbouss, laxoox, mauoloh, msir, mslalla, oilseed, ogi, ogili, ogiri
- **Americas:** chicha, elderberry wine, kombucha, pickling (pickled vegetables), sauerkraut, lupin seed, oilseed, chocolate, vanilla, tabasco, tibicos
- **Middle East:** kushuk, lamoun makbouss, mekhalel, torshi, boza
- **Europe:** rakfisk, sauerkraut, ogórek kiszony, surströmming, mead, elderberry wine, salami, prosciutto, cultured milk products such as quark, kefir, filmjölk, crème fraîche, smetana, skyr.
- **Oceania:** poi, kaanga pirau (rotten corn), sago

Fermented foods by type

Bean-based

Cheonggukjang (청국장), doenjang (된장), miso (味噌 (みそ)), natto (納豆 (なっとう)), soy sauce, stinky tofu (臭豆腐), tempeh, soybean paste (豆豉), Beijing mung bean milk (豆汁)

Grain-based

Batter made from Rice and Lentil (*Vigna mungo*) prepared and fermented for baking idlis and dosas

Amazake, beer, bread, choujiu, gamju (감주), injera, kvass, makgeolli, murri, ogi, sake, sikhye, sourdough, sowans, rice wine, malt whisky, grain whisky, vodka.

Vegetable-based

Kimchi (김치), mixed pickle, sauerkraut, Indian pickle

Fruit-based

Wine, vinegar, cider, perry, brandy

Honey-based

Mead, metheglin

Dairy-based

Cheese, kefir, kumis (mare milk), shubat (camel milk), cultured milk products such as quark, filmjölk, crème fraîche, smetana, skyr, yogurt

Fish-based

Bagoong, faseekh (خيسفة), fish sauce, Garum, Hákarl, jeotgal (젓갈), rakfisk, shrimp paste, surströmming, shidal

Meat-based

Jamón ibérico, Chorizo, Salami, pepperoni

Tea-based

Kombucha

Risks of consuming fermented foods

Alaska has witnessed a steady increase of cases of botulism since 1985. Despite its small population, it has more cases of botulism than any other state in the United States of America. This is caused by the traditional Eskimo practice of allowing animal products such as whole fish, fish heads, walrus, sea lion and whale flippers, beaver tails, seal oil, birds, etc., to ferment for an extended period of time before being consumed. The risk is exacerbated when a plastic container is used for this purpose instead of the old-fashioned method, a grass-lined hole, as the botulinum bacteria thrive in the anaerobic conditions created by the air-tight enclosure in plastic.

Chapter 11

Detection of Genetically Modified Organisms

The **detection of genetically modified organisms** in food or feed is possible by biochemical means. It can either be qualitative, showing which genetically modified organism (GMO) is present, or quantitative, measuring in which amount a certain GMO is present. Being able to detect a GMO is an important part of food safety, as without detection methods the traceability of GMOs would rely solely on documentation.

Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) is a biochemistry and molecular biology technique for isolating and exponentially amplifying a fragment of DNA, via enzymatic replication, without using a living organism. It enables the detection of specific strands of DNA by making millions of copies of a target genetic sequence. The target sequence is essentially photocopied at an exponential rate, and simple visualisation techniques can make the millions of copies easy to see.

The method works by pairing the targeted genetic sequence with custom designed complementary bits of DNA called primers. In the presence of the target sequence, the primers match with it and trigger a chain reaction. DNA replication enzymes use the primers as docking points and start doubling the target sequences. The process is repeated over and over again by sequential heating and cooling until doubling and redoubling has multiplied the target sequence several million-fold. The millions of identical fragments are then purified in a slab of gel, dyed, and can be seen with UV light.It is not prone to contamination.

Quantitative detection

Quantitative PCR (Q-PCR) is used to measure the quantity of a PCR product (preferably real-time, QRT-PCR). It is the method of choice to quantitatively measure amounts of transgene DNA in a food or feed sample. Q-PCR is commonly used to determine whether a DNA sequence is present in a sample and the number of its copies in the sample. The method with currently the highest level of accuracy is quantitative real-time PCR. QRT-

PCR methods use fluorescent dyes, such as Sybr Green, or fluorophore-containing DNA probes, such as TaqMan, to measure the amount of amplified product in real time. If the targeted genetic sequence is unique to a certain GMO, a positive PCR test proves that the GMO is present in the sample.

Qualitative detection

Whether or not a GMO is present in a sample can be tested by Q-PCR, but also by multiplex PCR. Multiplex PCR uses multiple, unique primer sets within a single PCR reaction to produce amplicons of varying sizes specific to different DNA sequences, i.e. different transgenes. By targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform. Annealing temperatures for each of the primer sets must be optimized to work correctly within a single reaction, and amplicon sizes, i.e., their base pair length, should be different enough to form distinct bands when visualized by gel electrophoresis.

Event-specific vs. construct-specific detection

When producers, importers or authorities test a sample for the unintended presence of GMOs, they usually do not know, which GMO to expect. While EU authorities prefer an event-specific approach to this problem, US authorities rely on construct-specific test schemes.

Event-specific detection

An event-specific detection searches for the presence of a DNA sequence unique to a certain GMO, usually the junction between the transgene and the organism's original DNA. This approach is ideal to precisely identify a GMO, yet highly similar GMOs will pass completely unnoticed. Event-specific detection is PCR-based.

Construct-specific detection

The construct-specific detection methods can either be DNA or protein based. DNA based detection looks for a part of the foreign DNA inserted in a GMO. For technical reasons, certain DNA sequences are shared by several GMOs. Protein-based methods detect the product of the transgene, for example the Bt toxin. Since different GMOs may produce the same protein, construct-specific detection can test a sample for several GMOs in one step, but is unable to tell precisely, which of the similar GMOs are present. Especially in the USA, protein-based detection is used for the construct-specific approach.

Shortcomings of current detection methods

Currently, it is highly unlikely that the presence of unexpected or even unknown GMOs will be detected, since either the DNA sequence of the transgene or its product, the

protein, must be known for detection. In addition, even testing for known GMOs is time-consuming and costly, as current reliable detection methods can test for only one GMO at a time. Therefore, research programmes such as Co-Extra are developing improved and alternative testing methods, for example DNA microarrays.

Alternative detection methods

Improving PCR based detection

Improving PCR based detection of GMOs is a further goal of the European research programme Co-Extra. Research is now underway to develop multiplex PCR methods that can simultaneously detect many different transgenic lines. Another major challenge is the increasing prevalence of transgenic crops with stacked traits. This refers to transgenic cultivars derived from crosses between transgenic parent lines, combining the transgenic traits of both parents. One GM maize variety now awaiting a decision by the European Commission, MON863 x MON810 x NK603, has three stacked traits. It is resistant to an herbicide and to two different kinds of insect pests. Some combined testing methods could give results that would triple the actual GM content of a sample containing this GMO.

Detecting unknown GMOs

Almost all transgenic plants contain a few common building blocks that make unknown GMOs easier to find. Even though detecting a novel gene in a GMO can be like finding a needle in a haystack, the fact that the needles are usually similar makes it much easier. To trigger gene expression, scientists couple the gene they want to add with what is known as a transcription promoter. The high-performing 35S promoter is a common feature to many GMOs. In addition, the stop signal for gene transcription in most GMOs is often the same: the NOS terminator. Researchers now compile a set of genetic sequences characteristic of GMOs. After genetic elements characteristic of GMOs are selected, methods and tools are developed for detecting them in test samples. Approaches being considered include microarrays and anchor PCR profiling.

Near infrared fluorescence (NIR)

Near infrared fluorescence (NIR) detection is a method that can reveal what kinds of chemicals are present in a sample based on their physical properties. By hitting a sample with near infrared light, chemical bonds in the sample vibrate and re-release the light energy at a wavelength characteristic for a specific molecule or chemical bond. It is not yet known if the differences between GMOs and conventional plants are large enough to detect with NIR imaging. Although the technique would require advanced machinery and data processing tools, a non-chemical approach could have some advantages such as lower costs and enhanced speed and mobility.

Chapter 12

Food Safety

Food safety is a scientific discipline describing handling, preparation, and storage of food in ways that prevent foodborne illness. This includes a number of routines that should be followed to avoid potentially severe health hazards. Food can transmit disease from person to person as well as serve as a growth medium for bacteria that can cause food poisoning. Debates on genetic food safety include such issues as impact of genetically modified food on health of further generations and genetic pollution of environment, which can destroy natural biological diversity. In developed countries there are intricate standards for food preparation, whereas in lesser developed countries the main issue is simply the availability of adequate safe water, which is usually a critical item. In theory food poisoning is 100% preventable.

Key principles

Five key principles

The five key principles of food hygiene, according to WHO, are:

1. Prevent contaminating food with pathogens spreading from people, pets, and pests.
2. Separate raw and cooked foods to prevent contaminating the cooked foods.
3. Cook foods for the appropriate length of time and at the appropriate temperature to kill pathogens.
4. Store food at the proper temperature.
5. Use safe water and raw materials

ISO 22000

ISO 22000 is a standard developed by the International Organization for Standardization dealing with food safety. This is a general derivative of ISO 9000. ISO 22000 standard:

The ISO 22000 international standard specifies the requirements for a food safety management system that involves **interactive communication, system management, prerequisite programs, HACCP principles.**

Incidence

A 2003 World Health Organization (WHO) report concluded that about 40% of reported food poisoning outbreaks in the WHO European Region occur in private homes. According to the WHO and CDC, in the USA alone, annually, there are 76 million cases of foodborne illness leading to 325,000 hospitalizations and 5,000 deaths.

Regulatory agencies

Australia

Australian Food Authority is working toward ensuring that all food businesses implement food safety systems to ensure food is safe to consume in a bid to halt the increasing incidence of food poisoning, this includes basic food safety training for at least one person in each business. Smart business operators know that basic food safety training improves the bottom line, staff take more pride in their work; there is less waste; and customers can have more confidence in the food they consume. Food Safety training in units of competence from a relevant training package, must be delivered by a Registered Training Organization (RTO) to enable staff to be issued with a nationally-recognised unit of competency code on their certificate. Generally this training can be completed in less than one day. Training options are available to suit the needs of everyone. Training may be carried out in-house for a group, in a public class, via correspondence or online. Basic food safety training includes:

- Understanding the hazards associated with the main types of food and the conditions to prevent the growth of bacteria which can cause food poisoning
- The problems associated with product packaging such as leaks in vacuum packs, damage to packaging or pest infestation, as well as problems and diseases spread by pests.
- Safe food handling. This includes safe procedures for each process such as receiving, re-packing, food storage, preparation and cooking, cooling and re-heating, displaying products, handling products when serving customers, packaging, cleaning and sanitizing, pest control, transport and delivery. Also the causes of cross contamination.
- Catering for customers who are particularly at risk of food-borne illness, including allergies and intolerance.
- Correct cleaning and sanitizing procedures, cleaning products and their correct use, and the storage of cleaning items such as brushes, mops and cloths.
- Personal hygiene, hand washing, illness, and protective clothing.

People responsible for serving unsafe food can be liable for heavy fines under this new leglislation, consumers are pleased that industry will be forced to take food safety seriously.

China

Food safety is a growing concern in Chinese agriculture. The Chinese government oversees agricultural production as well as the manufacture of food packaging, containers, chemical additives, drug production, and business regulation. In recent years, the Chinese government attempted to consolidate food regulation with the creation of the State Food and Drug Administration in 2003, and officials have also been under increasing public and international pressure to solve food safety problems. However, it appears that regulations are not well known by the trade. Labels used for "green" food, "organic" food and "pollution-free" food are not well recognized by traders and many are unclear about their meaning. A survey by the World Bank found that supermarket managers had difficulty in obtaining produce that met safety requirements and found that a high percentage of produce did not comply with established standards.

Traditional marketing systems, whether in China or the rest of Asia, presently provide little motivation or incentive for individual farmers to make improvements to either quality or safety as their produce tends to get grouped together with standard products as it progresses through the marketing channel. Direct linkages between farmer groups and traders or ultimate buyers, such as supermarkets, can help avoid this problem. Governments need to improve the condition of many markets through upgrading management and reinvesting market fees in physical infrastructure. Wholesale markets need to investigate the feasibility of developing separate sections to handle fruits and vegetables that meet defined safety and quality standards.

European Union

The parliament of the European Union (EU) makes legislation in the form of directives and regulations, many of which are mandatory for member states and which therefore must be incorporated into individual countries' national legislation. As a very large organisation that exists to remove barriers to trade between member states, and into which individual member states have only a proportional influence, the outcome is often seen as an excessively bureaucratic 'one size fits all' approach. However, in relation to food safety the tendency to err on the side of maximum protection for the consumer may be seen as a positive benefit. The EU parliament is informed on food safety matters by the European Food Safety Authority.

Individual member states may also have other legislation and controls in respect of food safety, provided that they do not prevent trade with other states, and can differ considerably in their internal structures and approaches to the regulatory control of food safety.

Germany

The Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) is a Federal Ministry of the Federal Republic of Germany. **History**: Founded as Federal Ministry of Food, Agriculture and Foresting in 1949, this name did not change until 2001. Then the name changed to Federal Ministry of Consumer Protection, Food and Agriculture. At the 22nd of November 2005, the name got changed again to its current state: Federal Ministry of Food, Agriculture and Consumer Protection. The reason for this last change was that all the resorts should get equal ranking which was achieved by sorting the resorts alphabetically. **Vision**: A balanced and healthy diet with safe food, distinct consumer rights and consumer information for various areas of life, and a strong and sustainable agriculture as well as perspectives for our rural areas are important goals of the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV). The Federal Office of Consumer Protection and Food Safety is under the control of the Federal Ministry of Food, Agriculture and Consumer Protection. It exercises several duties, with which it contributes to safer food and thereby intensifies health-based consumer protection in Germany. Food can be manufactured and sold within Germany without a special permission, as long as it does not cause any damage on consumers' health and meets the general standards set by the legislation. However, manufacturers, carriers, importers and retailers are responsible for the food they pass into circulation. They are obliged to ensure and document the safety and quality of their food with the use of in-house control mechanisms.

Hong Kong

In Hong Kong SAR, the Centre for Food Safety is in charge of ensuring food sold is safe and fit for consumption.

Pakistan

Pakistan does not have an integrated legal framework but has a set of laws, which deals with various aspects of food safety. These laws, despite the fact that they were enacted long time ago, have tremendous capacity to achieve at least minimum level of food safety. However, like many other laws, these laws remain very poorly enforced. There are four laws that specifically deal with food safety. Three of these laws directly focus issues related to food safety, while the fourth one namely Pakistan Standards and Quality Control Authority Act, is indirectly relevant to food safety. The Pure Food Ordinance, 1960 consolidates and amends the law in relation to the preparation and the sale of foods. All provinces and some northern areas have adopted this law with certain amendments. Its aim is to ensure purity of food being supplied to people in the market and, therefore, provides for preventing adulteration. The Pure Food Ordinance 1960 does not apply to cantonment areas. There is separate law for cantonments called "The Cantonment Pure Food Act, 1966". There is no substantial difference between the Pure Food Ordinance 1960 and The Cantonment Pure Food Act. Even the rules of operation are very much similar. Pakistan Hotels and Restaurant Act, 1976 applies to all hotels and restaurants in Pakistan and seeks to control and regulate the rates and standard of service(s) by hotels

and restaurants. In addition to other provisions, under section 22(2), the sale of food or beverages that are contaminated, not prepared hygienically or served in utensils that are not hygienic or clean is an offense. There are no express provisions for consumer complaints in the Pakistan Restaurants Act, 1976, Pakistan Penal Code, 1860 and Pakistan Standards and Quality Control Authority Act, 1996. However, the laws do not prevent citizens from lodging complaints with the concerned government officials. However, the consideration and handling of complaints is a matter of discretion of the officials.

South Korea

Korea Food & Drug Administration

Korea Food & Drug Administration (KFDA) is working for food safety since 1945. It is part of the Government of South Korea.

IOAS-Organic Certification Bodies Registered in KFDA: "Organic" or related claims can be labelled on food products when organic certificates are considered as valid by KFDA. KFDA admits organic certificates which can be issued by 1) IFOAM (International Federation of Organic Agriculture Movement) accredited certification bodies 2) Government accredited certification bodies - 328 bodies in 29 countries have been registered in KFDA.

Food Import Report: According to Food Import Report, it is supposed to report or register what you import. Competent authority is as followed:

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* Imported Agricultural Products, Processed Foods, Food Additives,
Utensils, Containers & Packages or Health Functional Foods
  → KFDA (Korea Food and Drug Administration)
* Imported Livestock, Livestock products (including Dairy products)
  → NVRQS (National Veterinary Research and Quarantine Service) -
  Packaged meat, milk & dairy products (butter, cheese), hamburger
patties, meat ball and other processed products
  which are stipulated by Livestock Sanitation Management Act
* Imported Marine products
  → NFIS (National Fisheries Products Quality Inspection Service) -
  Fresh, chilled, frozen, salted, dehydrated, eviscerated marine
produce which can be recognized its characteristics
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National Institute of Food and Drug Safety Evaluation

National Institute of Food and Drug Safety Evaluation (NIFDS) is functioning as well. The National Institute of Food and Drug Safety Evaluation is a national organization for toxicological tests and research. Under the Korea Food & Drug Administration, the Institute performs research on toxicology, pharmacology, and risk analysis of foods, drugs, and their additives. The Institute strives primarily to understand important biological triggering mechanisms and improve assessment methods of human exposure, sensitivities, and risk by (1) conducting basic, applied, and policy research that closely

examines biologically triggering harmful effects on the regulated products such as foods, food additives, and drugs, and (2) operating the national toxicology program for the toxicological test development and inspection of hazardous chemical ubstances assessments. The Institute ensures safety by (1) investigation and research on safety by its own researchers, (2) contract research by external academicians and research centers.

United States

The US food system is regulated by numerous federal, state and local officials. Although the US food safety system is one of the best in the world, it is lacking in "organization, regulatory tools, and resources to address food borne illness."

Federal level regulation

The Food and Drug Administration publishes the Food Code, a model set of guidelines and procedures that assists food control jurisdictions by providing a scientifically sound technical and legal basis for regulating the retail and food service industries, including restaurants, grocery stores and institutional foodservice providers such as nursing homes. Regulatory agencies at all levels of government in the United States use the FDA Food Code to develop or update food safety rules in their jurisdictions that are consistent with national food regulatory policy. According to the FDA, 48 of 56 states and territories, representing 79% of the U.S. population, have adopted food codes patterned after one of the five versions of the Food Code, beginning with the 1993 edition.

In the United States, federal regulations governing food safety are fragmented and complicated, according to a February 2007 report from the Government Accountability Office. There are 15 agencies sharing oversight responsibilities in the food safety system, although the two primary agencies are the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS), which is responsible for the safety of meat, poultry, and processed egg products, and the Food and Drug Administration (FDA), which is responsible for virtually all other foods.

The Food Safety and Inspection Service has approximately 7,800 inspection program personnel working in nearly 6,200 federally inspected meat, poultry and processed egg establishments. FSIS is charged with administering and enforcing the Federal Meat Inspection Act, the Poultry Products Inspection Act, the Egg Products Inspection Act, portions of the Agricultural Marketing Act, the Humane Slaughter Act, and the regulations that implement these laws. FSIS inspection program personnel inspect every animal before slaughter, and each carcass after slaughter to ensure public health requirements are met. In fiscal year (FY) 2008, this included about 50 billion pounds of livestock carcasses, about 59 billion pounds of poultry carcasses, and about 4.3 billion pounds of processed egg products. At U.S. borders, they also inspected 3.3 billion pounds of imported meat and poultry products.

Industry pressure

There have been concerns over the efficacy of safety practices and food industry pressure on U.S. regulators. A study reported by Reuters found that "the food industry is jeopardizing U.S. public health by withholding information from food safety investigators or pressuring regulators to withdraw or alter policy designed to protect consumers". A survey found that 25% of U.S. government inspectors and scientists surveyed have experienced during the past year corporate interests forcing their food safety agency to withdraw or to modify agency policy or action that protects consumers. Scientists have observed that management undercuts field inspectors who stand up for food safety against industry pressure. According to Dr. Dean Wyatt, a USDA veterinarian who oversees federal slaughter house inspectors, "Upper level management does not adequately support field inspectors and the actions they take to protect the food supply. Not only is there lack of support, but there's outright obstruction, retaliation and abuse of power."

State and local regulation

A number of U.S. states have their own meat inspection programs that substitute for USDA inspection for meats that are sold only in-state. Certain state programs have been criticized for undue leniency to bad practices.

However, other state food safety programs supplement, rather than replace, Federal inspections, generally with the goal of increasing consumer confidence in the state's produce. For example, state health departments have a role in investigating outbreaks of food-borne disease bacteria, as in the case of the 2006 outbreak of *Escherichia coli* O157:H7 (bad *E. coli* bacteria) from processed spinach. Health departments also promote better food processing practices to eliminate these threats.

In addition to the US Food and Drug Administration, several states that are major producers of fresh fruits and vegetables (including California, Arizona and Florida) have their own state programs to test produce for pesticide residues.

Restaurants and other retail food establishments fall under state law and are regulated by state or local health departments. Typically these regulations require official inspections of specific design features, best food-handling practices, and certification of food handlers. In some places a letter grade or numerical score must be prominently posted following each inspection. In some localities, inspection deficiencies and remedial action are posted on the Internet.

Manufacturing control

HACCP guidelines

The UK Food Standards Agency publishes recommendations as part of its Hazard Analysis and Critical Control Points (HACCP) programme. The relevant guidelines state that:

"**Cooking food until the CORE TEMPERATURE is 75 °C or above will ensure that harmful bacteria are destroyed.**
However, lower cooking temperatures are acceptable provided that the **CORE TEMPERATURE is maintained for a specified period of time** as follows :

- 60 °C for a minimum of 45 minutes
- 65 °C for a minimum of 10 minutes
- 70 °C for a minimum of 2 minutes"

Previous guidance from a leaflet produced by the UK Department Of Health “Handling Cooked Meats Safely A Ten Point Plan” also allowed for:

- "75 °C for a minimum of 30 seconds
- 80 °C for a minimum of 6 seconds"

as well as the above. Secondary references for the above may be found at

Note that recommended cooking conditions are only appropriate if initial bacterial numbers in the uncooked food are small. Cooking does not overcome poor hygiene.

Consumer labeling

United Kingdom

Food stuffs in the UK have one of two labels to indicate the nature of the deterioration of the product and any subsequent health issues. EHO Food Hygiene certification is required to prepare and distribute food. While there is no specified expiry date of such a qualification the changes in legislation it is suggested to update every five years.

Best before indicates a future date beyond which the food product *may* lose quality in terms of taste or texture amongst others, but does not imply any serious health problems if food is consumed beyond this date (within reasonable limits).

Use by indicates a legal date beyond which it is not permissible to sell a food product (usually one that deteriorates fairly rapidly after production) due to the potential serious nature of consumption of pathogens. Leeway is sometimes provided by producers in stating **display until** dates so that products are not at their limit of safe consumption on

the actual date stated (this latter is voluntary and not subject to regulatory control). This allows for the variability in production, storage and display methods.

United States

With the exception of infant formula and baby foods which must be withdrawn by their expiration date, Federal law does not require expiration dates. For all other foods, except dairy products in some states, freshness dating is strictly voluntary on the part of manufacturers. In response to consumer demand, perishable foods are typically labeled with a **Sell by** date. It is up to the consumer to decide how long after the Sell by date a package is usable. Other common dating statements are **Best if used by**, **Use-by date**, **Expiration date**, **Guaranteed fresh <date>**, and **Pack date**.

Australia and New Zealand

Guide to Food Labelling and Other Information Requirements: This guide provides background information on the general labelling requirements in the Code. The information in this guide applies both to food for retail sale and to food for catering purposes. Foods for catering purposes means those foods for use in restaurants, canteens, schools, caterers or self-catering institutions, where food is offered for immediate consumption. Labelling and information requirements in the new Code apply both to food sold or prepared for sale in Australia and New Zealand and food imported into Australia and New Zealand. Warning and Advisory Declarations, Ingredient Labelling, Date Marking, Nutrition Information Requirements, Legibility Requirements for Food Labels, Percentage Labelling, Information Requirements for Foods Exempt from Bearing a Label.

Issues associated with sell by / use by dates

According to the UK's Waste & Resources Action Programme, 33% percent of all food produced is wasted along the chill chain or at the consumer. At the same time, a large number of people get sick every year due to spoiled food.

UK government to replace sell by / use by dates?

According to the UK minister Hilary Benn the use by date and sell by dates are old technologies that are outdated and should be replaced by other solutions or disposed of altogether.

How to enhance food safety

There is a number of ways to enhance sell by and use by dates. These include better education of consumers on how to use, transport, and store fresh food products, but also by enhancing the use by and sell by dates by adding to the package smart indicators such as TTIs (Time Temperature Indicators). These show through a visible color change whether the product is still fresh. TTIs are already in use by retailers and food producers

in France (Monoprix and Carrefour), Switzerland (Kneuss), and other countries in western Europe.

Codex Alimentarius

In 2003, the WHO and FAO published the Codex Alimentarius which serves as a guideline to food safety.