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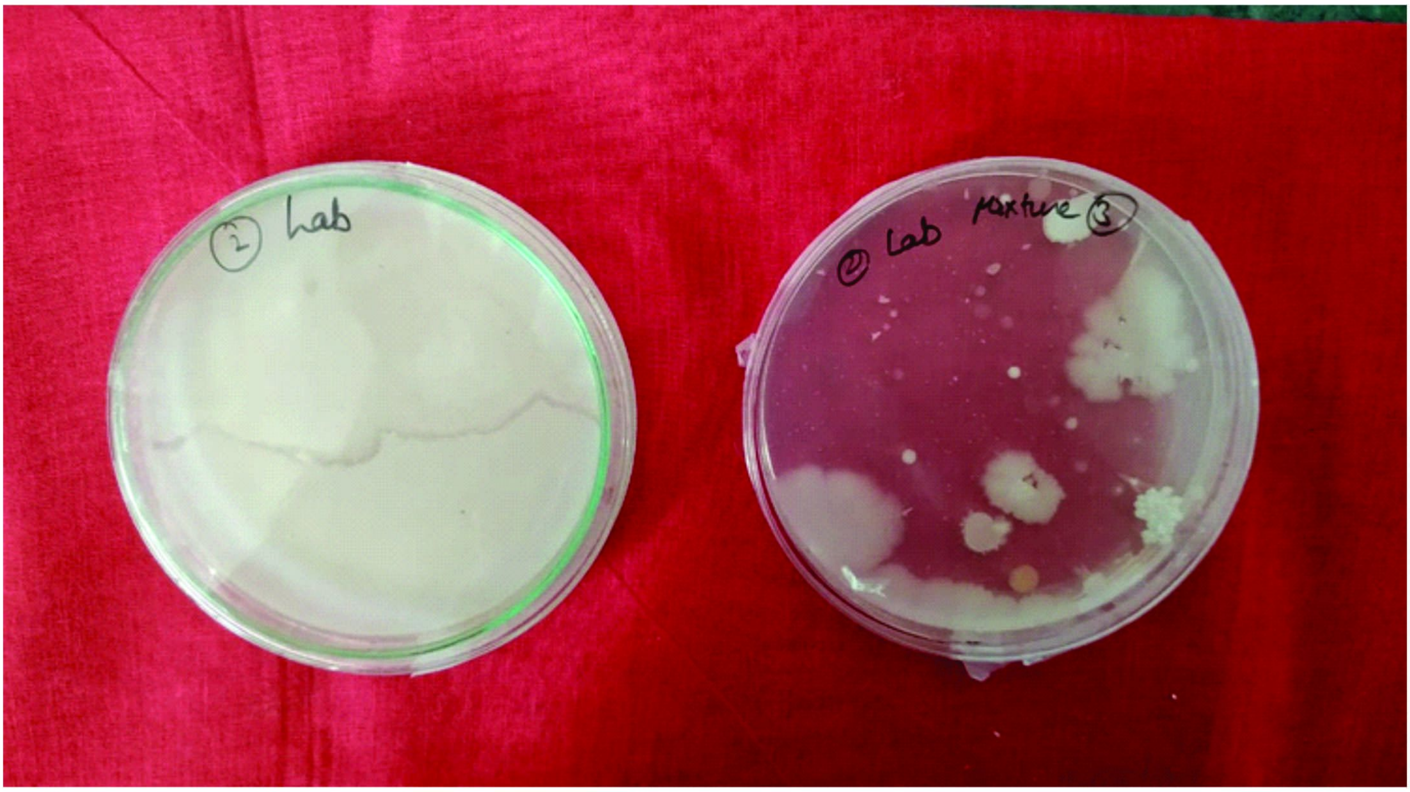
Food and Dairy technology
Part-1



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Chapter-1

Introduction to Food Microbiology

Food science is a discipline concerned with all aspects of food - beginning after harvesting, and ending with consumption by the consumer. It is considered one of the agricultural sciences, and it is a field which is entirely distinct from the field of nutrition. The field of food microbiology is a very broad one, encompassing the study of microorganisms which have both beneficial and deleterious effects on the quality and safety of raw and processed foods. It is important to understand the relationships among the various microorganisms making up the microflora of a food. Infact food microbiologists are concerned with the practical implications of the microflora of the food and the food microorganisms that can cause spoilage of food and disease in humans. The primary tool of microbiologists is the ability to identify and quantitate food-borne microorganisms. However, the inherent inaccuracies in enumeration processes, and the natural variation found in all bacterial populations complicate the microbiologist's job. Moreover, they may be important from the aesthetic point of view. Of course, some useful bacteria may be important because they change the functional properties of food stuffs resulting in new tastes, odors or textures. Microorganisms in food include bacteria, molds, yeasts, algae, viruses, parasitic worms and protozoa. These organisms differ in size and shape and in their biochemical and cultural characteristics.

The microorganisms described below are among the most important genera and species normally found in food products. Each microorganism has its own particular nutritional and environmental requirements.

1.2 Bacteria

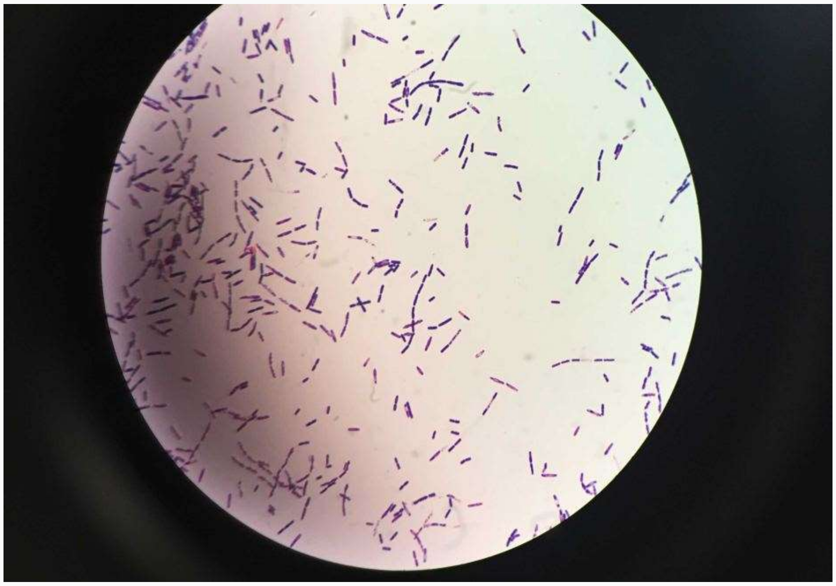
1.2.1 *Acinetobacter*

Acinetobacter is a genus of Gram-negative bacteria belonging to the Gammaproteobacteria . *Acinetobacter* species are non-motile and oxidase-negative, and occur in pairs as observed under magnification. Young cultures show rod shaped morphology. They are strict aerobes that do not reduce nitrates. They are important soil and water organisms and are also found on many foods especially refrigerated fresh products. *A. baumannii* is a frequent cause of nosocomial pneumonia, especially of late onset ventilator associated pneumonia. It can cause various other infections including skin and wound infections, bacteremia, and meningitis.

1.2.2 *Bacillus cereus*

B. cereus is a thick long rod-shaped Gram positive, catalase positive aerobic spore former and the organism is important in food borne illness. It is a normal inhabitant of soil and is isolated from a variety of foods. It is quite often a cause of diarrheal illness due to the consumption of desserts, meat, dishes, dairy products, rice, pasta etc that are cooked and kept at room temperature as it is thermoduric. Some of the *B. cereus* strains are psychrotrophic as they grow at refrigeration temperature.

B. cereus is spread from soil and grass to cows udders and into the raw milk. It is also capable of establishing in cans. It is also capable of producing proteolytic and amyloltic enzymes and also phoslipase C (lecithinase). The production of these enzymes by these organisms can lead to the spoilage of foods. The diarrheal illness is caused by an enterotoxin produced during the vegetative growth of *B. cereus* in small intestine. The bacterium has a maximum growth temperature around 48°C to 50°C and pH range 4.9 to 9.3. Like other spores of mesophilic *Bacillus* species, spores of *B. cereus* are also resistant to heat and survive pasteurization temperature.

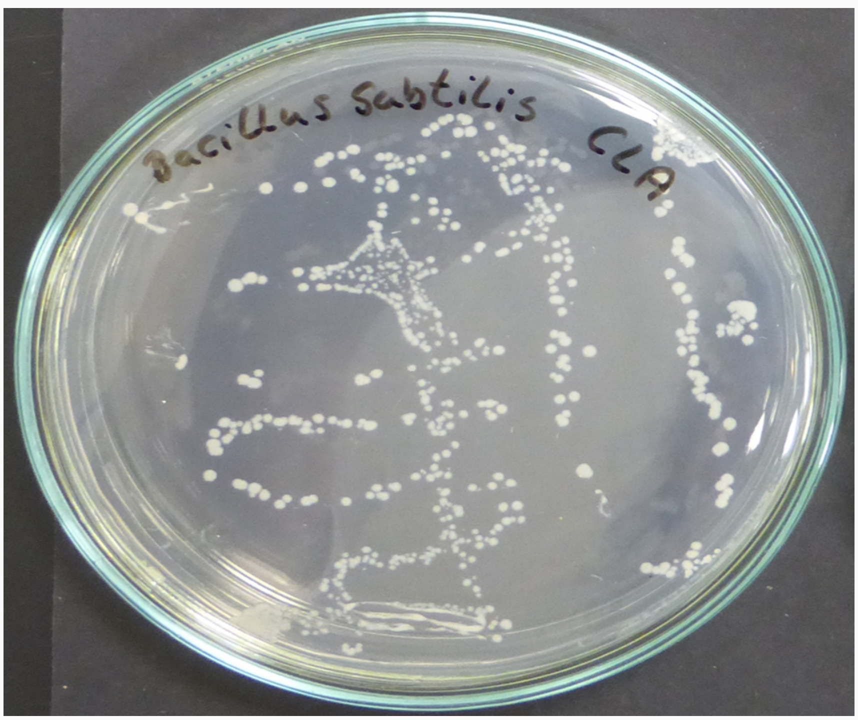


Source: Bacillus Cereus Bacteria - Bing images

Fig. 1.1 Bacillus cereus

1.2.3 *Bacillus subtilis*

Bacillus subtilis , known also as the hay bacillus or grass bacillus , is a Gram-positive, catalase-positive bacterium commonly found in soil. A member of the genus *Bacillus*, *B. subtilis* is thin short rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions. *B. subtilis* produces the proteolytic enzyme subtilisin. *B. subtilis* spores can survive the extreme heat during cooking. *B. subtilis* is responsible for causing ropiness a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides in spoiled bread dough. A strain of *B. subtilis* formerly known as *Bacillus natto* is used in the commercial production of the Japanese food *natto*, as well as the similar Korean food *cheonggukjang*. It is used to produce amylase and also used to produce hyaluronic acid, which is useful in the joint-care sector in healthcare.



Source: Bacillus Subtilis Colonies - Bing images

Fig. 1.2 Bacillus subtilis

1.2.4 *Carnobacterium*

Carnobacterium is a genus of Gram-positive bacteria within the family *Leuconostocaceae*. *C. divergens* and *C. maltaromaticum* are found in the wild and in food products and can grow anaerobically. These species are not known to be pathogenic in humans but may cause disease in fish. The genus *Carnobacterium* contains nine species, but only *C. divergens* and *C. maltaromaticum* are frequently isolated from natural environments and foods. They are tolerant to freezing/thawing and high pressure and able to grow at low temperatures, anaerobically. They metabolize arginine and various carbohydrates, including chitin, and this may improve their survival in the environment. *Carnobacteriumdivergens* and *C. maltaromaticum* have been extensively studied as protective cultures in order to inhibit growth of *Listeria monocytogenes* in fish and meat products. Several carnobacterial bacteriocins have been identified and described. Carnobacteria can spoil chilled foods, but spoilage activity shows intraspecies and interspecies variation. Their production of tyramine in foods is critical for susceptible individuals, but carnobacteria are not otherwise human pathogens.

1.2.5 *Corynebacterium*

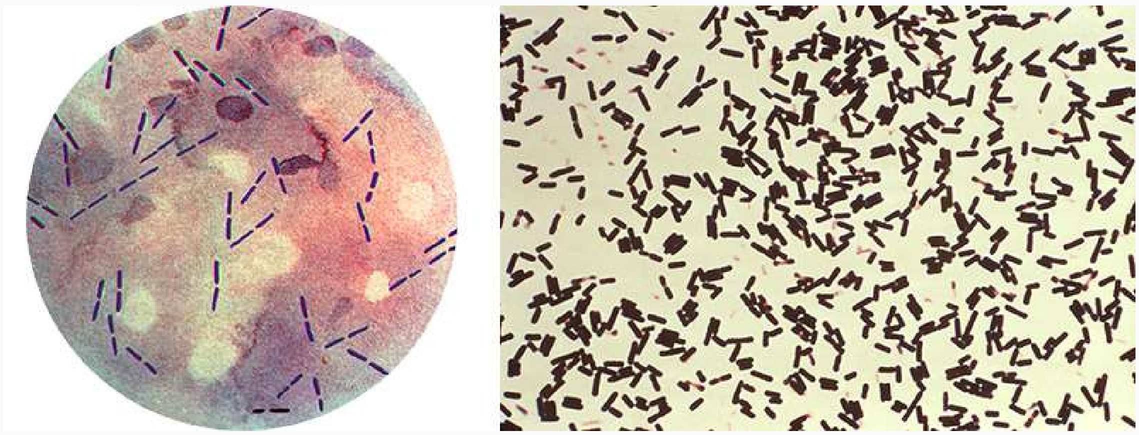
Corynebacterium is a genus of Gram-positive rod-shaped bacteria. They are widely distributed in nature and are mostly innocuous. Some are useful in industrial settings such as *C. glutamicum*. Others can cause human disease. *C. diphtheriae*, for example, is the pathogen responsible for diphtheria. Some species are known for their pathogenic effects in humans and other animals. Perhaps the most notable one is *C. diphtheriae*, which acquires the capacity to produce diphtheria toxin only after interacting with a bacteriophage. Diphtheria toxin is a single, 60,000 molecular weight protein composed of two peptide chains, fragment A and fragment B, held together by a disulfide bond.

1.2.6 *Clostridium perfringens*

C. perfringens is a Gram-positive encapsulated anaerobic non-motile bacterium commonly found on meat and meat products. It has the ability to cause food borne disease. It is a toxin producing organism-produces *C. perfringens* enterotoxin and β -toxin that are active on the human GI tract.

It multiplies very rapidly in food (doubling time < 10 min). Spores are resistant to radiation, desiccation and heat and thus survive in incompletely or inadequately cooked foods.

However, it tolerates moderate exposure to air. Vegetative cells of *C. perfringens* are also somewhat heat tolerant as they have relatively high growth temperature (43°C -45 °C) and can often grow at 50°C. They are not tolerant to refrigeration and freezing. No growth occurs at 6 °C . *C. perfringens* is present in soil and the other natural environment.

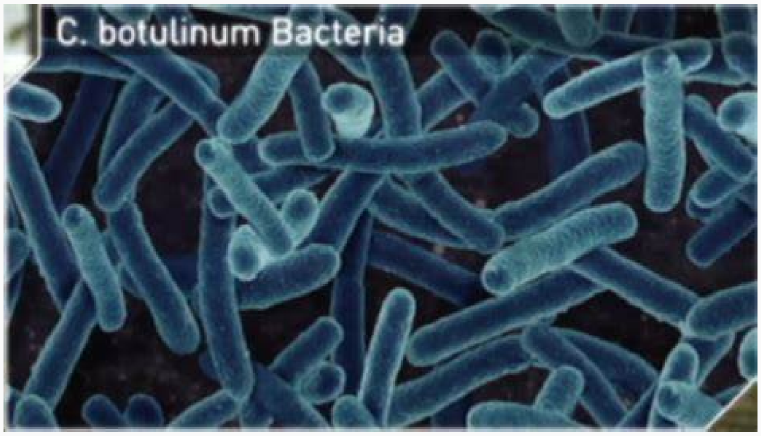


Source: c-perfringens-800px.jpg (800×300) (cdc.gov)

Fig. 1.3 Clostridium spp.

1.2.7 *Clostridium botulinum*

C. botulinum produces the most potent toxin known. It is a Gram-positive anaerobic rod shaped bacterium. Oval endospores are formed in stationary phase cultures. There are seven types of *C. botulinum* (A to G) based on the serological specificity of the neurotoxin produced. Botulism is a rare but very serious disease. The ingestion of neurotoxin produced by the organism in foods can lead to death. However, the toxin (a protein) is easily inactivated by heat. The organism can grow at temperature ranging from 10-48 °C with optimum growth temperature at 37°C. Spores are highly heat resistant. The outgrowth of spores is inhibited at pH < 4.6, NaCl> 10% or water activity< 0.94. Botulinum spores are probably the most radiation resistant spores of public health concern. Contamination of foods is through soil and sediments where they are commonly present. The organism grows under obligate anaerobic conditions and produces toxin in under processed (improper canning) low acid foods at ambient temperature.



Source: Bot_bacteria.png (200×114) (nocookie.net)

Fig. 1.4 Clostridium botulinum

1.2.8 *Campylobacter*

Campylobacter are Gram negative nonspore forming rods. *Campyloleacterjejuni* is an important food borne pathogen. It is one of the many species within the genus *Campylobacter*. *Campylobacter* species *C. jejuni* and *C. coli* cause diarrhea in humans. The organism is heat sensitive (destroyed by milk pasteurization temperature). It is also sensitive to freezing. The organism belongs to the family *Campylobactereaceae*. The organisms are curved, S-shaped, or spiral rods that may form spherical or coccoids forms in old cultures or cultures exposed to air for prolonged periods. Most of the species are microaerophilic. It is oxidase and catalase positive and does not grow in the presence of 3.5% NaCl or at 25 °C or below. The incidence reported for gastro enteritis by this organism are as high as in case of *Salmonella*.

The organism is commonly present in raw milk, poultry products, fresh meats, pork sausages and ground beef. The infective dose of *C.jejuni* may be <1,000 organisms.

Source: campylobacter_2.jpg (1280×960) (dxline.info)

Fig. 1.5 Campylobacter

1.2.9 *Erwinia*

Erwinia is a genus of the family *Enterobacteriaceae* bacteria containing mostly plant pathogenic species. The organisms was named after the first phytobacteriologist, Erwin Smith. It is a Gram negative bacterium related to *E. coli*, *Shigella*, *Salmonella* and *Yersinia*. It is primarily a rod-shaped bacterium. A well-known member of this genus is the species *E. amylovora*, which causes fire blight on apple, pear and other Rosaceous crops. *Erwinia carotovora* (also known as *Pectobacteriumcarotovorum*) is another species, which causes diseases in many plants. These species produce pectolytic enzymes that hydrolyze pectin between individual plant cells. Decay caused by *E. carotovora* is often referred to as bacterial soft rot (BSR). Most plants or plant parts can resist invasion by the bacteria, unless some type of wound is present. High humidity and temperatures around 30°C favor development of decay.

1.2.10 *Enterococcus* (*E. faecium*, *E. faecalis*)

Enterococcus is a genus of lactic acid bacteria. Enterococci are Gram positive cocci that often occur in pairs (diplococci) or short chains and are difficult to distinguish from streptococci on physical characters mentioned above. The two species are commensal organisms in the intestine of humans.

The Enterococci are facultative anaerobic organisms non spore forming that grows optimally at 35°C . However, they tolerate wide range of environmental conditions (10-45°C) pH (4.5 to 10.5) quite high NaCl concentration (.6.5%) and can survive heating at 60°C for 30 min.

Source: https://images.fineartamerica.com/images-medium-large-5/1-enterococcus-faecalis-bacteria-science-photo-library.jpg

Fig. 1.6 Enterococcus spp.

Catalase-negative, oxidase negative-bacteria of the genes *Enterococcus* are ubiquitous organisms that often occur in large numbers on vegetables, plant materials and foods especially those of animal origin such as meat and dairy products. Enterococci also constitute a large preparation of autochthonous bacteria associated with the mammalian gastro-intestinal tract.

The resistance of enterococci to pasteurization temperatures and their adaptability to different substrates and growth conditions in food products manufactured from raw materials and in heat treated food products is of great significance.

Enterococci may constitute an important part of the microflora of fermented cheese and meats.

1.2.11 *Escherichia coli*

E. coli strains are associated with food borne gastroenteritis. These are Gram-negative asprogeneous rods that ferment lactose and produce dark colonies with a metallic sheen on Endo agar. The organism grows well on a large number of media and in many foods. They grow over a wide range of temperature (4 to 46 °C) and pH (4.4 to 9.0).

However, they grow very slowly in foods held at refrigerator temp. (5 °C). They belong to the family *Enterobacteriaceae*. The organism is also an indicator of fecal pollution. The organism is also capable of producing acid and gas and off-flavours in foods. *E. coli* strains involved in foodborne-illness can be placed into five groups: enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC) and facultatively enteropathogenic (FEEC).

The organism also grows in the presence of bile salts. The primary habitat of *E.coli* is the intestinal tract of most warm blooded animals. *E.coli* 0157: H7 strains are unusually tolerant of acidic environments.

Source: https://images.medicinenet.com/images/article/main_image/escherichia-coli.jpg

Fig. 1.7 E. coli

1.2.12 *Lactococcus*

L.lactis subsp. *lactis*

L.lactis subsp. *cremoris*

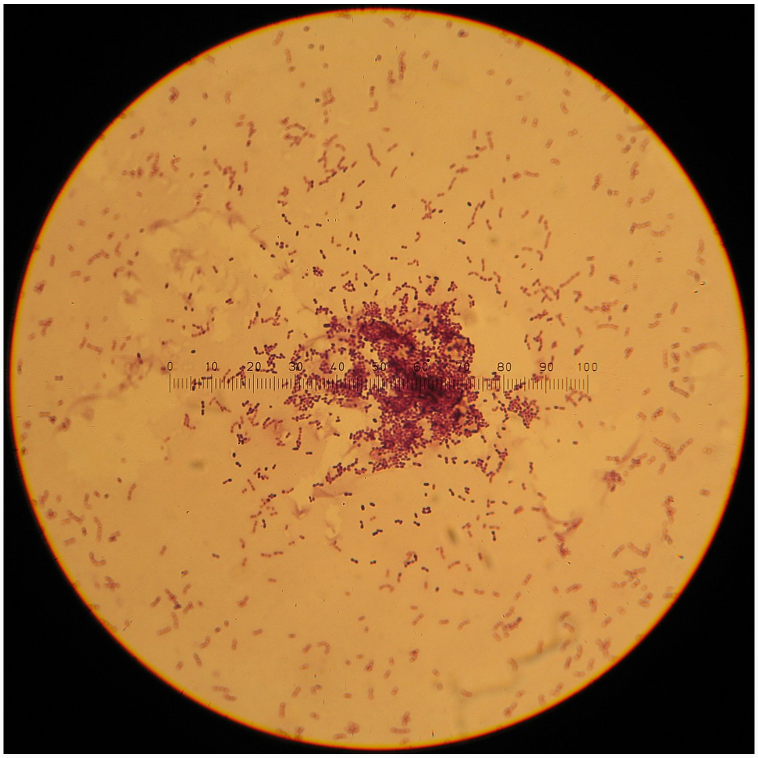
L.lactis subsp.*lactis biovar diaectylactis*

Lactococcus is a genus of lactic acid bacteria that were formerly included in the genus Streptococcus Group N (Group N Streptococci). They are known as homofermentors meaning that they produce a single product of glucose fermentation. They are Gram-positive, catalase negative, non-motile coccus that are found singly, in pairs or in chains. Some of the strains of lactococci are known to grow at or below 7 °C.

Lactococci are intimately associated with dairy products. These organisms are commonly used in the dairy industry in the manufacture of fermented dairy products like cheeses. They can be used in single strain starter cultures or in mixed strain cultures with other lactic acid bacteria such as *Lactobacillus* and *Streptococcus*. Their main purpose in dairy production is the rapid acidification of milk. This causes drop in the pH of fermented product which prevents the growth of spoilage and pathogenic bacteria. These bacteria also play a role in the flavor of the final product. Dairy lactococci have also been exploited for several industrial fermentations in the biotechnology industry. They are easily grown at industrial scale up on cheap whey based media.

Lactococcus lactis subsp. *lactis* includes species formerly designated as *S. lactis* subsp. *lactis*. *L. lactis* subsp. *cremoris* is distinguished from *L.*

Lactis subsp. *lactis* by the inability to (i) grow at 40 °C (ii) grow in 4% NaCl (iii) hydrolyse arginine and (iv) ferment ribose.



Source:
https://upload.wikimedia.org/wikipedia/commons/thumb/4/4f/Lactococcus_lactis.jpg/1200px-Lactococcus_lactis.jpg

Fig. 1.8 Lactococcus spp.

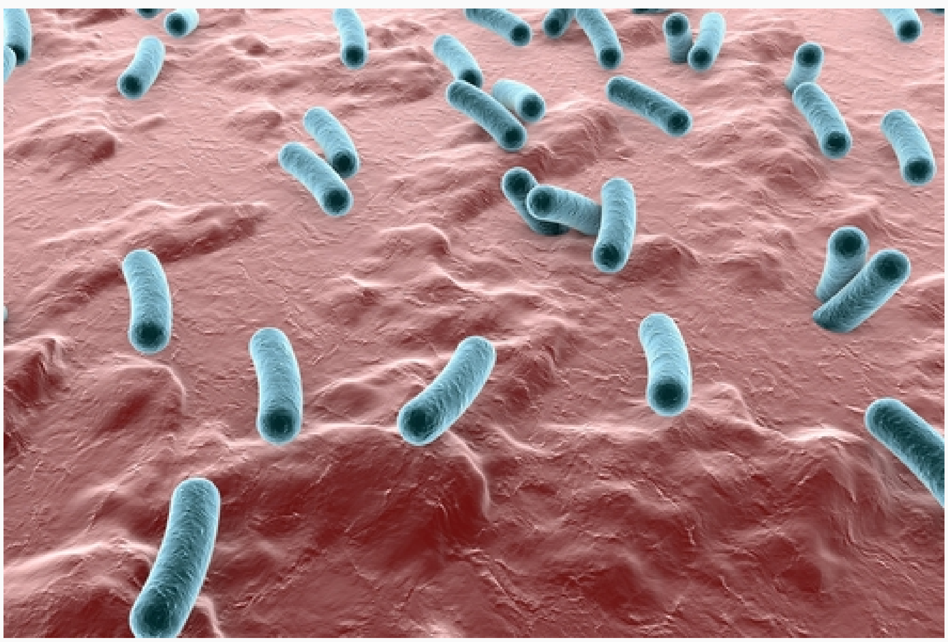
1.2.13 *Lactobacillus* (*L. bulgaricus, L. helveticus. L. plantarum,L. acidophilus, L. casei, L. lactis, L. fermentum*)

The organisms belonging to this important genus are rods usually long and slender and in some of the species form chains. They are aerotolerant/microaerophilic but some ferment sugars chiefly to lactic acids if they are homofermentative. The hetero fermentative species, besides lactic acid, also produce small amount of acetic acid, carbon dioxide and trace amounts of volatile compounds such acetaldehyde and alcohol. The homofermentative species of *Lactobacillus* include *L. bulgaricus, L. casei, L. helveticus, L. lactis, L. acidophilus* and grow optimally at 37 °C. *L. fermentum*, *L. brevis* are the typical example of hetero fermentative *Lactobacillus* and grow well at higher temperatures.

Lactobacilli are of considerable importance in foods as they ferment sugar to lactic acid and other desirable flavouring compounds and are thus used in the production of fermented plant dairy and meat products. However, they are also implicated in the spoilage of wine and beer.

The organism normally occurs on plant surfaces silage, manure and dairy products. They are quite fastidious in their nutritional requirements as they are unable to synthesize certain vitamins they require and, therefore, media need to be supplemented with these vitamins for their growth.

Some of the strains are psychotrophic in nature and are thus involved in the spoilage of refrigerated meats. On the other hand thermoduric properties (resistance to pasteurization temperature) of some of the thermophilic strains of lactobacilli are quite useful in the manufacture of certain varieties of cheeses e.g. Swiss cheese. Some strains of lactobacilli also show probiotic attributes and are finding application in functional probiotic foods and in pharmaceutical preparations.



Source: https://nootriment.com/wp-content/uploads/2016/01/lactobacillus-bacteria.jpg

Fig. 1.9 Lactobacillus spp.

1.2.14 *Leuconostoc*

Leuconostoc is a genus of Gram-positive bacteria, placed within the family of *Leuconostocaceae*. They are generally ovoid cocci often forming chains. *Leuconostoc* spp.

are intrinsically resistant to vancomycin and are catalase-negative (which distinguishes them from staphylococci). All species within this genus are heterofermentative and are able to produce dextran from sucrose. They are generally slime-forming. Blamed for causing the 'stink' when creating a sourdough starter, some species are also capable of causing human infection.

Leuconostoc spp. along with other lactic acid bacteria such as *Pediococcus* and *Lactobacillus* spp , is responsible for the fermentation of cabbage, to sauerkraut. In this process the sugars in fresh cabbage are transformed to lactic acid which give it a sour flavour and good keeping qualities.

1.2.15 *Listeria monocytogenes*

Listeria monocytogenes in foods has attracted worldwide attention due to the serious illness it causes in human beings. The Listeria are Gram positive non spore forming, nonacid-fast rods. The organism is catalase positive and produces lactic acid from glucose and other fermentable sugars. The organism grows well in brain heart infusion (BHI), trypticase soy, and tryptose broths. However, the medium should be fortified with B. vitamins and the amino acids. It is a mesophilic organism with optimal growth temperature 37°C but it can grow at refrigerator temperature also. Strains grows over the temperature range of 1°C to 45°C and pH range 4.1 to 9.6.

Listeria monocytogenes is widely distributed in nature and can be isolated from decaying vegetation, soil, animal feces, sewage, silage and water. The organism has been found in raw milk, pork, raw poultry, ground beef and vegetables. The HTST treatment of pasteurization is good enough to destroy the organism in milk.

The most significant virulence factor associated with *L. monocytogenes* is listeriolysin O. The virulent strains produce β-hemolysis on blood agar and acid from rhamnose.

L. monocytogenes grows well in moderate salt concentrations (6.5%).

L. monocytogenes is unique among foodborne pathogens while other pathogens excrete toxins or multiply in the blood stream, *L. monocytogenes* enters the host's cells and grows inside the cell. In humans it crosses the intestinal barrier after entering by the oral route.

Ready to Eat (RTE) foods that are preserved by refrigeration pose a special challenge with regard to *L. monocytogenes* infection.

Source: https://thefoodpoisoninglawyers.com/wp-content/uploads/2014/04/o-LISTERIA-facebook.jpg

Fig. 1.10 Listeria spp.

1.2.16 *Micrococcus*

Micrococcus occurs in a wide range of environments, including water, dust, and soil. Micrococci are Gram-positive spherical cells ranging from about 0.5 to 3 micrometers in diameter and typically appear in tetrads. *Micrococcus* has a substantial cell wall, which may comprise as much as 50% of the cell mass. Some species of *Micrococcus*, such as *M. luteusM. roseus* (red) produce yellow or pink colonies when grown on mannitol salt agar. *Micrococcus* is generally thought to be a saprophytic or commensal organism, though it can be an opportunistic pathogen, particularly in hosts with compromised immune systems, such as HIV patients.

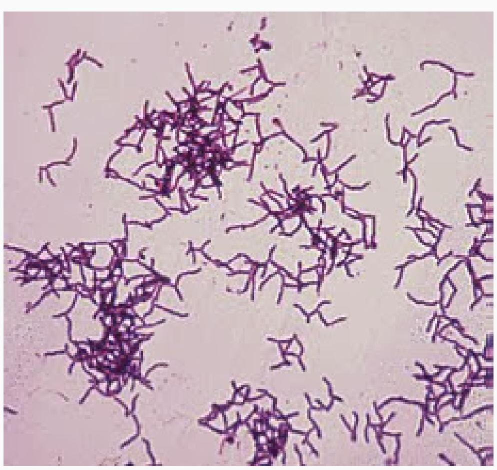
1.2.17 *Proteus*

Since it belongs to the family of *Enterobacteriaceae*, general characters are applied on this genus: It is oxidase-negative, but catalase and nitrate reductase positive. Three species *P. vulgaris*, *P. mirabilis*, and *P. penneri* are opportunistic human pathogens. *Proteus* includes pathogens responsible for many human urinary tract infections. *P. mirabilis* causes wound and urinary tract infections. Most strains of *P. mirabilis* are sensitive to ampicillin and cephalosporins. *P. vulgaris* is not sensitive to these antibiotics. However, this organism is isolated less often in the laboratory and usually only targets immune suppressed individuals. *P. vulgaris* occurs naturally in the intestines of humans and a wide variety of animals; alsomanure, soil and polluted waters. *P. mirabilis*, once attached to urinary tract, infects the kidney more commonly than *E. coli*. *P. mirabili*s are often found as free-living organisms in soil and water.

1.2.18 *Propionibacterium* spp. (*P. freudenreichii*)

Historically, *Propionibacterium* spp. are of interest because of their use as dairy starters (especially in the production of Swiss-type cheese) and their ability to produce propionic acid during growth. The genus *Propionibacterium* is generally split into "cutaneous" and "dairy" groups. The dairy *Propionibacterium* spp. can also be isolated primarily from dairy foods and silage. The species in dairy products include *P. jensenii, P. acidipropionici, P. theoniiP. freudenreichii*. Propionibacteria have a role in the production of flavour compounds in cheese by proteolysis and propionic acid production. Dairy strains of propionibacteria are autolytic under environmental conditions found in cheese and degrade peptides and amino acids that are present in the cheese. and

The dairy species offer an interesting opportunity as novel probiotic organisms with the most obvious advantage being that they are considered safe for ingestion.



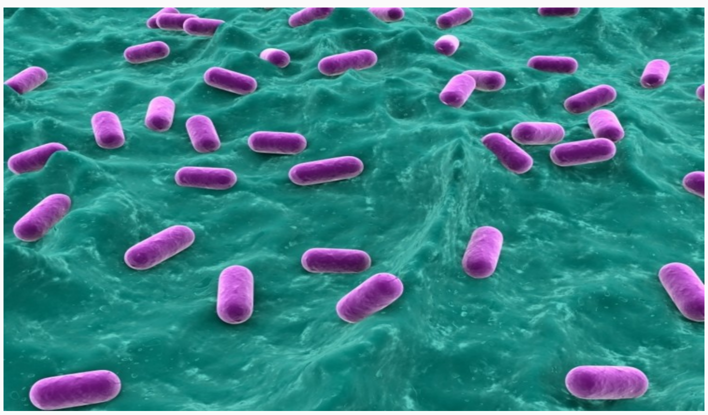
Source:https://lh5.googleusercontent.com/OuPP032mQcs/UtFLPaeqdxI/AAAAAAAAQ68/BalmljnQn2g/s400/preview_html_m55fbddcc.jpg

Fig. 1.11 Propionibacterium spp.

1.2.19 *Pediococcus* spp. (*Pediococcuspentosaceus*, *P. acidilactici*)

Pediococci compromise a group of bacteria that are of economic importance in the brewing and food industries. Several species and strains of pediococci have been used as starter cultures in the fermentation of vegetables, meats, sausage products, fermented milks and associated with the development of flavor in Cheddar and other related cheese varieties. Some strains form capsular material that causes beer to become ropy and viscous.

They are catalase negative and exhibit a homolactic type of fermentation and produce optically inactive lactic acid i.e. a mixture of the L(+) and D(-) type. They generally appear in tetrads.



Source: https://cdn-a.william-reed.com/var/wrbm_gb_food_pharma/storage/images/9/9/8/5/3015899-3-eng-GB/New-pediococcus-acidilactici-probiotic-s-origin-in-nature-confers-its-high-stability-supplier-says_wrbm_large.jpg

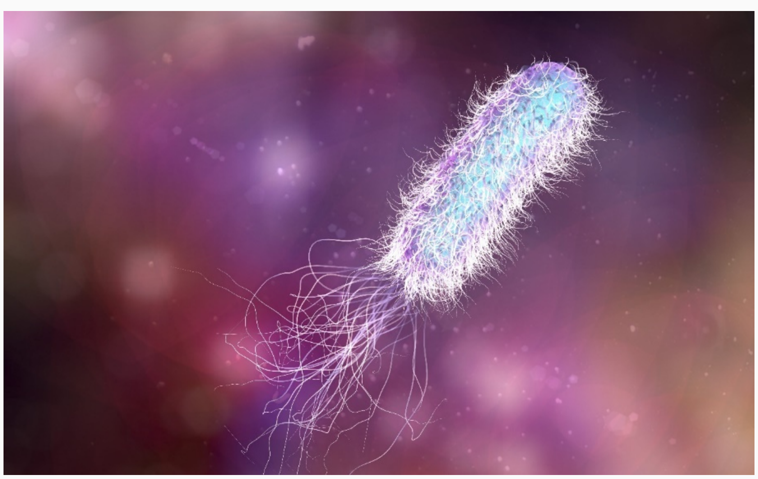
Fig. 1.12 Pediococcus spp.

1.2.20 *Pseudomonas fluorescens*

Pseudomonas fluorescens is a common Gram-negative, rod-shaped, motile bacterium. The organism is psychrotrophic in nature and grows at refrigeration temperature (7°C). It has an extremely versatile metabolism, and can be found in the soil and in water. It is an obligate aerobe, but certain strains are capable of using nitrate instead of oxygen as a final electron acceptor during cellular respiration. Optimal temperature for growth of *Pseudomonas fluorescens* is 25-30 °C. It tests positive for the oxidase. *Pseudomonas fluorescens* is also a nonsaccharolytic organism. Heat-stable lipases and proteases are produced by *Pseudomonas fluorescens* and other similar pseudomonads. These enzymes cause milk to spoil, by causing bitterness, casein breakdown, and ropiness due to the production of slime and coagulation of proteins.

1.2.21 *Pseudomans aeruginosa*

It is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility. An opportunistic human pathogen, *P. aeruginosa* is also an opportunistic pathogen of plants. *P. aeruginosa* is the type species of the genus *Pseudomonas* (Migula). Gram-stained *Pseudomonas aeruginosa* bacteria (pink-red rods) secretes a variety of pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown). *P. aeruginosa* is often preliminarily identified by its fluroscence and grape-like or tortilla-like odor *in vitro*. Definitive clinical identification of *P. aeruginosa* often includes identifying the production of pyocyanin and fluorescein, as well as its ability to grow at 42°C. *P. aeruginosa* is capable of growth in diesel and jet fuel, where it is known as a hydrocarbon-using microorganism (or "HUM bug"), causing microbial corrosion. *P. aeruginosa* is considered by many as a facultative anaerobe



Source: https://www.id-hub.com/wp-content/uploads/2017/02/shutterstock_386499940-bacterium-flagella-e1551112634738.jpg

Fig. 1.13 Pseudomonas aeruginosa

1.2.22 *Salmonella* (*S. typhimurium*, *S. typhi*, *S.enteritidis*)

Salmonella spp. have been reported to be a leading cause of foodborne illnesses in humans. Foodborne salmonellosis scores over all other foodborne bacterial illnesses in humans. Enteric fever is a serious human disease associated with typhoid and paratyphoid strains. *SalmonellaEnterobacteriaceae*. The optimum growth temperature is 37-45 °C. The organism can also grow at about 7°C in foods. I t ferments carbohydrates with its production of acid and gas. Salmonella are oxidase negative, catalase positive and grow on citrate as a sole carbon source and produce H_2S. Some *Salmonella* strains can grow at higher temperatures (54 °C) while others exhibit psychrotrophic properties. The organism has the ability to grow at pH values ranging from 4.5 to 9.5, with an optimum pH growth at 6.5 to 7.5. spp. are facultatively anaerobic, small Gram-negative, non spore forming, rod-shaped (2-4 m m) bacteria belonging to the family

Milk, meat and poultry are principle vehicles of human foodborne salmonellosis. Ingestion of only a few salmonella cells can be infectious. Low levels of salmonellae in a finished food products may, therefore, be of serious public health consequence.

Source: https://cdn.arstechnica.net/wp-content/uploads/2017/02/salmonella_750px.jpg

Fig. 1.14 Salmonella spp.

1.2.23 *Serratia*

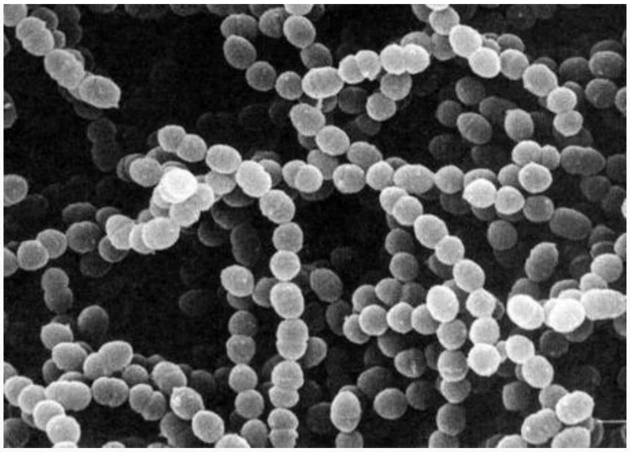
Serratia is a genus of Gram-negative, facultatively anaerobic, rod-shaped bacteria of the *Enterobacteriaceae* family. The most common species in the genus, *S. marcescens*, is normally the only pathogen and usually causes nosocomial infections. However, rare strains of *S. plymuthica*, *S. liquefaciens*, *S. rubidaea*, and *S. odoriferae* have caused diseases through infection. Members of this genus produce characteristic red pigment, prodigiosin.

1.2.24 *Streptococcus thermophilus*

The only *streptococcus* species that is associated with food technology is *S. thermophilus* which is used in the manufacture of yoghurt (in co culture with *L. bulgari*cus and Dahi).

S. thermophilus is a Gram positive facultative anaerobe and belongs to the family *Streptococcaceae*. It is catalase negative organism that is non-motile, non-spore forming and homofermentative and occurs in pairs to long chains. The spherical to avoid cells are with a diameter in the range of 0.7 to 0.9 µm. The optimum temperature for the growth of this organism is between 39°C to 45°C, although most species in the genus are able to grow at temperature ranging from 45-60°C. They do not grow at temperature below 20°C, but they can survive at 65°C for 30 min. They ferment sugars with L (+) lactic acid as the major end product and produce around 0.6 to 0.8% lactic acid. They are able to grow in broth with 2.5% NaCl but fail to grow in 6.5% NaCl at pH 9.6 or in milk with 0.1% methylene blue (Bergey's Manual 1994). It is also classified as lactic acid bacteria (LAB). It is a very versatile organism. *S. thermophilus* has properties that make it one of the commercially most important lactic acid organism. *S. thermophilus* is used along with *Lactobacillus* spp., as a starter culture to manufacture several important fermented dairy foods including yoghurt and mozzarella cheese.

Though the natural habitat of *S. thermophilus* is yet to be established, most strains have been isolated from milk environments.



Source: https://5.imimg.com/data5/GX/YD/MY-22272522/streptococcus-thermophilus-500x500.jpg

Fig. 1.15 Streptococcus thermophilus

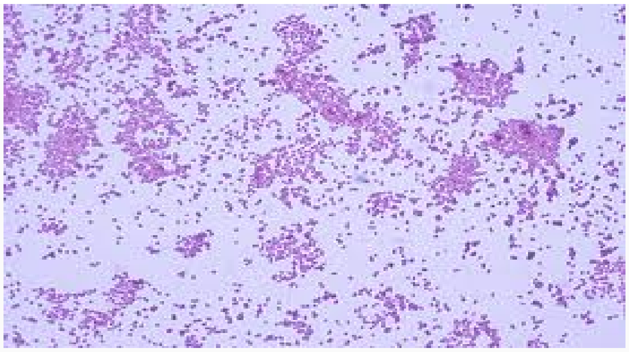
1.2.25 *Staphylococcus aureus*

Staphylococcus aureus is commonly associated with humans. It is a Gram-positive catalase-positive coccus. *Staphylococcus aureus* is the common cause of foodborne gastroenteritis known as staphylococcal food poisoning. Staphylococcal gastroenteritis is caused by the ingestion of food that contains one or more enterotoxin which are produced by some strains of *S. aureus*.

Although enterotoxin production is believed generally to be associated with coagulase and thermo nuclease producing *S. aureus* strains, many species of *Staphylococcus* that produce neither coagulase nor TNase are also known to produce enterotoxin.

The main reservoir of *S. aureus* is the nasal cavity of human beings from where they find their way to the skin and wounds. Mastitis in animals due to *S. aureus* is quite common and from the infected udder the organism finds its way to the milk.

The organism can grow well in NaCl concentrations of 7 to 10%. Though the optimum growth temperature of the organism is 37 °C , some strains can grow at a temperature as low as 6.7 °C. The organism can grow to water activity as low as 0.86.



Source: https://4.bp.blogspot.com/_Uf5f7SzC8gE/TRgN7j_nkJI/AAAAAAAABdk/z4-q2LSKtA0/s1600/v.jpg

Fig. 1.16 Staphylococcus spp.

1.2.26 *Shigella*

Bacillary dysentery, or shigellosis, is caused by *Shigella* species. *Shigella* is a member of the family *Enterobacteriaceae*. The growth temperature varies from 10 to 48 °C. *Shigella*$_2$S. *Shigella* does not usually survive well in low pH foods. *Shigella* is sensitive to ionizing radiations. species are non-motile, oxidase negative produce acid only from sugars; do not grow on citrate as sole carbon source, do not grow on KCN agar, and unlike Salmonellae do not produce H

Shigellosis is an important disease in developed and developing countries. Disease is caused by ingestion of contaminated foods, and in some instances it subsequently leads to rapid dissemination through contaminated feces from infected individuals. The infective dose may be as low as 100 cells. Contamination of foods usually does not occur at the processing plant but rather through an infected food handler. Humans are the natural reservoir of Shigella. The organism is spread through the fecal-oral route.

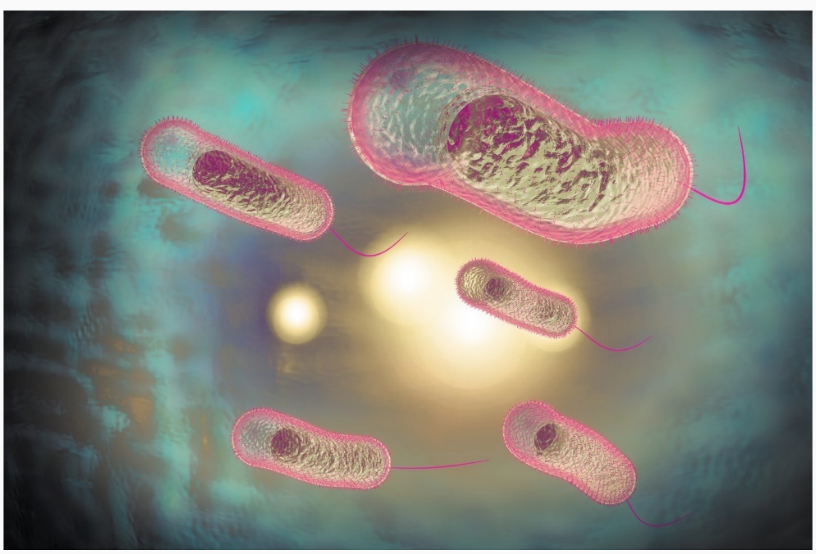
Source:
https://fm.cnbc.com/applications/cnbc.com/resources/img/editorial/2015/04/02/102557636-Shigella.1910x1000.jpg

Fig. 1.17 Shigella spp.

1.2.27 *Vibrio*

Vibrio cholerae and *V. parahaemolyticus* are the two important species of the genus *Vibrio*. *Vibrio cholerae* O1 causes cholera, one of the few food borne illnesses with epidemic and pandemic potential. *Vibrio cholerae* are Gram-negative straight or curved rods and belong to the family *Vibrionaceae*. Important distinctions within the species are made on the basis of productions of cholera enterotoxin (CT) and serogroup.

Vibrio cholerae is part of the normal free living bacterial flora in estuarine areas. Amongst the many different enrichment broths described for the isolation of vibrios alkaline peptone water is the most commonly used. Though *V. parahaemolyticus* can grow in the presence of 1-8% NaCl, the best growth occurs in the salt concentration 2 to 4%.



Source: https://waterandhealth.org/wp-content/uploads/2017/05/AdobeStock_91517814-e1494945250451.jpeg

Fig. 1.18 Vibrio spp.

1.2.28 *Yersinia*

Yersinia enterocolitica and *Yersinia pestis* are the two important human pathogens while *Y. enterocolitica* causes food borne gastroenteritis, *Y. pestis* is an agent of human plague. *Y. enterocolitica* also known as newly emerging human pathogen is a heterogeneous species that is divisible into a large number of subgroups.

Y. enterocolitica is unusual because it can grow at temperatures below 4 °C. The generations time at the 28-30 °C (Optimum growth temperature) is almost 34 min. It also survives in frozen foods. It grows better in processed foods such as pasteurized milk, vacuum packed meat, boiled eggs, boiled fish, and cottage cheese.

Both the species can grow over a pH range of 4 to 10 (optimum pH is 7.6) and tolerate alkaline environment well. They can motile at a temperature < 30 °C. However, both these organisms are susceptible to pasteurization, ionizing and ultraviolet (UV) irradiation. The organism can also tolerate upto 5% NaCl.

Infections with *Yersinia* species are due to transmittance of the organism from animals to humans. The organism is frequently present in pork, lamb, poultry and dairy products.

2.1 Food Borne Yeasts

Yeasts have been associated with foods since earliest times, both as beneficial agents and as major causes of spoilage and economic loss. Current losses to the food and dairy industry caused by yeast spoilage are estimated at several billion dollars. As new food ingredients and new food manufacturing technologies are introduced, novel food spoilage yeasts are emerging to present additional problems. To date over 70 biological species of yeasts have been described and thousands of different varieties have been shown to exist in all kinds of natural and artificial habitats.

Yeasts may be viewed as being unicellular fungi in contrast to the molds, which are multi-cellular. Yeasts can be differentiated from bacteria by their larger cell size and their oval, elongate, elliptical, or spherical cell shapes. Typical yeast cells range from 5 to 8 um in diameter, with some being even larger. Older yeast cultures tend to have smaller cells. Most of those of importance in foods divide by budding or fission.

Yeasts can grow in presence of various types of organic acids such as lactic, citric and tartaric acid etc and also over a wide range of acid pH and in up to 18% ethanol. Many grow in the presence of 55-60% sucrose. Many colours are produced by yeasts, ranging from creamy to pink to red. The asco- and arthrospores of some are quite heat resistant.

2.1.1 *Candida*

Members of the *Candida* genus form shining white colonies and cells contain no carotenoid pigments. *Candida tropicalis* is the most prevalent in foods in general Some members are involved in the fermentation of cocoa beans, as a component of kefir grains, and many other products, including beers, and fruit juices.

2.1.2 *Debaromyces*

Debaromyces is one of the most prevalent yeast genera in the dairy products. It can grow in 24% NaCl and at an a_w as low as 0.65.

2.1.3 *Kluyveromyces*

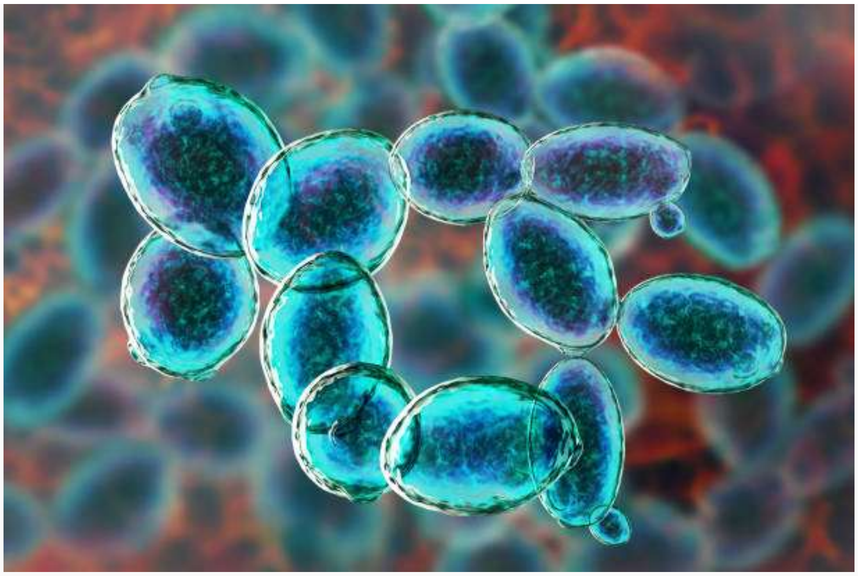
Kluyveromyces spp. produces β-galctosidase and are vigorous fermenters of sugars including lactose. *K. marxianus* is one of the two most prevalent yeasts in dairy products, kefir grain and causes cheese spoilage.

2.1.4 *Rhodotorula*

The genus *Rhodotorula* contains many psychrotrophic species that are found on fresh poultry, shrimp, fish and beef. Some grow on the surface of butter.

2.1.5 S*accharomyces*

Saccharomyces are ascosporogenous yeasts that multiply by lateral budding and produce spherical spores in asci. They are diploid and do not ferment lactose. All bakers' brewers', wine and champagne yeasts are *S. cerevisiae*. They are found in Kefir grains and can be isolated from wide range of foods. *S. cerevisiae* rarely causes spoilage.



Source: https://media.istockphoto.com/photos/saccharomyces-cerevisiae-yeast-picture-id1040455800?k=6&m=1040455800&s=612x612&w=0&h=RLjAY8eOYuilkZihzrToTA4bS6-t8bhFCLp-nIOaX28=

Source: https://nutritionreview.org/wp-content/uploads/2019/01/shutterstock_400086184-Candida-700.jpeg

Fig. 1.19 Food Borne Yeasts (a) *Saccharomyces* spp. (b) *Candida* spp.

2.1.6 *Torulaspora*

Torulaspora multiplies by lateral budding. They are strong fermenters of sugars. *Torula delbrueckii* is the most prevalent species.

2.2 Food-Borne Molds

Molds are filamentous fungi that grow in the form of tangled mass that spreads rapidly and may cover several inches of area in a very short period. It is also referred to as mycelial growth. Mycelium is composed of branches of filaments referred to as hyphae. The molds of great importance in foods multiply by ascospores or conidia. The ascospores of some of the mold genera are notable for their extreme degrees of heat resistance.

2.2.1 *Alternaria*

Alternaria spp. form septate mycelia with conidiophores and large brown conidia are produced. They cause brown to black rots of fruits, apples, and figs. Some species produce mycotoxins.

2.2.2 *Aspergillus*

The *Aspergillus* spp. appear yellow to green to black on a large number of foods. Some species cause spoilage of oils. *A. niger* produces β-galactosidase, glucoamylase, invertase, lipase and pectinase. *A. oryzae* produces a -amylase. Two species *A. flavus* and *A. parasiticus* produce aflatoxins, and others produce ochratoxin A and sterigmatocystin.

2.2.3 *Geotrichum*

The yeast like fungi, *Geotrichum* are also referred to as dairy mold.

2.2.4 *Mucor and Rhizopus*

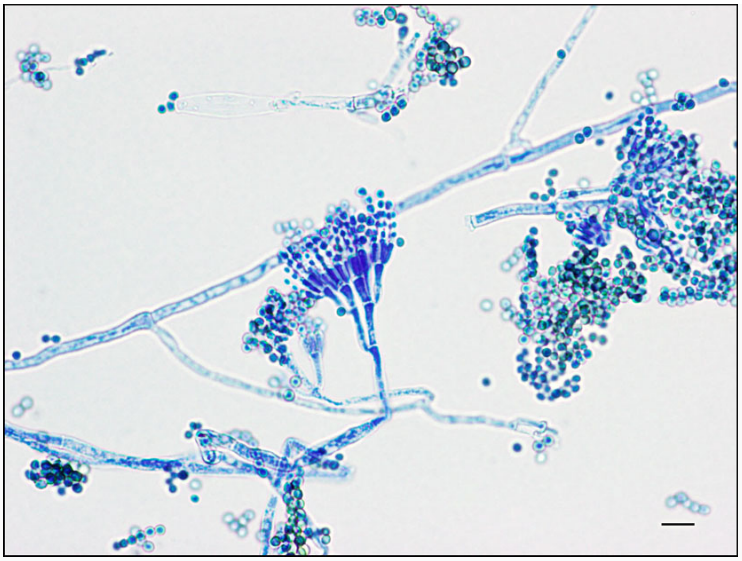
Mucor species that produce non-septate hyphae are prominent food spoilers. Similarly, *Rhizopus* spp. also produce non septate hyphae but give rise to stolons and rhizoids. *R. stolonifer* is by far the most common species in foods and is also referred to as "bread mold".

Other important genera of molds related to spoilage of foods are *Neurospora, Thamnidium, Trichothecium, Penicillium* and *Cladosporium* etc.

Source:https://th.bing.com/th/id/OIP.ZhSxtr5bcNIuINm-2GHQBQHaFw?w=213&h=180&c=7&o=5&dpr=1.25&pid=1.7

Source: https://th.bing.com/th/id/OIP.N1ga5GONZV7TFTq-IZlLmQHaFj?pid=Api&rs=1

Fig. 1.20 Food borne molds (a) *Aspergillus* spp. and (b) *Rhizopus* spp.



Source: https://propertyrecovery911.com/wp-content/uploads/2015/02/Penicillium.jpg

Fig. 1.21 Food borne molds (a) Penicillium spp.

2.3 Food Borne Viruses

Viruses are filterable, ultra microscopic particles and can be cultivated only on live tissues. Viruses consist of a core of nucleic acid (DNA or RNA) and a protein coat. It is commonly believed that some of the viruses are responsible for food borne diseases in humans, particularly some non bacterial gastroenteritis due to enteroviruses. Contaminated water and food are important carriers of hepatitis viruses. Foot and mouth disease (FMD) causing virus in cattle can be transmitted to human beings through foods. Similarly, viral diseases of poultry have also been source of ailments in humans.

Chapter 2

History of Food Microbiology

Although processes of food spoilage and methods of food preservation and food fermentation have been recognized since ancient times. It was not until the 1800s that the relationship between foods and micro-organisms was established. In 1837, Schwann proposed that the yeast which appeared during alcoholic fermentation was a microscopic plant, and between 1857 and 1876, Pasteur showed that micro-organisms were responsible for the chemical changes that take place in foods and beverages. The observations laid the foundation for the development of food microbiology. Knowledge in terms of the role that micro-organisms play in food preservation, food spoilage and food preservation, food spoilage and food poisoning accelerated rapidly until food microbiology gradually emerged as a discipline. Not all groups of micro-organisms are of equal interest to the food microbiologist. Bacteria come on top of the list with molds and yeasts also of considerable importance and viruses.

The history and scope of food microbiology has been classified during various time periods and these have been stated as follows:

7000 BC – Evidence that the Babylonians manufactured beer (fermentation). Wine appeared in about 3500 BC. In early civilizations and even in the present world in underdeveloped countries, where modern sanitation is lacking, alcoholic beverages like beer and wine were much safe to consume than the local water supply. The reason being, the water was often contaminated with intestinal micro-organisms that caused cholera, dysentery and other serious diseases.

6000 BC – The first apparent reference to food spoilage in recorded history.

3000 BC – Egyptians manufactured cheese (fermentation) and butter (fermentation, low aw). Fermented foods, such as cheese and sour milk (yogurt) were safe to consume and repelled damage better as compared to their raw agricultural counterparts. Several cultures also learned to use salt (low aw) to preserve meat and other foods around this time.

1000 BC – Romans made use of snow to preserve shrimp. As it has to be preserved in low temperature, records of smoked and fermented meats also appeared. Even though early human cultures revealed effective ways to preserve food, i.e. fermentation, salt, ice, drying and smoking, they did not understood how these practices, inhibited food spoilage or food borne disease. Their unawareness was compounded by a confidence that living things formed spontaneously from non-living matter.

1665 – An Italian physician, Franceso Redi established that maggots on putrefying meat did not arise unexpectedly but were instead the larval stages of flies. This was the first step away from the doctrine of spontaneous generation.

1683 – Anton van Leeuwenhoek from the Netherlands scrutinized and researched upon bacteria through a microscope. At about the same time, the Royal Society was established in England to communicate and publish scientific work. They invited Leeuwenhoek to impart information regarding his observations. He did so for nearly 50 years until his death in 1723. As a result, Leeuwenhoek's reports were comprehensively disseminated and he is justly regarded as the person, who discovered the microbial world.

1765 – Italian named Spallanzani tried to disprove the theory of spontaneous generation of life by demonstrating that beef broth, which was boiled and then sealed remained sterile. His work was criticized because they believed O_2 was excluded, which they thought was vital for generation that was unstructured.

1795 – The French government offered 12,000 francs to anyone who could develop a practical way to preserve food. A French confectioner, named Nicholas Appert was issued the patent after showing that meat could be preserved when it was placed in glass bottles and boiled. This was the beginning of food preservation by canning.

1837 – Schwann validates that healed infusions remain sterile in the presence of air, which he passed in through heated coils, again to invalidate spontaneous generation. Critics suggest heating somehow changed the effect of air as it was needed for spontaneous generation.

The first person to actually acknowledge and understand the causal relationship between micro-organisms in infusions and the chemical changes that took place in those infusions was Louis Pasteur. Through his experiments, Pasteur convinced the scientific world that all fermentative processes were caused by micro-organisms and that explicit types of fermentations e.g., alcoholic, lactic or butyric were the result of precise types of micro-organisms.

In 1857, he showed that souring milk was caused by microbes and in 1860, he demonstrated that heat destroyed unwanted microbes in wine and beer. The latter process is now used for a variety of foods and is called pasteurization. Because of the importance of his work, Pasteur is known as the founder of food microbiology and microbiological science. He demonstrated that air doesn't have to be heated to remain sterile using his famous swan-necked flasks that finally invalidated spontaneous generation.

In the United States (U.S.), many food industries hesitated to adopt industry wide microbiological standards until they were economically endangered by the publicity which surrounded outbreaks of food borne disease. Several unpleasant occurrences of botulism in the early 1920s finally prompted the U.S. canning industry to adopt a conservative heat treatment, known as the 12D process, that causes a reduction in the probability of survival of the most heat resistant *C*. botulinumspores to one in a billion. This practice continues in the present existence, and since 1925, the canning industry has produced more than a trillion containers with only five to six known incidents of botulism. Most of these incidents involved faulty containers, which are not under processing.

Chapter-3

Primary Sources of Micro-organisms to Food

The primary sources of micro-organisms to food have been stated as follows:

Soil and Water - In soil and water, organisms are generally encountered which are often food-borne bacteria, i.e.Alcaligenes, Bacillus, Citrobacter, Clostridium, Corynebacterium, Enterobacter, Micrococcus, Proteus, Pseudomonas, Serratia and Streptomyces among others. For molds, some of the most commonly encountered in soils are Aspergillus, Rhizopus, Penicillium, Trichothecium, Botrytis, Fusarium, and others. In the case of yeast, a large number of yeast genera are found in the soil, but their number are generally low in water.

Plants and Plant Products - Bacteria often found to be associated with plants and plant products are, Acetobacter, Erwinia, Flavobacterium, Kurthia, Lactobacillus, Leuconostoc, Pediococcus and Streptococcus. As for molds, the most important plant-borne genera are those that cause the damage of vegetables and fruits and these include, Fusarium, Aspergillus, Botrytis, Alternaria etc. The commonly encountered genera of yeast in plant products are the genus Saccharomyces, Rhodotorulaand Torulas.

Food Utensils - The types of food borne microbes that are found in food utensils depend on types of food to a large extent. The care of these utensils, storage and other factors need to be taken into consideration. For example, utensils used in handling vegetables would be expected to have organisms that are associated with vegetables. Also utensils which have been cleaned with hot or boiling water will only as its micro-flora those organisms that are able to withstand the management. Utensils stored in open place, where dust might gather should be expected to have air-borne bacteria, yeast and molds.

Intestinal Tract of Man and Animals - Organisms usually found in the intestinal tract of man are, Bacteroides, Escherichia, Lactobacillus, Proteus, Salmonella, Shigella, Staphylococcus, and Streptococcus. Others include, Clostridium, Nitrobacteria, Enterobacter, and Pseudomonas. These organisms, through fecal dissemination in man found their way directly into soil and water. From soil they may find their way to food utensils. The most encountered yeast in this case is the Candida.

Food Handlers - The organisms usually found on the hands and outer garments of food handlers are a function of the environment and the habit of food handlers. Apart from these, there are some bacteria that are explicitly associated with hands, nasal cavities and mouth. These include Micrococcus and Staphylococcus. The genera Salmonella and Shigella are basically found in the intestine of man. They may be deposited into foods and utensils by food handlers. If sanitary practices are not followed by each individual. Yeast and Molds may be found on the hands and garments of individuals depending upon their direct history.

Animal Feeds – The types of organisms to be found in animal feed would depend on the source of the feeds, the treatment given to them damage micro-organisms, the container in which they are stored and the like. Any one of the above-mentioned genera of bacteria, yeast, and mold may be found in animal feeds. The micro-organism, which is of particular importance is Salmonella sp. which causes food poisoning.

Animal Hides - Generally organisms in soils, water, animal feeds, dust and faecal matter are often found on the hides of animals. From the animal hides, the microbes may find their ways into the air, hands of workers and directly into foods. Some members of the hide flora find their way into the lymphatic system of slaughtered animals from which they migrate into the muscle tissue of the slaughtered animals.

Air and Dust - Majority of genera of bacteria mentioned above except for some pathogens are found in air and dust. Also, many genera of molds and yeast are found. Bacillus and Micrococcus spp. are some of the notable bacteria often found in air and dust because of their ability to sustain aridity to changing degrees.

Chapter-4

Intrinsic & Extrinsic factors affecting the growth of microorganisms

The interaction between microorganisms and other living things in the earth is natural, constant and which plays a significant role in maintaining the ecological balance and stability of biogeochemical cycling. As microorganisms are associated with living things in nature they play a significant role for survival of plants and animals.

Majority of food materials are obtained from plants and animals and it is rich in different type of microorganisms. Some microorganisms serve as food for human and animal, e.g. mushrooms, some present in food are helpful and some others are harmful to our health. Microorganisms use food as the substrate for their growth and colonization.

Depending on the type of microorganisms the growth of many organisms in food can result in improving overall quality of food and in some cases they can deteriorate the quality also. Growth of harmful microorganisms especially bacteria and fungi in food constitutes food spoilage and sometimes cause several diseases on consumption of such food.

The major reason for food spoilage is due to increase in number of microorganisms, utilization of nutrients, causing enzymatic changes resulting in bad flavors due to breakdown of some food materials or synthesis of new compounds. Food becomes unfit for human consumption because of such microbial activities. Microorganisms can oxidize reduced carbon; nitrogen and sulfur compounds present in dead plants and animals and can contribute the minerals to the biogeochemical cycling.

Food acts as good medium for transmission of many diseases and infections. If the food is contaminated by pathogenic microorganisms or their spores, they can grow and increase their population and can produce various types of toxins which may leads to several diseases.

Sometime microorganisms may not grow in food but food can act as transmission route of many diseases. Therefore, food act as good medium for spread of diseases. Several food borne diseases are the result of microorganism present in food or their growth in them.

Growth of microorganisms in food is dependent on various parameters. The factors influencing the growth of microorganisms are physical, chemical and biological in nature. The factors can be generally classified as intrinsic and extrinsic factors.

The intrinsic and extrinsic factors affecting the growth of microorganisms in food are explained below:

Intrinsic Parameters in Food:

The parameters present in substrates in which the microorganisms are growing, that are internal parts of the substrate are called as intrinsic parameters.

The most important types of internal factors in food are:

1. Hydrogen Ion Concentration (pH):

All the microorganisms have a minimal, maximal and optimal pH for their growth, survival and activity of their enzymes. Growth of microorganisms is affected by the pH of growth environments in food (growth medium) resulting large number of enzymes responsible for metabolism and growth. Influence of pH of food not only has effect on growth of microorganisms but also on processing conditions. Food having acidic contents promotes growth of acid loving microorganisms such as yeasts, moulds and some acidophilic bacteria.

Mould can grow over a wider range of acidic pH than bacteria and yeast. Most of the fermentative yeasts can grow at pH of about 4.0 to 4.5, as in fruit juices and acid food such as sauerkraut and pickles. A food with an acid pH would tend to be more microbiologically stable than neutral or alkaline food. Because of this restrictive pH the food such as fruits, soft drinks, fermented milks, sauerkraut and pickles are stable against bacterial spoilage.

Most of the bacteria, except acid fermenters are favored alkaline or neutral pH. Most of the bacteria preferred a pH range between 7.0-7.5 but some proteolytic bacteria can grow on food substrate with high pH. The buffer content in the food is important to maintain the stability against microbial spoilage.

Buffers permit an acid (or alkali) fermentation to go on longer with a greater yield of products and organisms. Vegetable juices have low buffering capacity permitting a decrease in pH with the production of only small amount of acid by the lactic acid bacteria during the early stage of sauerkraut and pickle fermentation. This helps to inhibit the growth of pectin hydrolyzing and proteolytic competing bacteria in food.

Food acidification by fermentation in home food preparations is the oldest practice man has been doing. It is due to production of organic acids in food by growth and fermentation of microorganisms such as lactic and acetic acid bacteria. The inhibitory properties of many of the organic acids such as citric acid, lactic acid, benzoic acid, propionic acid, sorbic acids, etc. can be used as effective acidulants or chemical preservatives against food spoilage bacteria.

2. Water Activity or Moisture Content (a_w):

Water is an excellent solvent for all life processes in every living organism for biocatalytic activity. The amount of water required varies for different organisms. Water requirement of microorganisms is expressed as available water or water activity a_w. Water activity is the vapor pressure of the solution (of solutes in water in most food) divided by the vapor pressure of the solvent (usually water).

In other words it is defined by the ratio of the water vapor pressure of food substrate to the vapor pressure p of pure water at the same temperature – $a_w = p/p_o$, where P is the vapor pressure of the solution and Po is the vapor pressure of the solvent (usually water). The a_w content is very well related to relative humidity (RH) in the following way: RH = 100 x a_w.

Pure water has an a_w of 1.00, a 22% NaCl solution (w/v) has an a_w of 0.86, and a saturated solution of NaCl has an a_w of 0.75. The water activity (a_w) of most fresh foods is above 0.99. In general, bacteria require more water activity than moulds and yeasts. Gram-negative bacteria have higher water requirements than gram-positive bacteria.

Most of the food spoilage bacteria do not grow below a_w 0.91, while spoilage moulds can grow even at a_w 0.80. The aerobic food poisoning bacterium Staphylococcus aureus is found to grow at a_w as low as 0.86 while anaerobic Clostridium botulinum does not grow below a_K 0.94. Moulds differ considerably in optimal a_w for vegetative growth and spore germination.

The lowest a_w value for foodborne bacteria is 0.75 for halophiles ("salt-loving"), whereas xerophilic ("dry-loving") moulds and osmophilic (preferring high osmotic pressures) yeasts have been reported to grow at a_w values of 0.65 and 0.61.

Table: 4.1: Minimal water activity values for different types of microorganisms spoiling food

Group of Microorganism	**Minimal (aw) value**
Bacteria	0.91
Yeasts	0.88
Moulds	0.80
Halophilic bacteria	0.75
Xerophilic fungi	0.65
Osmophilic yeasts	0.60

The effect of lowering a_w below optimum is to increase the length of the lag phase of growth and to decrease the growth rate and size of final population of microorganisms. This is due to adverse influences of lowered water on all metabolic activities in microorganisms since all chemical reactions in cells require an aqueous environment.

The a_w is influenced by other environmental parameters such as pH, Eh (redox potential) and growth temperature required for microorganisms. The other factors which influence the water activity are the kinds of solute employed to reduce water activity, the nutritive significance of culture medium, temperature, supply of oxygen, hydrogen ion concentration and presence of inhibitors.

3. Redox Potential (Eh):

The reducing and oxidizing power of the food will influence the type of organism and chemical changes produced in the food. The concentration of oxygen in food, chemical composition and type of microorganisms associated contribute to the oxidation-reduction (O-R) potential of food and affect growth of microorganisms in them. The O-R potential of a food may be defined as the ease with which the substrate loses or gains electrons.

The Redox potential of food is determined by characters such as:

(a) Oxygen tension of atmosphere above the food,

(b) Access of atmosphere to the food,

(c) Resistance of food to the changes occurring and

(d) O-R state of materials present in food.

On the basis of the ability of microorganism to utilize oxygen, organisms are classified as aerobic, anaerobic and facultative anaerobes. Aerobes require free oxygen and anaerobes don't prefer oxygen as it is toxic to them, hence, it is grow in the absence of molecular oxygen. Facultative may grow both aerobic and anaerobic conditions.

Generally fungi- mould and yeasts are aerobic. But bacteria are variables of these aspects. Some are aerobic, some are anaerobics and others are facultative anaerobes. If oxidation potential is high then aerobes will grow better than anaerobes, but if conditions become more reduced then anaerobes will be the predominant organisms.

The O-R potential is written as Eh and measured and expressed as millivolts (mV). If the substrate is highly oxidized would have a positive Eh and substrate is reduced is a negative Eh. Aerobic microorganisms such as bacilli, cocci, micrococci, pseudomonas, acinetobacters require and grow at positive O-R potential and anaerobe such as Clostridia and bacteriodes require negative O-R potential for their growth.

Most of the fresh plant and animal food have low redox potential because of reducing substances present in them. Fresh vegetables and fruits contain reducing substances such as ascorbic acid, reducing sugars and animal tissues have sulfhydryl (-SH) and other reducing group compounds considered as antioxidants.

Fresh vegetables, fruits and meat promote growth of aerobic microorganisms in the surface regions because of positive redox potential. However, the anaerobic microorganisms grow in inner parts of vegetables, fruits and meat because of negative redox potential. Most of processed plant and animal food gain positive redox potential therefore promote growth of aerobic organisms.

4. Composition of Nutrients:

Nutrients are one of the most important compounds for the growth and functioning of microorganism. Nutritional quality of food depends on the chemical composition, nutritive value or nutrients, their proportion and growth promoting ability to the microorganisms.

The most important factors which have to be considered are the energy substances in food, nitrogen substances, growth promoting substances, accessory food substances or vitamins, minerals, and water content which all are very essential for growth or energy production of organisms.

The most energy sources of organisms are carbohydrates. Complex carbohydrates such as cellulose, hemicelluloses, starch, pectin, etc. can be utilized by various types of microorganisms. At the same time other carbon compounds such as esters, alcohols, peptides, amino acids, organic acid and their salt are also serving as energy sources for many organisms.

Bacteria are identified and classified based on their ability or inability to utilize various sugars and alcohols. Most organisms can hydrolyses complex carbohydrates and can use glucose as energy source. Some organisms have the ability to hydrolyze pectin by producing the enzyme pectinase.

Some microorganism can hydrolyze triglycerides and other types of fats by microbial lipase and produces glycerol and smaller fatty acid. In this step triglycerides are hydrolyzed in to diglycerides then monoglycerides and glycerols under alkaline condition by microbial lipase. The glycerol and fatty acids are excellent sources of carbon and energy sources of many aerobic organisms.

Hydrolytic products of proteins and peptides serves as sources of nitrogen for many proteolytic bacteria such as Pseudomonas sps. The primary nitrogen sources utilized by heterotrophic microorganisms are amino acids. A large number of other nitrogenous compounds may serve this function for various types of organisms. Some microbes are able to utilize nucleotides and free amino acids, whereas others are able to utilize peptides and proteins.

In general, simple compounds such as amino acids will be utilized by almost all organisms before any attack is made on the more complex compounds such as high-molecular-weight proteins. Protein rich food promotes more growth of bacteria than moulds and yeasts.

Some microorganisms require vitamins and other growth factors for their growth and that has to be supplied with the growth medium. Such microorganisms are called fastidious organisms. Food contains different vitamins, minerals and other growth factors and their composition and content may vary.

Fresh plant and animal food contain vitamin B complex and fruits are low, but fruits are high in ascorbic acid. Processing of food often reduces the vitamin content. Thiamine, pantothenic acid, folic acid and ascorbic acid are heat-labile and drying cause's loss in vitamins such as thiamine and ascorbic acid.

Storage of food for long may also result in decrease in vitamins and other growth factors. Some microorganisms produce vitamins and other growth factors which support growth of others organisms present in food. Each kind of microorganisms has a range of food requirements.

Water is another very important component for food nutrients. The water requirement of organisms will depend on the type of organisms. Generally moulds have the lowest requirement, followed by gram-negative bacteria, yeasts, and gram-positive bacteria.

5. Inhibitory Substances:

Inhibitory substances are present in the food as its own origin, or added purposely by preventing or inhibiting the growth of organisms. The stability of certain foods against attack by microorganisms is due to the presence of certain naturally occurring substances that possess and express antimicrobial activity.

Some plant species are known to contain essential oils that possess antimicrobial activity. Eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allyl isothiocyanate in mustard, eugenol and thymol in sage and carvacrol (isothymol) and thymol in oregano are some of the best studied examples. Milk contains several antimicrobial substances, including lactoferrin, conglutinin and the lactoperoxidase system.

Milk casein and some fatty acids have been shown to be antimicrobial property against some organisms. Lactoferrin is an iron-binding glycoprotein that is inhibitory to a number of foodborne bacteria and its use as a microbial blocking agent on beef carcasses. Eggs contain lysozyme; ovotransferrin and conalbumin have shown some antimicrobial properties.

6. Biological Structures:

The natural covering of some foods provides excellent protection against the entry and subsequent damage by spoilage organisms. The inner part of healthy tissues of living plants and animals are sterile and contains less microbial count. The protective covering of food such as the skin of eggs, the skin on poultry, rind on fruits and vegetables, shell on nuts and artificial coating helps to protect its inner structures from microbial contamination and spoilage.

The physical protection of the food my help for preservation and determination of kind, rate and course of spoilage. Layers of fat over meat may protect that part of the flesh, or scales may protect the outer part of fish. In the case of nuts such as pecans and walnuts, the shell or covering is sufficient to prevent the entry of all organisms.

Once cracked nut meats are subject to spoilage by moulds. The outer shell and membranes of eggs prevent the entry of all microorganisms when stored under the proper conditions of humidity and temperature. Fruits and vegetables with damaged covering undergo spoilage much faster than those not damaged.

Extrinsic Parameters:

The extrinsic parameters are substrate independent and in this case the storage environment that affect both the food and their microorganisms.

The main extrinsic parameters influence the foods are:

1. Relative Humidity (RH):

The relative humidity of the storage environment is important extrinsic parameter both from the standpoint of a_w within foods and the growth of microorganisms at the surfaces. When foods with low a_w contents are placed in high RH environments, the foods takes up more moisture until equilibrium has been established.

Similarly foods with a high a_w lose moisture when placed in an environment of low RH. There is a relationship between RH and temperature that should be borne in mind in selecting proper storage environments for foods. Generally, if the temperature high then the RH low and vice versa.

2. Atmospheric Gases:

Like O_2, Carbon dioxide (CO_2) is also most important atmospheric gas that is used to control microorganisms in foods. Modified atmosphere packaged (MAP) foods are make use of this types of gases. Ozone (O_3) is the other atmospheric gas that has high antimicrobial properties; it has strong oxidizing property hence it should not use for fat rich food as it will undergo auto-oxidation. It has been noticed that ozone extend the shelf life of many foods and it has shown to be effective against a variety of microorganisms.

3. Temperature:

Microorganisms can grow over a wide range of temperatures. The lowest temperature at which a microorganism has been reported to grow is -34°C; the highest is somewhere in excess of 100°C.But some spore producing bacteria such as Bacillus stearothermophilus, Clostridium tetani and Clostridium perfringens can grow above 100°C.

Based on the temperature range microorganisms are classified as three groups –

i. Psychrophiles (Psychrotrophs), those organisms are grown between the temperature ranges of 2°C to 20-30°C.

ii. Mesophiles, the organism preferably grow at the temperature between 20°C and 45°C and

iii. Thermophiles, the organisms grow better in range of 55°C-65°C.

The most important psychrotrophs include Alcaligenes, Shewanella, Brochothrix, Corynebacterium, Flavobacterium, Lactobacillus, Micrococcus, Pectobacterium, Pseudomonas, Psychrobacter, Enterococcus and others. The psychrotrophs found most commonly on foods are those that belong to the genera Pseudomonas and Enterococcus.

These organisms grow well at refrigerator temperature and cause spoilage at 5-7°C of meats, fish, poultry, eggs, and other foods normally held at this temperature. Mesophilic species and strains are known bacteria among all genera and may be found on food held at refrigerator temperatures. Most important thermophilic bacteria in food belong to the genera Bacillus, Paenibacillus, Clostridium, Geobacillus, Alicyclobacillus and Thermoanaerobacter.

Like bacteria fungi are also able to grow over wide ranges of temperature. Many moulds are able to grow at refrigerator temperatures, especially some strains of Aspergillus, Cladosporium, and Thamnidium, which may be found growing on eggs, sides of beef and fruits. Yeasts prefer psychrotrophic and mesophilic temperature ranges but generally not within the thermophilic range.

4. Other Microbial Flora:

Microorganisms present in the food can undergo various types of negative interactions. These kinds of interaction cause inhibition of some microorganisms as they are undergoing competitions and antibiosis. Some organisms especially moulds can produce various types of secondary metabolites such as antibiotics that are toxic to many bacteria. Some foodborne organisms produce substances that are either inhibitory or lethal to others; these include bacteriocins, hydrogen peroxide and organic acids.

Chapter- 5

Bacterial growth curve

Bacteria are prokaryotic organisms that most commonly replicate by the asexual process of **binary fission**. These microbes reproduce rapidly at an exponential rate under favorable conditions. When grown in culture, a predictable pattern of growth in a bacterial population occurs. This pattern can be graphically represented as the number of living cells in a population over time and is known as a **bacterial growth curve**. Bacterial growth cycles in a growth curve consist of four phases: lag, exponential (log), stationary, and death.

Bacteria require certain conditions for growth, and these conditions are not the same for all bacteria. Factors such as oxygen, pH, temperature, and light influence microbial growth. Additional factors include osmotic pressure, atmospheric pressure, and moisture availability. A bacterial population's **generation time**, or time it takes for a population to double, varies between species and depends on how well growth requirements are met.

In nature, bacteria do not experience perfect environmental conditions for growth. As such, the species that populate an environment change over time. In a laboratory, however, optimal conditions can be met by growing bacteria in a closed culture environment. It is under these conditions that the curve pattern of bacterial growth can be observed.

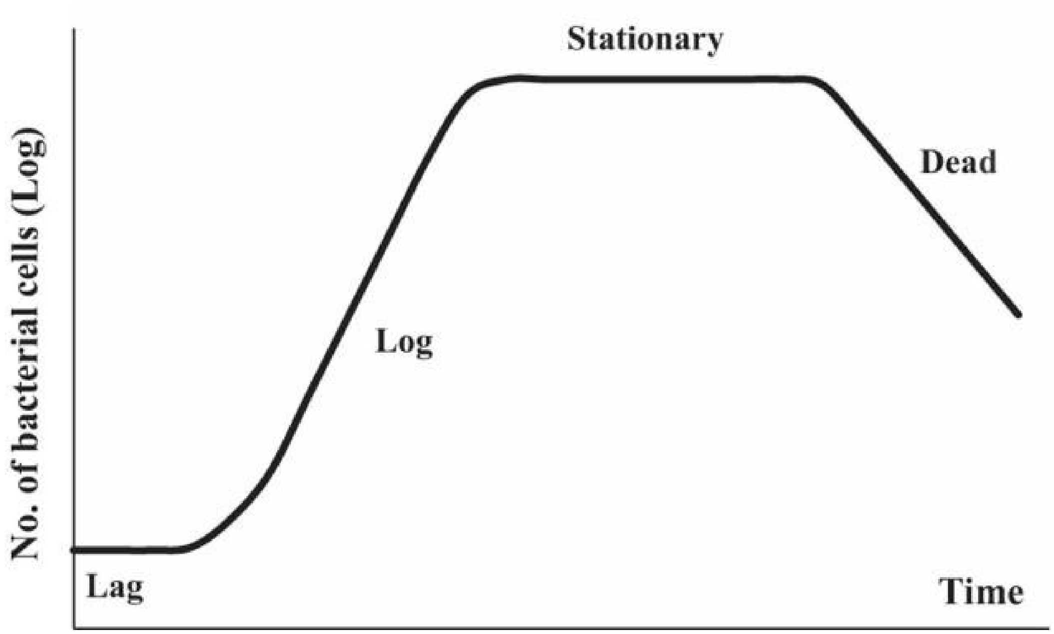


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Fig: Bacterial growth curve

The **bacterial growth curve** represents the number of live cells in a bacterial population over a period of time.

- **Lag Phase:** This initial phase is characterized by cellular activity but not growth. A small group of cells are placed in a nutrient rich medium that allows them to synthesize proteins and other molecules necessary for replication. These cells increase in size, but no cell division occurs in the phase.
- **Exponential (Log) Phase:** After the lag phase, bacterial cells enter the exponential or log phase. This is the time when the cells are dividing by binary fission and doubling in numbers after each generation time. Metabolic activity is high as DNA, RNA, cell wall components, and other substances necessary for growth are generated for division. It is in this growth phase that antibiotics and disinfectants are most effective as these substances typically target bacteria cell walls or the protein synthesis processes of DNA transcription and RNA translation.
- **Stationary Phase:** Eventually, the population growth experienced in the log phase begins to decline as the available nutrients become depleted and waste products start to accumulate. Bacterial cell growth reaches a plateau, or stationary phase, where the number of dividing cells equal the number of dying cells. This results in no overall population growth. Under the less favorable conditions, competition for nutrients increases and the cells become less metabolically active. Spore forming bacteria produce endospores in this phase and pathogenic bacteria begin to generate substances (virulence factors) that help them survive harsh conditions and consequently cause disease.
- **Death Phase:** As nutrients become less available and waste products increase, the number of dying cells continues to rise. In the death phase, the number of living cells decreases exponentially and population growth experiences a sharp decline. As dying cells lyse or break open, they spill their contents into the environment making these nutrients available to other bacteria. This helps spore producing bacteria to survive long enough for spore production. Spores are able to survive the harsh conditions of

the death phase and become growing bacteria when placed in an environment that supports life.

Bacterial Growth and Oxygen

Bacteria, like all living organisms, require an environment that is suitable for growth. This environment must meet several different factors that support bacterial growth. Such factors include oxygen, pH, temperature, and light requirements. Each of these factors may be different for different bacteria and limit the types of microbes that populate a particular environment.

Bacteria can be categorized based on their **oxygen requirement** or tolerance levels. Bacteria that can not survive without oxygen are known as **obligate aerobes**. These microbes are dependent upon oxygen, as they convert oxygen to energy during cellular respiration. Unlike bacteria that require oxygen, other bacteria can not live in its presence. These microbes are called **obligate anaerobes** and their metabolic processes for energy production are halted in the presence of oxygen.

Other bacteria are **facultative anaerobes** and can grow with or without oxygen. In the absence of oxygen, they utilize either fermentation or anaerobic respiration for energy production. **Aerotolerant anerobes** utilize anaerobic respiration but are not harmed in the presence of oxygen. **Microaerophilic bacteria** require oxygen but only grow where oxygen concentration levels are low. *Campylobacter jejuni* is an example of a microaerophilic bacterium that lives in the digestive tract of animals and is a major cause of foodborne illness in humans.

Bacterial Growth and pH

Another important factor for bacterial growth is pH. Acidic environments have pH values that are less that 7, neutral environments have values at or near 7, and basic environments have pH values greater than 7. Bacteria that are **acidophiles** thrive in areas where the pH is less than 5, with an optimal growth value close to a pH of 3. These microbes can be found in locations such as hot springs and in the human body in acidic areas such as the vagina.

The majority of bacteria are **neutrophiles** and grow best in sites with pH values close to 7. *Helicobacter pylori* is an example of a neutrophile that lives in the acidic environment of the stomach. This bacterium survives by secreting an enzyme that neutralizes stomach acid in the surrounding area.

Alkaliphiles grow optimally at pH ranges between 8 and 10. These microbes thrive in basic environments such as alkaline soils and lakes.

Bacterial Growth and Temperature

Temperature is another important factor for bacterial growth. Bacteria that grow best in cooler environments are called **psycrophiles**. These microbes prefer temperatures ranging between 4°C and 25°C (39°F and 77°F). Extreme psycrophiles thrive in temperatures below 0°C/32°F and can be found in places such as arctic lakes and deep ocean waters.

Bacteria that thrive in moderate temperatures (20-45°C/68-113°F) are called **mesophiles**. These include bacteria that are part of the human microbiome which experience optimum growth at or near body temperature (37°C/98.6°F).

Thermophiles grow best in hot temperatures (50-80°C/122-176°F) and can be found in hot springs and geothermal soils. Bacteria that favor extremely hot temperatures (80°C-110°C/122-230°F) are called **hyperthermophiles**.

Bacterial Growth and Light

Some bacteria require light for growth. These microbes have light-capturing pigments that are able to gather light energy at certain wavelengths and convert it to chemical energy. **Cyanobacteria** are examples of photoautotrophs that require light for photosynthesis. These microbes contain the pigment **chlorophyll** for light absorption and oxygen production through photosynthesis. Cyanobacteria live in both land and aquatic environments and can also exist as phytoplankton living in symbiotic relationships with fungi (lichen), protists, and plants.

Other bacteria, such as **purple and green bacteria**, do not produce oxygen and utilize sulfide or sulfur for photosynthesis. These bacteria contain **bacteriochlorophyll**, a pigment capable of absorbing shorter wavelengths of light than chlorophyll. Purple and green bacteria inhabit deep aquatic zones.

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