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Basic Aspects, History, Scope and Microbes (Bacteria, Yeasts and Molds) Associated with Food



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Introduction to Food Microbiology

- 1. Food microbiology will provide brief information on the genera of different microorganisms (bacteria and fungi) encountered in foods.**
- 2. Forms the basis of the study of microorganisms and their interactions to food.**
- 3. Major objective of relating interaction of microorganisms and food is in relation to food bioprocessing, food spoilage and foodborne diseases.**

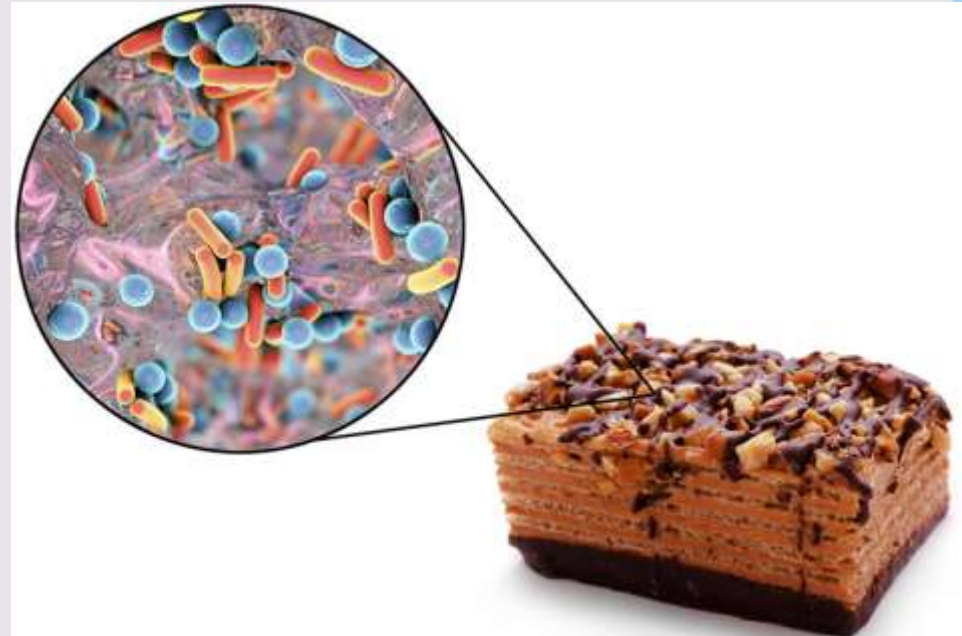


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History of Microorganisms

1658	Athanasius Kircher using a microscope had seen minute living worms in putrid meat and milk
1664	Robert Hooke described structure of molds
1676-1683	Antony van Leeuwenhoek observed bacteria (animalcules) in saliva, rainwater, vinegar, and other materials as spheroids or cocci, cylindrical rods/bacilli, spiral/spirilla)
1875	Ferdinand Cohn had developed the preliminary classification system of bacteria
1940	Existence of submicroscopic viruses





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What are the importance of microorganisms in food?

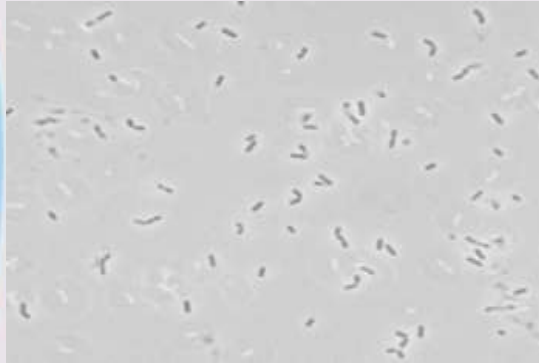
Good (desirable)	Bad (undesirable)
Food bioprocessing	Foodborne disease
Food biopreservation	Food spoilage
Probiotics	

Spoilage bacteria



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Brochothrix



Pseudomonas



Alcaligenes

Food poisoning bacteria



Salmonella
Found in animals,
raw poultry and
birds



**Staphylococcus
Aureus**
Found in human
nose and throat
(also skin)



**Clostridium
Perfringens**
Found in animals
and birds



Bacillus Cereus
Found in soil,
vegetation,
cereals and
spices



**Clostridium
Botulinum**
Found in the soil
and associated
with vegetables
and meats



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Important bacterial groups in food

- **Bacteria constitute major important groups.**
- **Highly characterized because of their rapid growth rate, ability to utilize food nutrients, and ability to grow under a wide range of temperatures, aerobiosis, pH, and water activity, as well as to better survive adverse situations, such as survival of spores at high temperature.**



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Acinetobacter:

- **Genus of Gram-negative bacteria belonging to the Gammaproteobacteria.**
- **Non-motile, oxidase-negative, occur in pairs under magnification.**
- **Young cultures show rod shaped morphology.**
- **Strict aerobes that do not reduce nitrates.**
- **Important soil and water organisms and found especially refrigerated fresh products.**
- ***A. baumannii*: Nosocomial pneumonia, skin and wound infections, bacteremia, and meningitis.**



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Bacillus cereus:

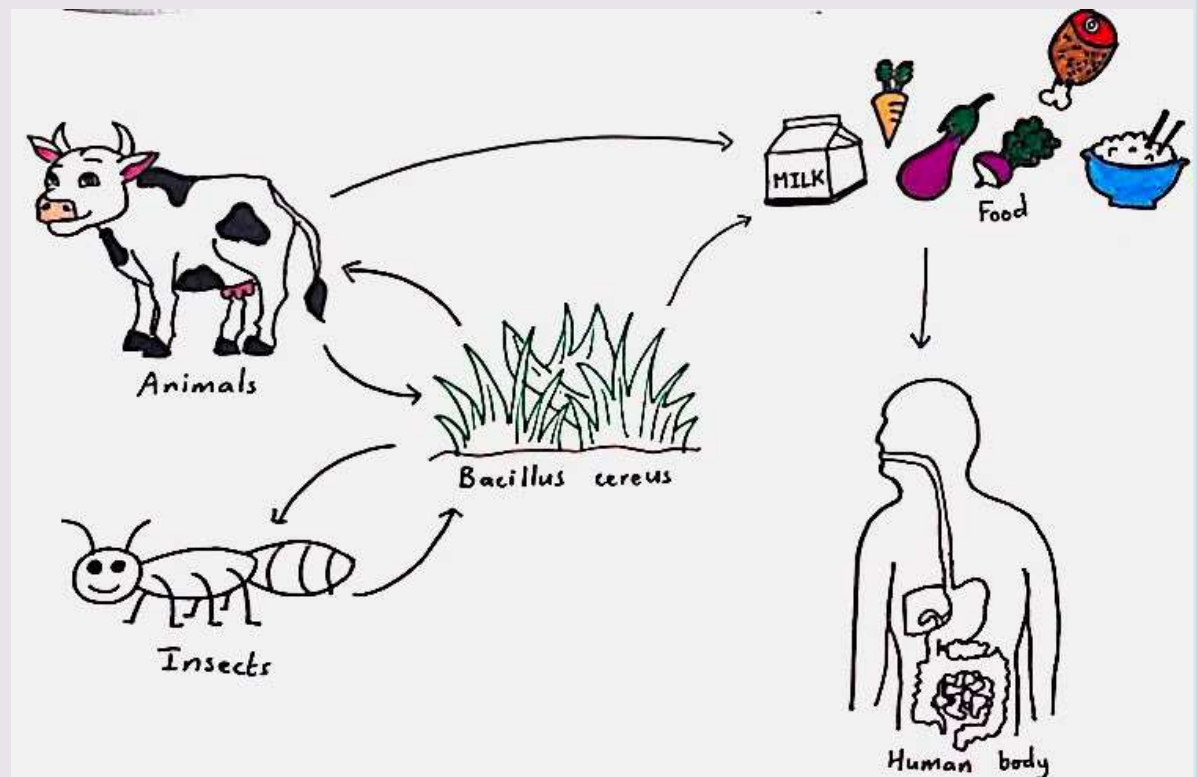


- **Thick, long rod shaped, Gram positive, catalase positive, aerobic spore former and important in food borne illness.**
- **Normal inhabitant of soil and isolated from variety of foods.**
- **Cause of diarrheal illness due to the consumption of desserts, meat, dishes, dairy products, cooked rice and pasta etc kept at room temperature.**
- **Spread from soil and grass to cows udders and into the raw milk.**



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- Capable of producing proteolytic, amylolytic enzymes and phospholipase C which can lead to food spoilage.
- Diarrheal illness is caused by an enterotoxin produced during vegetative growth of *B. cereus* in small intestine.
- Maximum growth temperature: 48-50°C and pH range 4.9-9.3.
- Spores are resistant to heat and survive pasteurization temperature.





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Bacillus subtilis: (Hay Bacillus/Grass Bacillus)

- **Gram-positive, catalase-positive commonly found in soil.**
- **Thin short rod-shaped, ability to form tough, protective endospore, allowing organism to tolerate extreme environmental conditions.**
- **Produces proteolytic enzyme subtilisin.**
- **Responsible for causing ropiness, sticky, stringy consistency caused by bacterial production of long-chain polysaccharides in spoiled bread dough.**
- ***Bacillus natto* is used in commercial production of Japanese food natto.**



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Carnobacterium:

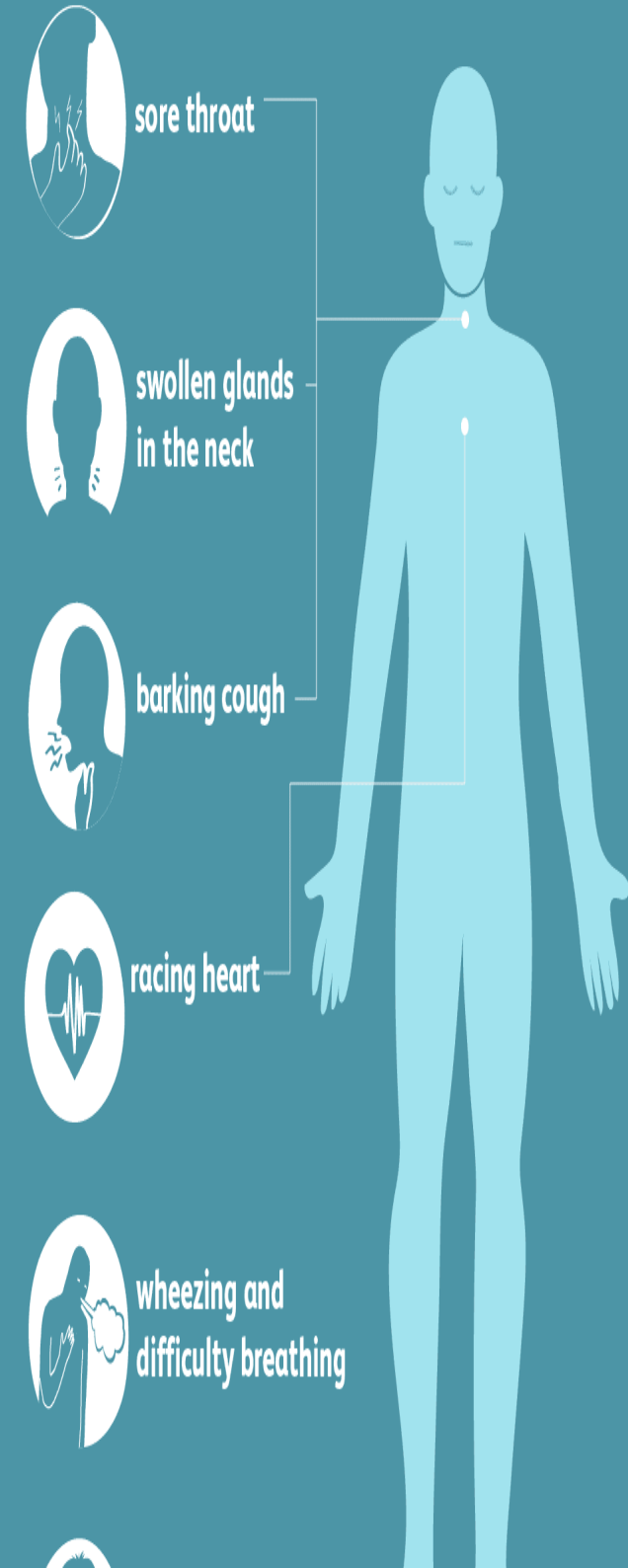
- Gram-positive within Leuconostocaceae.
- *C. divergens* and *C. maltaromaticum* are found in food products and can grow anaerobically.
- Not pathogenic in humans but may cause disease in fish.
- Nine species, but only *C. divergens* and *C. maltaromaticum* are frequently isolated from natural environments and foods. Tolerant to freezing/thawing, high pressure.
- Both are protective cultures in order to inhibit growth of *Listeria monocytogenes* in fish and meat products.
- Can spoil chilled foods.
- Production of tyramine in foods is critical for susceptible individuals.



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Corynebacterium:

- Gram-positive, rod-shaped.
- Some useful in industries: *C. glutamicum*.
- *C. diphtheriae*: Diphtheria.
- *C. diphtheriae* (pathogenic effect) acquires capacity to produce diphtheria toxin only after interacting with 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.





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Clostridium perfringens:

- Gram-positive, encapsulated, anaerobic, non-motile bacterium commonly found on meat and meat products.
- Toxin producing organism produces *C. perfringens* enterotoxin and β -toxin that are active on human GI tract.
- Multiplies very rapidly in food (doubling time < 10 min).
- Spores are resistant to radiation, desiccation and heat thus, survive incompletely in cooked foods.
- Tolerates moderate exposure to air.
- Vegetative cells are heat tolerant (43-45°C) and can often grow at 50°C.
- Not tolerant to refrigeration and freezing.



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Clostridium botulinum:

- Gram-positive, obligate anaerobic, rod shaped.
- Oval endospores are formed in stationary phase cultures.
- Seven types of *C. botulinum* (A to G) based on the serological specificity of neurotoxin.
- Botulism is a serious disease (ingestion of neurotoxin produced by organism in foods can lead to death). Toxin is easily inactivated by heat.
- Growth temperature: 10-48°C with optimum at 37°C. Spores are highly heat resistant.
- Outgrowth of spores is inhibited at pH<4.6, NaCl> 10% or water activity< 0.94.
- *Botulinum* spores are radiation resistant.
- Produces toxin in under processed (improper canning) low acid foods at ambient temperature.



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***Campylobacter* :**

- Gram-negative, microaerophilic, non-spore forming rods.
- *C. jejuni* is an important food borne pathogen.
- *C. jejuni* and *C. coli* cause diarrhea in humans.
- Destroyed by milk pasteurization temperature).
- Sensitive to freezing.
- Curved, S-shaped/spiral rods that may form spherical/cocoid forms in old cultures or cultures exposed to air for prolonged periods.
- Oxidase and catalase positive and does not grow in the presence of 3.5% NaCl.
- Present in raw milk, poultry, fresh meats, pork sausages and ground beef.
- Infective dose: <1,000 organisms





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Erwinia:

- Family *Enterobacteriaceae* containing mostly plant pathogenic species.
- Organisms was named after the first phytobacteriologist, Erwin Smith.
- Rod-shaped, Gram negative related to *E. coli*, *Shigella*, *Salmonella* and *Yersinia*.
- *E. amylovora* causes fire blight on apple and pear.
- *E. carotovora* (*Pectobacterium carotovorum*) causes diseases in many plants.
- Species produce pectolytic enzymes that hydrolyze pectin between individual plant cells.
- Decay caused by *E. carotovora* is often referred as bacterial soft rot (BSR).
- High humidity and temperatures (30°C) favor development of decay.



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Enterococcus (E. faecium, E. faecalis):

- **Genus of LAB, Gram positive, cocci that often occur in pairs (diplococci)/short chains.**
- **Commensal organisms in human intestine.**
- **Facultative anaerobic, non-spore forming, optimum growth at 35°C (tolerate wide range 10-45°C, pH 4.5-10.5, high NaCl concentration 6.5%) and can survive heating at 60°C for 30 min.**
- **Catalase-negative, oxidase-negative occurs in vegetables, plant materials, meat and dairy products.**
- **Resists pasteurization temperatures and adapts to different substrates and growth conditions in food products.**
- **Constitute an important microflora in fermented cheese and meats.**



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E. coli:

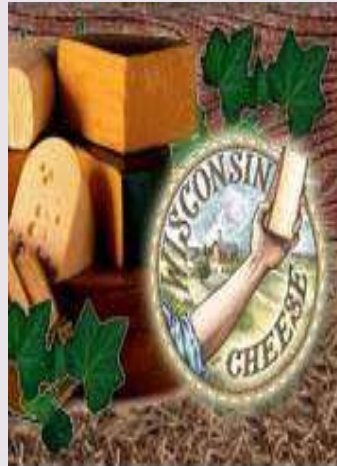
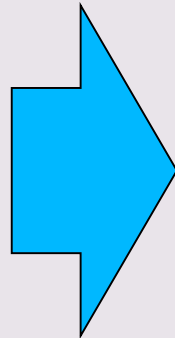
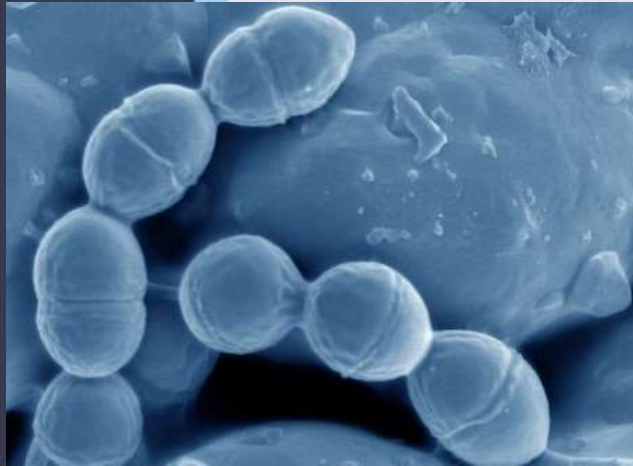
- Associated with food borne gastroenteritis.
- Gram-negative rods, ferment lactose and produce dark colonies with a metallic sheen on Endo agar.
- Growth temperature (4-46 °C) and pH (4.4-9.0).
- Indicator of fecal pollution.
- Capable of producing acid, gas and off-flavours in foods.
- Five groups: **enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC) and facultatively enteropathogenic (FEEC).**
- Grows in presence of bile salts.
- Primary habitat: Intestinal tract of most warm blooded animals.
- *E. coli* 0157:H7: Unusually tolerant of acidic environments.



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Lactococcus:

- Formerly included in genus *Streptococcus* Group N (Group N Streptococci).
- Homofermentors (produce lactic acid).
- Gram-positive, catalase negative, non-motile coccus that are found singly, in pairs or in chains.
- Commonly used in dairy industry for manufacture of fermented dairy products (cheeses).
- Used in single strain starter cultures or in mixed strain cultures with *Lactobacillus* or *Streptococcus*.
- Rapid acidification of milk causes pH drop of fermented product that prevents growth of spoilage and pathogenic bacteria.





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- Play role in flavor of the final product.
- Dairy lactococci exploited for several industrial fermentations in the biotechnology industry.
- *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.



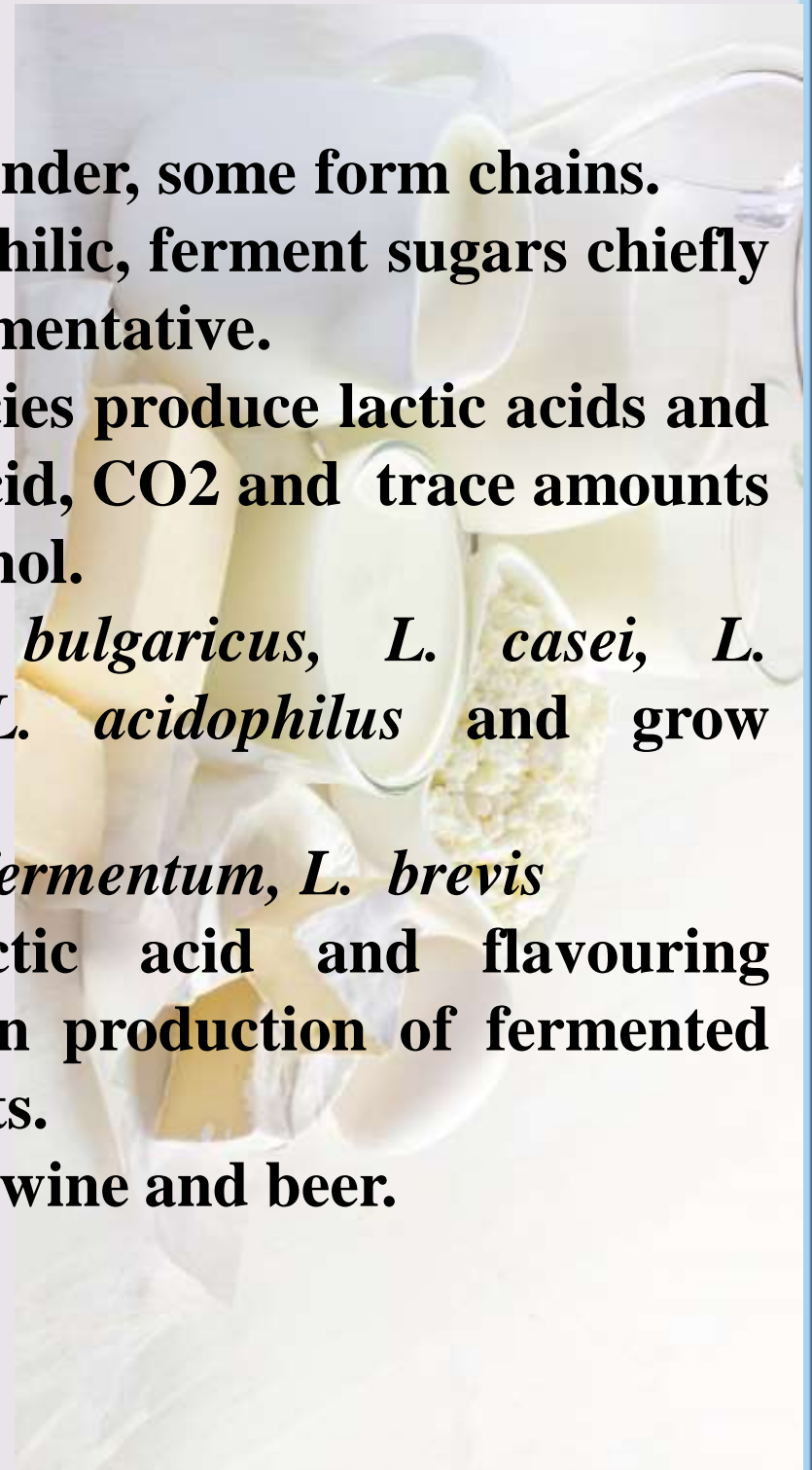


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Lactobacillus:

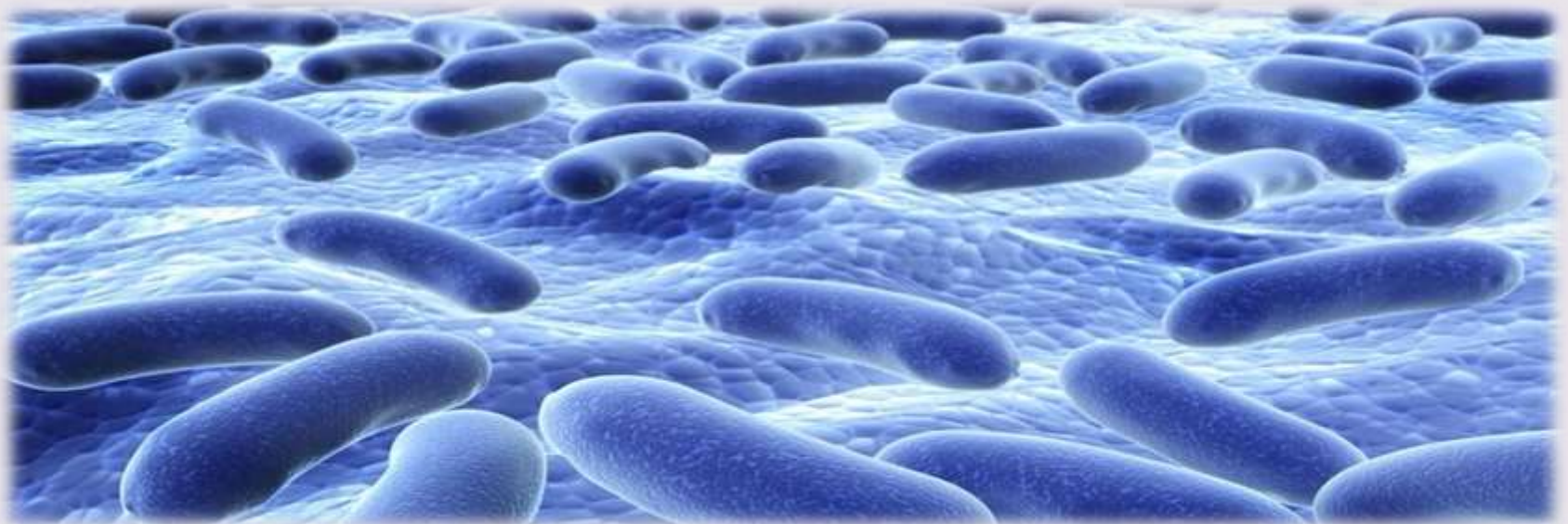
- Rods usually long and slender, some form chains.
- Aerotolerant/microaerophilic, ferment sugars chiefly to lactic acids if homofermentative.
- Hetero fermentative species produce lactic acids and small amount of acetic acid, CO₂ and trace amounts of acetaldehyde and alcohol.
- Homofermentative: *L. bulgaricus*, *L. casei*, *L. helveticus*, *L. lactis*, *L. acidophilus* and grow optimally at 37 °C.
- Heterofermentative: *L. fermentum*, *L. brevis*
- Ferment sugar to lactic acid and flavouring compounds thus, used in production of fermented dairy and meat products.
- Implicated in spoilage of wine and beer.





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- **Occurs on plant surfaces silage, manure and dairy products.**
- **Unable to synthesize certain vitamins therefore, media needs to be supplemented with vitamins.**
- **Involved in the spoilage of refrigerated meats.**
- **Thermotolerant properties leads to some of the thermophilic strains in manufacture of Swiss cheese.**
- **Probiotic attributes and application in functional probiotic foods and in pharmaceutical preparations.**





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Leuconostoc:

- **Gram-positive, ovoid cocci often forming chains, resistant to vancomycin, catalase-negative (distinguishes from staphylococci).**
- **Heterofermentative and produce dextran from sucrose.**
- **Slime -forming.**
- **Causes 'stink' when creating a sourdough starter.**
- **Some species causes human infection.**
- ***Leuconostoc* spp. with *Pediococcus* and *Lactobacillus* spp. causes fermentation of cabbage to sauerkraut (sugars in fresh cabbage are transformed to lactic acid giving sour flavour and good keeping qualities).**



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Listeria monocytogenes:

- **Serious illness in human beings.**
- **Gram-positive, non-spore forming, nonacid-fast rods, catalase positive and produces lactic acid from glucose and other fermentable sugars.**
- **Grows well in brain heart infusion (BHI), trypticase soy and tryptose broths.**
- **Optimal growth temperature: 37°C**
- **Temperature range: 1-45°C and pH range 4.1-9.6.**
- **Isolated from decaying vegetation, soil, animal feces, sewage, silage and water. Also found in raw milk, pork, raw poultry, ground beef and vegetables.**
- **HTST pasteurization destroy the organism in milk.**



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- **Virulence factor is listeriolysin O.**
- **Virulent strains produce β -hemolysis on blood agar and acid from rhamnose.**
- **Grows well in moderate salt concentrations (6.5%).**
- **This is foodborne pathogen while other pathogens excrete toxins or multiply in blood stream. It enters host cells and grows inside cell. In humans, it crosses intestinal barrier after entering by oral route.**
- **Ready to Eat (RTE) foods preserved by refrigeration pose special challenge with *L. monocytogenes* infection.**

A microscopic image showing numerous small, spherical, purple-stained bacteria (Micrococcus) arranged in clusters and chains. The background is a light, pinkish-white color.

Micrococcus:

- Occurs in water, dust, and soil.
- Gram positive, spherical cells 0.5-3 micrometers in diameter.
- *M. luteus*, *M. roseus* (red) produce yellow or pink colonies when grown on mannitol salt agar.
- Saprophytic or commensal organism.



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Proteus:

- **Oxidase negative, catalase and nitrate reductase positive.**
- ***P. vulgaris*, *P. mirabilis* and *P. penneri* are opportunistic human pathogens responsible for human urinary tract infections.**
- ***P. mirabilis* causes wound and urinary tract infections.**
- ***P. mirabilis* sensitive to ampicillin and cephalosporins.**
- ***P. vulgaris* occurs naturally in human intestine, manure, soil and polluted waters.**
- ***P. mirabilis* found as free-living organisms in soil and water.**



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Propionibacterium:

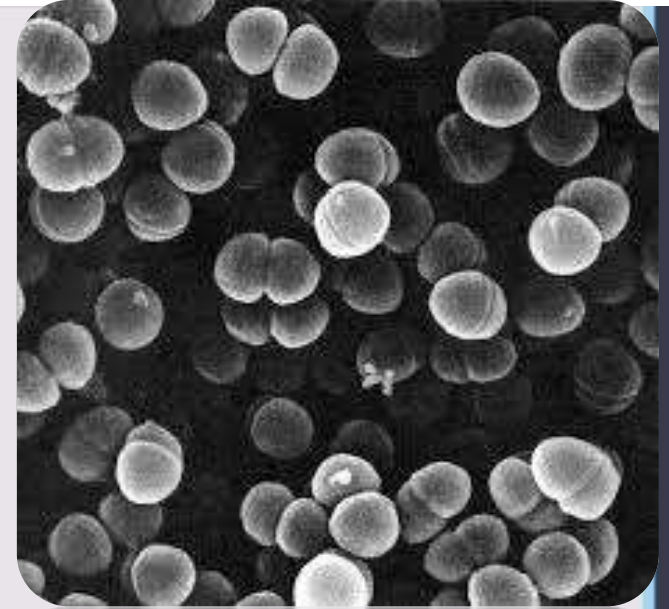
- Dairy starters (Swiss-type cheese) and their ability to produce propionic acid during growth.
- Split into **cutaneous** and **dairy** groups.
- Isolated primarily from dairy foods and silage.
- Species in dairy products: *P. jensenii*, *P. acidipropionici*, *P. theonii*, *P. freudenreichii*.
- Propionibacteria produce flavour compounds in cheese by proteolysis and propionic acid production.
- Dairy species offer an interesting opportunity as novel probiotic organisms.





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Pediococcus:

- **Economic importance in brewing and food industries.**
- **Used as starter cultures in fermentation of vegetables, meats, sausage products, fermented milks and associated with development of flavor in Cheddar.**
- **Form capsular material that causes beer to become ropy and viscous.**
- **Catalase negative, homolactic fermentation and produce optically inactive lactic acid i.e. mixture of the L(+) and D(-) type.**
- **Appear in tetrads.**



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Pseudomonas fluorescens:

- **Gram-negative, rod-shaped, motile.**
- **Psychrotrophic in nature and grows at refrigeration temperature (7°C).**
- **Obligate aerobe, but certain strains are capable of using nitrate instead of oxygen as final electron acceptor during cellular respiration.**
- **Optimal temperature: 25-30 °C.**
- **Oxidase positive, non-saccharolytic.**
- **Heat-stable lipases and proteases are produced that cause milk to spoil causing bitterness, casein breakdown and ropiness due to production of slime and coagulation of proteins.**

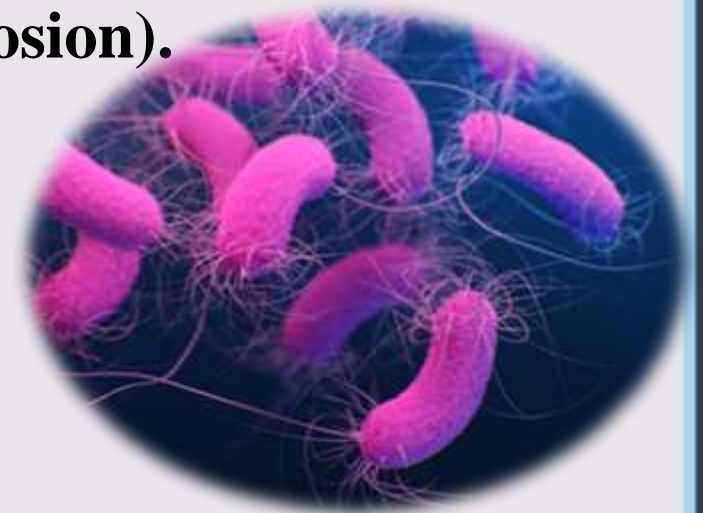


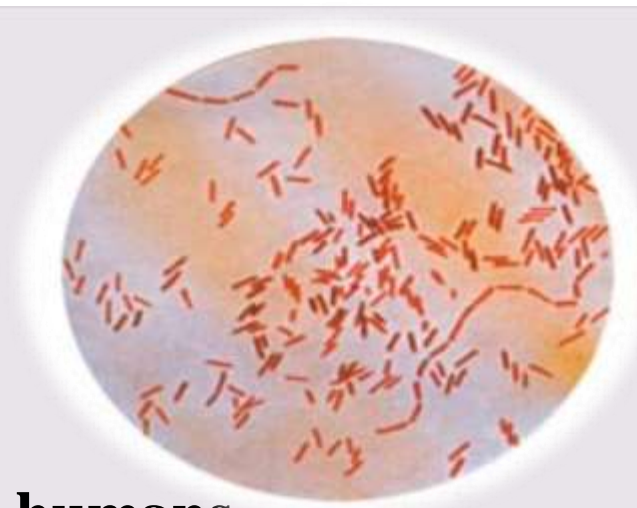
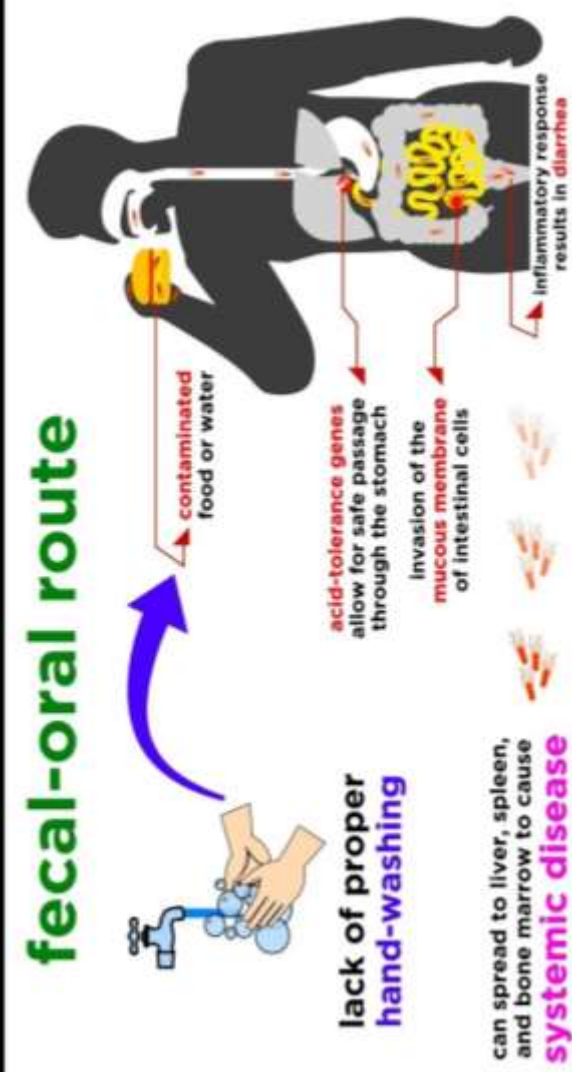
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Pseudomonas aeruginosa:

- **Gram-negative, aerobic, rod-shaped with unipolar motility.**
- **Opportunistic human pathogen.**
- **Secretes pigments (pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent) and pyorubin (red-brown)).**
- **Preliminarily identified by its fluorescence and grape-like or tortilla-like odor.**
- **Capable of growth in diesel and jet fuel (known as hydrocarbon-using microorganism (or "HUM bug")), causing microbial corrosion).**





Salmonella:

- Foodborne illnesses in humans.
- Enteric fever associated with typhoid and paratyphoid strains.
- Optimum growth temperature: 37-45°C.
- Ferments carbohydrates with its production of acid and gas.
- Oxidase negative, catalase positive, grow on citrate as sole carbon source and produce H₂S.
- Some exhibit psychrotrophic properties, ability to grow at pH values: 4.5-9.5.
- Milk, meat and poultry are principle vehicles of human foodborne salmonellosis.



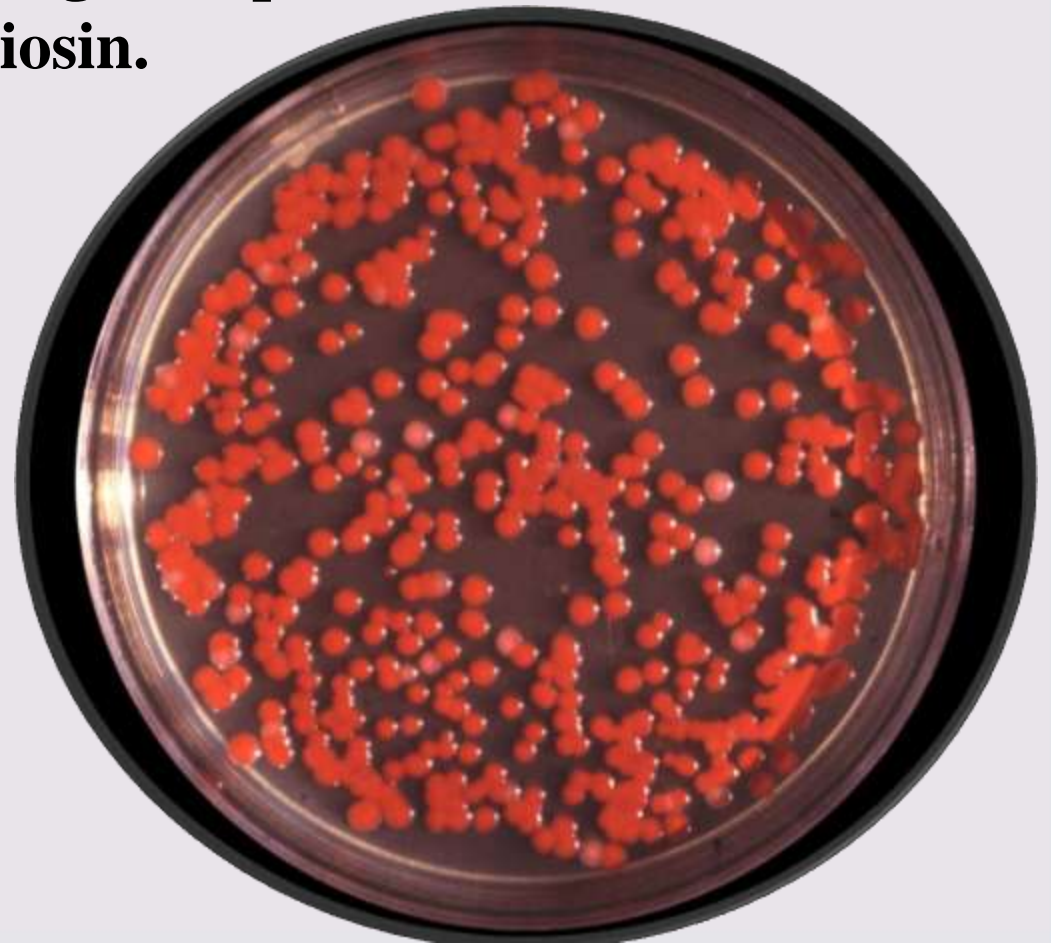


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Serratia:

- **Gram-negative, facultatively anaerobic, rod-shaped.**
- ***S. marcescens* is the only pathogen that causes nosocomial infections.**
- **Members of this genus produce characteristic red pigment, prodigiosin.**





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Streptococcus thermophilus:

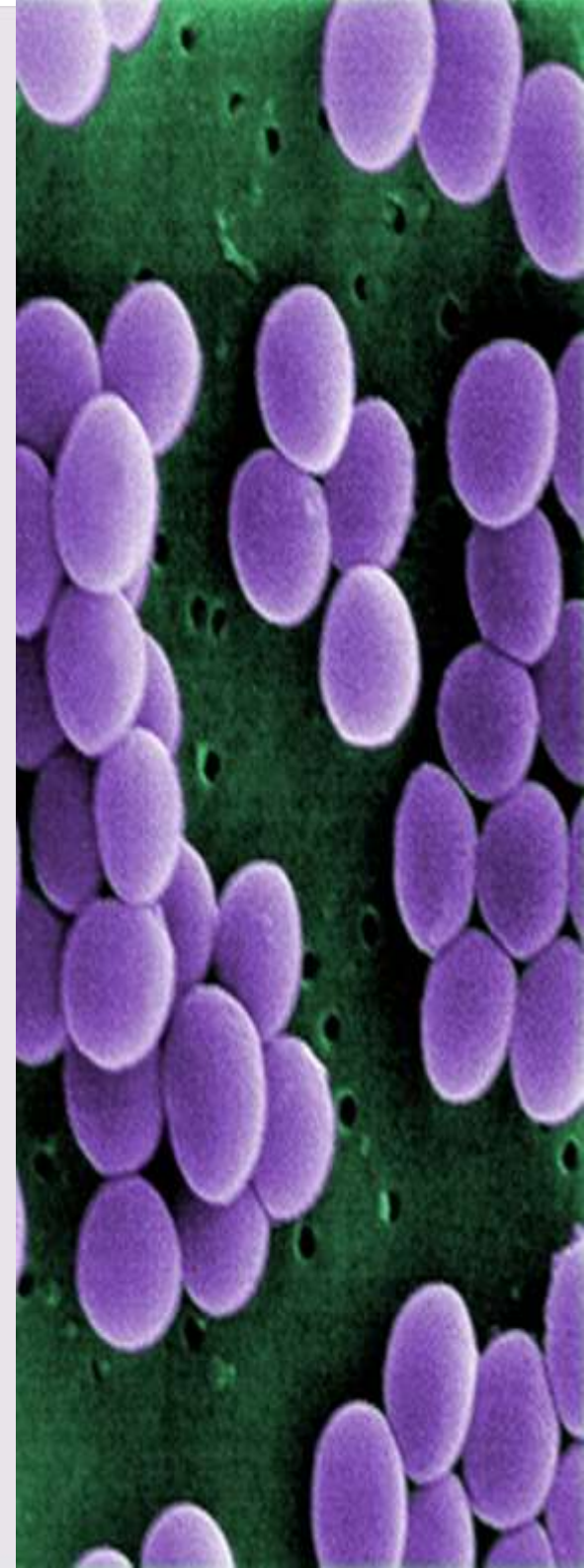
- **Gram-positive, facultative anaerobe, catalase negative, non-motile, non-spore forming, homofermentative and occurs in pairs to long chains.**
- **Spherical to ovoid cells, diameter in range of 0.7-0.9 μm .**
- **Optimum growth temperature: 39-45°C.**
- **Ferment sugars with L (+) lactic acid as major end product and produce around 0.6-0.8% lactic acid.**
- **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.**
- ***S. thermophilus* and *Lactobacillus* spp. used to manufacture fermented dairy foods (yoghurt and mozzarella cheese).**



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Staphylococcus aureus:

- **Gram-positive, catalase-positive coccus.**
- **Cause of foodborne gastroenteritis (staphylococcal food poisoning- ingestion of food that contains one or more enterotoxin).**
- **Enterotoxin production is associated with coagulase and thermo nuclease producing *S. aureus* strains.**
- **Reservoir: Nasal cavity of human beings, where they find their way to skin and wounds. Mastitis in animals occurs and from infected udder, organism finds its way to milk.**
- **Grows well in NaCl concentrations of 7-10% and water activity as low as 0.86.**





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Shigella:

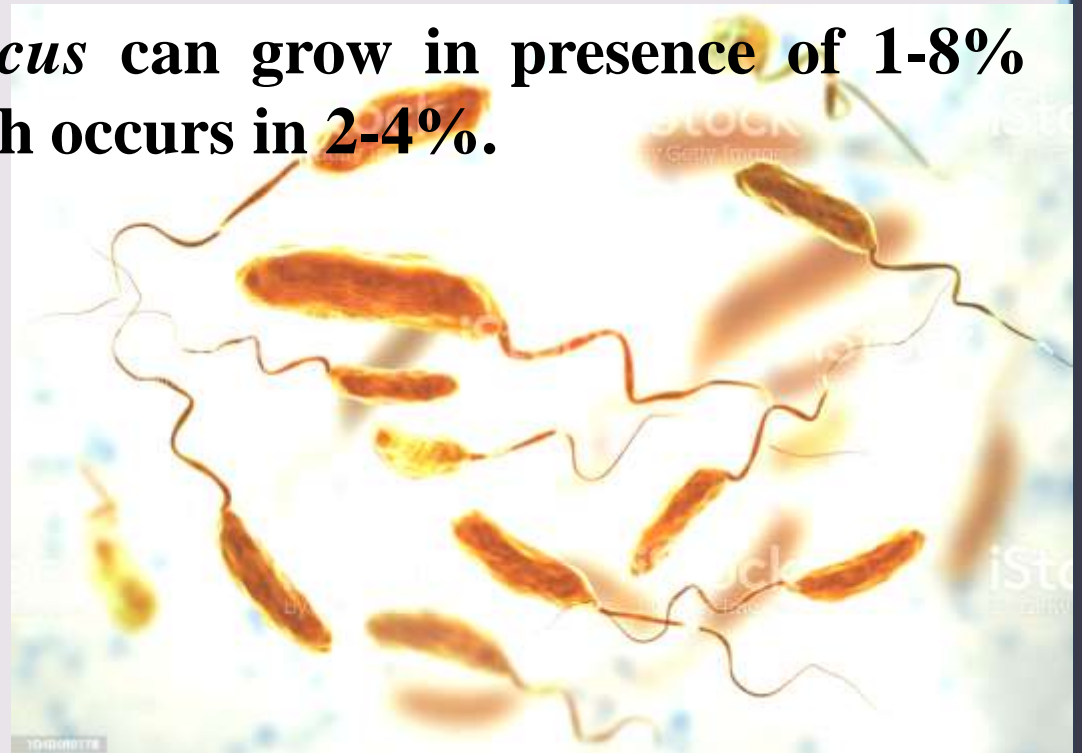
- **Growth temperature varies from 10-48 °C.**
- **Sensitive to ionizing radiations.**
- **Non-motile, oxidase negative produce acid only from sugars; do not grow on citrate as sole carbon source, do not grow on KCN agar.**
- **Disease: Shigellosis**
- **Infective dose may be as low as 100 cells. Contamination of foods does not occur at processing plant but through an infected food handler.**
- **Humans are natural reservoir of Shigella. Spread through fecal-oral route.**



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Vibrio:

- ***V. cholerae* and *V. parahaemolyticus*: Two important species.**
- ***V. cholerae* are Gram-negative straight/curved rods.**
- **Important distinctions within species are made on basis of production of cholera enterotoxin (CT) and serogroup.**
- **Alkaline peptone water is enrichment broth for isolation of vibrio.**
- ***V. parahaemolyticus* can grow in presence of 1-8% NaCl, best growth occurs in 2-4%.**



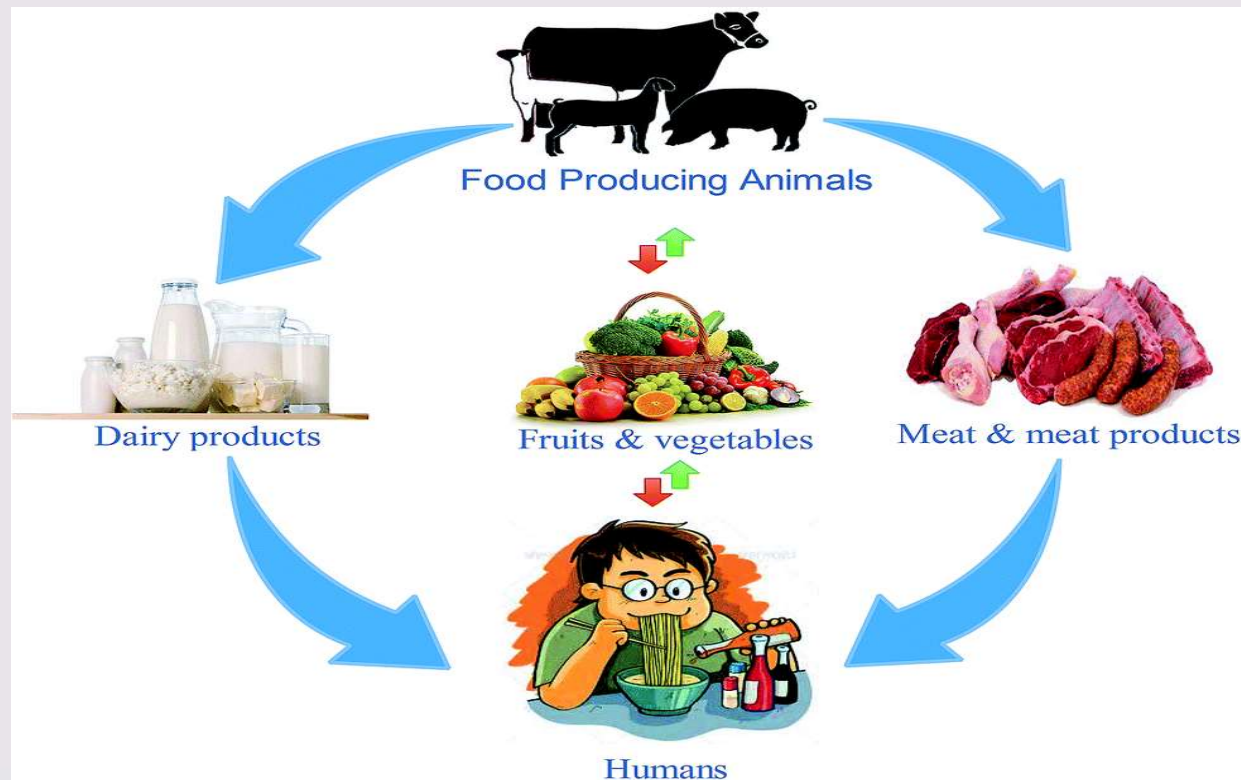


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Yersinia:

- *Y. enterocolitica* and *Y. pestis*-Two important human pathogens.
- *Y. enterocolitica* causes food borne gastroenteritis, *Y. pestis* is an agent of human plague.
- *Y. enterocolitica* can grow at temperatures $<4^{\circ}\text{C}$.
- Grows better in processed foods (pasteurized milk, vacuum packed meat, boiled eggs, boiled fish, cottage cheese).
- Susceptible to pasteurization, ionizing and UV irradiation.
- Transmittance of organism from animals (pork, lamb, poultry and dairy products) to humans.

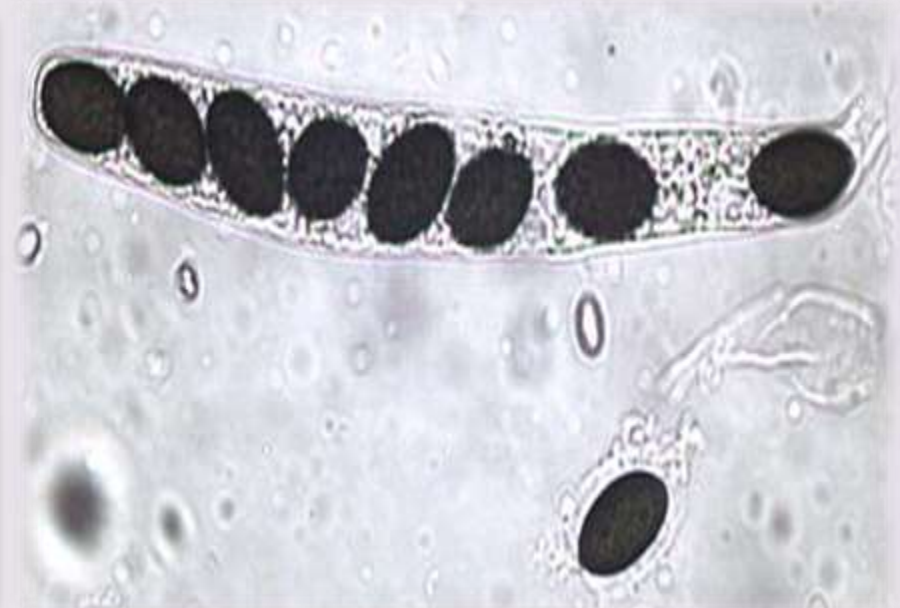
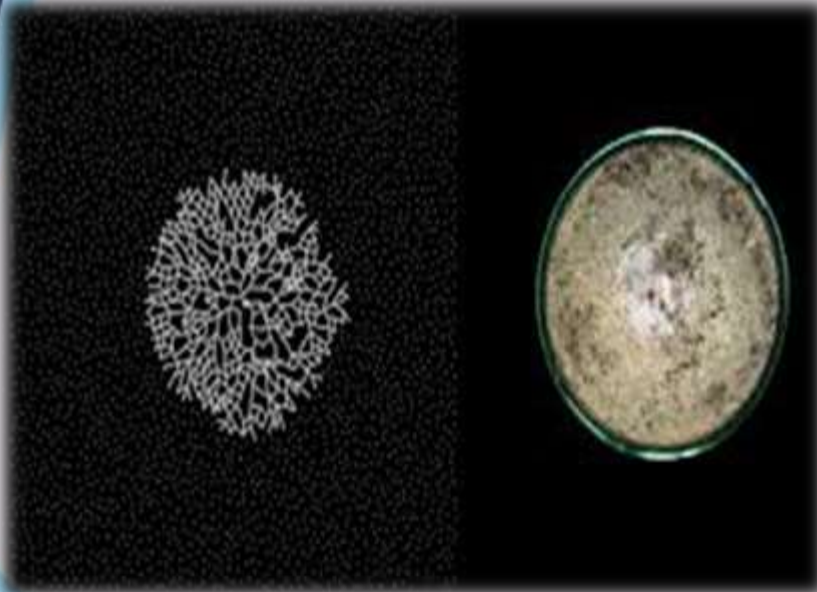




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Food Borne Molds

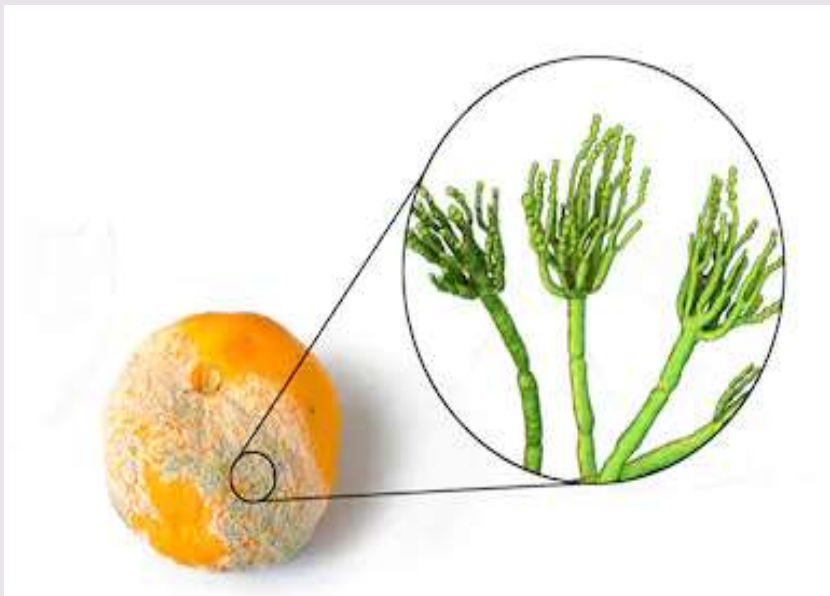
- **Filamentous fungi that grow in form of tangled mass that spreads rapidly and may cover several inches of area in short period (referred as mycelial growth).**
- **Mycelium is composed of branches of filaments referred to as hyphae.**
- **Molds of importance in foods multiply by ascospores/conidia.**
- **Ascospores are notable for their extreme degrees of heat resistance.**





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- **Important in food because they can grow even in conditions in which many bacteria cannot grow, such as low pH, low water activity (a_w), and high osmotic pressure.**
- **Many types of molds are found in foods.**
- **They are important spoilage microorganisms. Many strains also produce mycotoxins (*Aspergillus flavus*) and have been implicated in foodborne intoxication.**
- **Many are used in food bioprocessing as well as used to produce food additives and enzymes (*A. oryzae*).**





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Alternaria:

- Form septate mycelia with conidiophores and large brown conidia are produced.
- Cause brown to black rots of apples, and figs.
- Some species produce mycotoxins.

Aspergillus:

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





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Geotrichum:

- Referred as **dairy mold**.

Mucor and Rhizopus

- *Mucor* produce non-septate hyphae and are prominent food spoilers.
- *Rhizopus* spp. also produce non septate hyphae but give rise to stolons and rhizoids.
- *R. stolonifer* referred to as “**bread mold**”.

Molds related to spoilage of foods:

Neurospora, Thamnidium,

Trichothecium,

Penicillium and Cladosporium

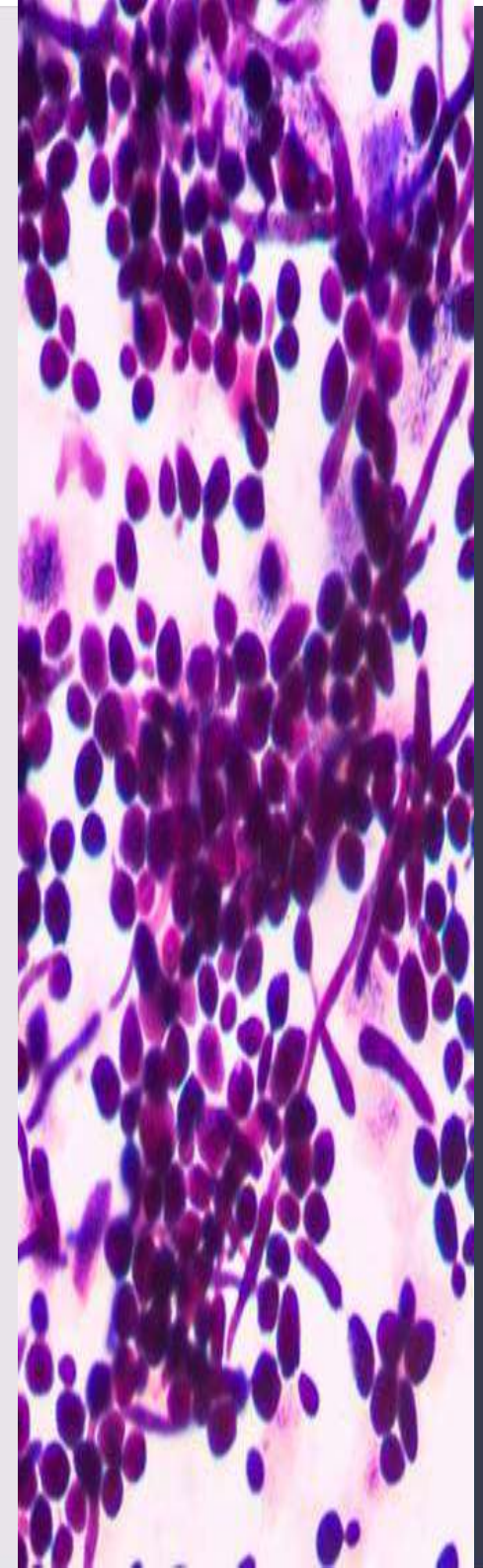


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Food Borne Yeasts

- **Unicellular fungi**
- **Yeasts can be differentiated from bacteria by their larger cell size and their oval, elongate, elliptical, or spherical shape.**
- **Yeast cells range from 5-8 um in diameter.**
- **Divide by budding/fission.**
- **Yeasts can grow in presence of lactic, citric and tartaric acid, wide range of acid pH and upto 18% ethanol.**
- **Grow in presence of 55-60% sucrose.**





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- **Yeasts are important in food because of their ability to cause spoilage. eg., *Torulopsis***
- **Many are also used in food bioprocessing and some for food additives. eg., *Saccharomyces cerevisiae***

Using Yeast in Cooking

- An important ingredient in baking and beer brewing
- Two types of yeast: brewer's yeast (a wet yeast for beer making), and baker's yeast (a leavening agent)
- Two types of baker's yeast: fresh yeast and active dry yeast





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Candida:

- Form shining white colonies and cells contain no carotenoid pigments.
- *Candida tropicalis* is the most prevalent in foods.
- Some members are involved in the fermentation of cocoa beans, component of kefir grains and many other products (beers and fruit juices).

Debaromyces:

- Most prevalent yeast genera in the dairy products.
- Can grow in 24% NaCl and aw as low as 0.65.

Kluyveromyces:

- Produces β -galactosidase 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.



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Rhodotorula:

- Contains many psychrotrophic species that are found on fresh poultry, shrimp, fish and beef.
- Some grow on surface of butter.

Saccharomyces:

- Ascosporogenous yeasts that multiply by lateral budding and produce spherical spores in asci.
- Diploid and do not ferment lactose.
- All bakers' brewers', wine and champagne yeasts are *S. cerevisiae*.
- Found in Kefir grains.

Torulasporea:

- Multiplies by lateral budding.
- Strong fermenters of sugars.
- *Torula delbrueckii* : Prevalent sp.





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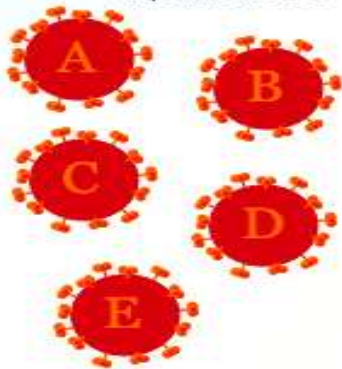
Viruses in Food

- Filterable, ultra microscopic particles and can be cultivated only on live tissues.
- Consist of core of nucleic acid (DNA or RNA) and protein coat.
- Some are responsible for food borne diseases in humans (non-bacterial gastroenteritis due to enterovirus).
- 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.

What is hepatitis?

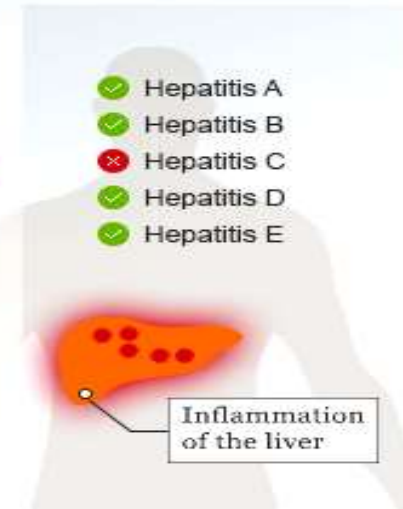
The A, B, C, D and E of hepatitis

There are five main hepatitis viruses



What vaccines are available for which types of hepatitis?

- ✓ Hepatitis A
- ✓ Hepatitis B
- ✗ Hepatitis C
- ✓ Hepatitis D
- ✓ Hepatitis E



Estimated cases world-wide (per year)

Hepatitis **B and C**:
400 million cases

Gradual death:
1.4 million people die worldwide from hepatitis every year

Treatment:
90% of hepatitis C patients can be healed within three to six months

How does the virus spread?



Hepatitis **A and E**:
Lack of food hygiene, contaminated water and sub-standard sanitary facilities



Hepatitis **B, C and D**:
Blood, sperm and other bodily fluids