

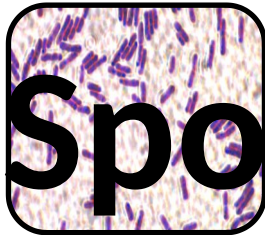


Microorganisms associated with raw milk and their significance

Milk contains bacteria that can be classified into three categories



Beneficial bacteria



Spoilage bacteria



Pathogenic bacteria

Beneficial bacteria: Help in making food products (cheese, yogurt, and buttermilk).

Spoilage bacteria: Produces bad smell/taste and poor look of the product. Most bacteria in fresh milk from healthy animals are usually harmless but rapid changes in the health of animal or of the handler, or contaminants from polluted water, dirt, manure, air, cuts and wounds can make raw milk potentially dangerous. Milk is sterile, when it is secreted in udder but gets contaminated before it leaves udder. Contaminating bacteria are usually harmless but can lead to different spoilage problems. Microorganisms causing spoilage may be psychrotrophs, mesophiles or thermophiles which are of great significance in milk:

- Information on microbial counts of milk can be a diagnostic criteria for the judgement of its overall quality
- If multiply, bacteria can cause spoilage of milk much faster
- Certain types of microbes produce chemical changes that are desirable in the production of different dairy products, for example cheese, yogurt etc.

Pathogenic bacteria:

- 1. Consumption of raw milk was prevalent even prior to the industrial revolution and discovery of pasteurization.**
- 2. Before that period, it was unknown about the infectious microbes and their association with water, milk and milk based products and the cause of various diseases.**
- 3. Modern scientific techniques made milk free from harmful microbes but many problems may still persist like eradication of their toxic metabolites.**
- 4. Pasteurization may be an effective method in control of problems like enforcement of sanitation and testing procedures at farm level and implantation of various practices at commercial level.**
- 5. Nowadays, microorganisms can be identified at genomic level to establish preventive measures effectively.**

Microflora of Raw Milk

1. Milk provides a wealth of nutritional benefits, but raw milk can harbour dangerous microorganisms posing serious health risks to the consumers.
2. Raw, unpasteurized milk can carry dangerous bacteria (*Salmonella*, *E. coli*, and *Listeria*) responsible for causing numerous food-borne illnesses as it contains all essential nutrients, pH and water activity for the growth of microbes. Hence, there is a high risk for these products to get contaminated with spoilage causing microbes.
3. Milk when secreted into the udder of an animal is considered to be as sterile; and it gets contaminated from the microflora residing in the udder tissues. These contaminants are non-infectious except in case of mastitis.

4. Milk becomes more contaminated, when it comes in contact with an external environment during milking, transportation and storage. Utensils, machines, handlers, water, air and soil, also contributes greatly to the micro flora of milk. This way bacteria, yeast and molds get entry into milk constituting the normal microflora.
5. The presence of these non-pathogenic microbes in milk is not very serious, but multiplication may cause souring, putrefaction and development of different taints, undesirable flavours and colours of milk and milk products.
6. Suitable preventative measures are required to check spoilage of highly nutritious perishable, food commodities.

Name of micro-organism	Activity associated
Pseudomonas	Spoilage
Brucella	Pathogenic
Enterobacteriaceae	Pathogenic and spoilage both
Staphylococci	
<i>Staphylococcus aureus</i>	Pathogenic
Streptococcus	
<i>S. agalactiae</i>	Pathogenic
<i>S. thermophilus</i> ; <i>L. lactis</i> ssp. <i>lactis</i>	Acid fermentation
<i>L. lactis</i> ssp. <i>lactis</i> biovar <i>diacetylactis</i>	Flavour production
<i>L. lactis</i> ssp. <i>cremoris</i> ; <i>Leuconostoc lactis</i>	Acid production
<i>Bacillus cereus</i>	Spoilage
Lactobacillus	
<i>L. lactis</i> ; <i>L. bulgaricus</i> ; <i>L. acidophilus</i>	Acid production
<i>Propionibacterium</i>	Acid production
<i>Mycobacterium tuberculosis</i>	Pathogenic

Microbial Contaminants from the Surroundings

Animal and its udder

•The animal and its teats contribute greatly to the microflora of milk that includes those resided on skin and present in the niche of animal, where it is reared or milked.

Utensils and sanitary practices

•Milk residues left behind on equipments may support the growth of different microbes that on subsequent milking may lead to spoilage milk.

Psychrotrophic Bacteria

- 1. Psychrotrophs are the microorganisms that have the ability to grow near freezing temperature in the range of 0-7°C. The typical representatives of this class are both Gram negative and positive microorganisms.**
- 2. Gram negative includes *Pseudomonas*, *Achromobacter*, *Aeromonas*, *Serratia*, *Alcaligenes*, *Chromobacterium* and *Flavobacterium* spp.**
- 3. Gram positive includes *Bacillus*, *Clostridium*, *Corynebacterium*, *Streptococcus*, *Lactobacillus* and *Microbacterium* spp.**
- 4. The quality of raw milk has been considerably maintained by refrigeration on farms, and in processing plants. Current practices in India for collection and storage of the raw milk favor growth of psychrotrophs that are able to grow below 7°C.**

4. Besides, their rapid growth ability in refrigerated milk, psychrotrophs produces heat stable extracellular proteases, lipases, phospholipases etc.
5. Proteases and lipases contribute to the spoilage of dairy products. The extracellular enzymes can resist pasteurization (72°C for 15 s) and even ultra-high temperature (138°C for 2 s) processing.
6. The lipases hydrolyze triglycerides causing flavour defects associated with fat breakdown in cream, butter and cheese.
7. Proteases are associated with bitterness in milk, gelation of sterilized milk, and reduced yields of soft cheese. Most proteases can degrade caseins and are highly heat stable.
8. Psychrotrophs play a greater role in spoilage of refrigerated milk. The numbers of psychrotrophs that develop after milk collection depend on the storage temperature and time. Under sanitary conditions, <10% of the total microflora are psychrotrophs in contrast to >75% under unsanitary conditions.
9. *Pseudomonas* is the most prevalent psychrotroph in raw milk.

Significance of psychrotrophs in milk:

- ❑ Apart from psychrotrophic spoilage bacteria, psychrotrophic pathogens (*Listeria monocytogenes*, *Yersinia enterocolitica*) has made situation more severe. Listeriosis has become a major food-borne disease, as *L. monocytogenes* can cause meningitis, septicemia, and abortion. So, control of psychrotrophs is important to stop spoilage.
- ❑ Cold storage of milk favours the growth of psychrotrophs. Following pasteurization/other heat treatment of raw milk to destroy microbes, spore germination or recontamination can still cause quality deterioration; furthermore, heat-resistant extracellular proteinases and lipases produced by psychrotrophs before processing are a major spoilage factors during storage of milk.
- ❑ The detection and control of psychrotrophs and their heat resistant enzymes are major concerns of quality control in dairy industry.
- ❑ Thermoresistant psychrotrophs survive pasteurization.
- ❑ *Arthrobacter*, *Microbacterium*, *Flavobacterium*, *Acinetobacter* and *Alcaligenes*. *Bacillus* spp. (sporeformer) dominates and secretes extracellular heat resistant proteinases, lipases and phospholipases.

Control of psychrotrophs and their enzymes in milk can be achieved by:

- ✓ Hygienic practices in different aspects of milk handling
- ✓ Minimizing storage time of milk
- ✓ Following effective HACCP system
- ✓ Strict maintenance of refrigeration at 4°C
- ✓ Heat treatment with aseptic filling or packaging
- ✓ Use of natural preservation by bacteriocins (proteinaceous or peptidic toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain)
- ✓ Treatment of milk with carbon dioxide or nitrogen
- ✓ Thermization of milk before chilling and storage
- ✓ The suggested standards for psychrotrophs in raw milk:
 - 10^4 cfu/ml - Satisfactory
 - >math>10^4</math> cfu/ml - Unsatisfactory

Mesophilic Bacteria

Mesophiles are microorganism that grows best in moderate temperature, optimally between 25-40°C. These microorganisms have important applications in food preparation, especially in cheese and yogurt making.

Group	Genera
Micrococci	<i>M. varians</i> , <i>M. luteus</i> , <i>M. roseus</i>
Streptococci	<i>S. agalactiae</i> , <i>S. dysgalactiae</i> , <i>S. uberis</i> , <i>S. faecalis</i> , <i>S. zymogenes</i> , etc.
Gram positive rods	<i>L. casei</i> , <i>L. plantarum</i> , <i>Corynebacterium</i> spp., <i>Microbacterium</i> spp., <i>Arthrobacter</i> spp., <i>Bacillus</i> spp.
Gram negative rods	<i>Pseudomonas</i> spp., <i>Alcaligenes</i> spp., <i>Coliforms</i> , <i>Flavobacterium</i> etc.

Microbiological standards for raw milk (BIS Standards)

Direct Microscopic Clump Counts/ml	Grading of milk
<5,00,000	Good
5,00,001 - 4,000,000	Fair
4,000,001 - 20,000,000	Poor

Thermotolerant Bacteria

- ❑ Microorganisms that can survive pasteurization but cannot grow at high temperatures are called thermotolerants. Hence, these are carried into the pasteurized product, where these may cause defect and promote health hazard risks when standard growth conditions are prevalent.
- ❑ Widely distributed in animal feeds, bedding, soil and feces. These contaminate animal's teats and milking equipments. Therefore, it is difficult to completely eradicate these microbes from the milk processing system. eg., *Micrococcus*, *Microbacterium*, *Bacillus* and some *Coryneforms*.
- ❑ **Control measures:**
 1. Implementation of hygienic milking practices
 2. Use of clean and hygienic milk equipments
 3. Proper fecal disposal system
 4. Maintenance of clean environment

Thermotolerant counts (BIS standards)

Counts cfu/ ml	Remarks
Below 10,000	Good
10,000 to 30,000	Fair
Above 30,000	Poor

Genus	Important Features
<i>Microbacterium</i>	<i>M. lacticum</i> shows 100% survival during pasteurization. <i>Microbacterium</i> is derived from milking equipment.
<i>Bacillus</i> (spores)	<i>Bacillus</i> spore count is higher in winter than in summer because spores are mainly derived from the teat surfaces spoiled with bedding materials. Eg., <i>B. licheniformis</i>
<i>Clostridium</i> (spores)	Spore count of raw milk is higher in winter because these are derived from silage and bedding materials.eg., <i>C. tyrobutyricum</i>
<i>Micrococcus</i> (100%)	Exclusively derived from milking equipment and utensils.eg., <i>M. freudenreichii</i>
<i>Alcaligenes</i> (1-10%)	1-10% survive pasteurization. Eg., <i>A. tolerans</i>

Coliforms

- ❑ Gram-negative, rod shaped, non-spore former that ferment lactose to produce acid and gas at 35-37°C/ 24-48 h. Their presence in food indicates the sanitary quality of production. Therefore, sometimes these are also called as an 'index of direct fecal contamination' in product.
- ❑ These microbes produce different toxins in foods that create major health hazard issues for consumers.
- ❑ A toxin producing strain has been identified as *E. coli* O157: H7 that presents serious health issues, especially in children.
- ❑ **Sources:** Faeces of healthy livestock including dairy cattle, utensils, contaminated water, and unhygienic milk production practices, animal suffering with coliform mastitis, handlers and contaminated utensils are major sources of coliforms.
- ❑ The example includes: *E. coli*, *Salmonella*, *Enterobacter aerogenes*.
- ❑ Coliforms count for raw milk should not be more than 10/ml.

Significance of Coliforms in Raw Milk:

- ❑ Presence of coliforms in milk indicates a possible fecal contamination, especially when these are handled under unsanitary conditions.
- ❑ The presence of these microorganisms is considered undesirable because they produce acid, gas and taints in milk products.
- ❑ Coliforms are destroyed during pasteurization and hence, their presence in pasteurized milk indicates a **post pasteurization contamination**.
- ❑ Absence of coliforms in 1:100 dilution in raw milk and 1:10 dilution of pasteurized milk is accepted as a criteria of satisfactory quality.
- ❑ The presence of faecal *E. coli* proves contamination from excreta has taken place.

Thermophilic Bacteria

- ❑ Obligate thermophiles have a temperature range as high as 85°C, while some facultative thermophiles grow as low as 20°C and as high as 60°C.
- ❑ Thermophiles are a heterogeneous group of microbes which not only survive pasteurization but also grow at high temperatures.
- ❑ Among the thermophiles, the common members belong to aerobic or facultatively anaerobic sporeforming rods eg., *Bacillus* sp. (*B. stearothermophilus*, *B. circulans*, *B. thermoacidurans*) and *Lactobacillus thermophilus*.
- ❑ The standard method of enumerating this group of organisms is to incubate agar plates at 55 C.
- ❑ **Sources:** Thermophiles may gain access into raw milk mainly from soil, bedding, feeds, and rarely from water supplies. The number increases due to their multiplication if raw milk is stored under warmer ambient conditions. Thermophiles may also multiply in pasteurizer, when milk is not maintained at proper temperature.

- In pasteurizing plant, the plant may be receiving an unusual contamination; the walls of the pasteurizer may have a thin layer of cooked milk where thermophiles may grow; the construction of the pasteurizer may be defective so that some of the milk fails to reach the required temperature; there may be an excessive amount of foam that is not heated properly; or the vats may not be cleaned between the successive runs.

There are no reasons that the thermophiles are more harmful in milk than microbes growing at lower temperatures. In fact, most of these grow slowly or not at all.



Pathogenic Microbes in Raw Milk

Microbial pathogens may get entry into raw milk either from the surroundings (i.e. contaminated utensils, feces, water, handlers) or from the infected udders/ tissues of the producing animals.

Different pathogenic microbes and disease associated

Pathogenic microbial agent	Disease associated with microbe
<i>Campylobacter jejuni</i>	Campylobacteriosis
<i>Corynebacterium diphtheria</i>	Diphtheria
<i>Coxiella burnetii</i>	'Q' fever
<i>Escherichia coli</i> O157: H7	EHEC - Enterohaemorrhagic <i>E. coli</i>
Hepatitis A	Jaundice
<i>Listeria monocytogenes</i>	Listeriosis
<i>Mycobacterium tuberculosis</i>	Tuberculosis
<i>Salmonella typhi</i>	Typhoid fever
<i>Salmonella</i> spp.	Salmonellosis
<i>Shigella dysenteriae</i>	Shigellosis
<i>Yersinia enterocolitica</i>	Yersinosis

Psychrophile	Grows well at 0°C and has an optimum growth temperature of 15°C or lower	<i>Bacillus psychrophilus</i> , <i>Chlamydomonas nivalis</i>
Psychrotroph	Can grow at 0–7°C; has an optimum between 20 and 30°C and a maximum around 35°C	<i>Listeria monocytogenes</i> , <i>Pseudomonas fluorescens</i>
Mesophile	Has growth optimum around 20–45°C	<i>Escherichia coli</i> , <i>Neisseria gonorrhoeae</i> , <i>Trichomonas vaginalis</i>
Thermophile	Can grow at 55°C or higher; optimum often between 55 and 65°C	<i>Geobacillus stearothermophilus</i> , <i>Thermus aquaticus</i> , <i>Cyanidium caldarium</i> , <i>Chaetomium thermophile</i>