Cornell University
Department of Food Science
Stocking Hall, Ithaca, NY 14853
Phone: 607-255-2893
E-mail: scm4@cornell.edu

Dairy Foods Science Notes

Version 06-01-10

BASIC DAIRY BACTERIOLOGY

Microorganisms play important roles in the quality and safety of dairy products. This document provides basic information on bacteria, perhaps the most important group of microorganisms in dairy. Other microorganisms such as yeast, molds and starter cultures are discussed briefly; more detailed information can be found in other *Dairy Foods Science Notes* publications.

DEFINITION - BACTERIA

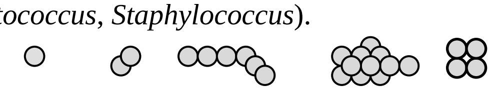
Bacteria are single celled organisms that can only be seen with a microscope ("microorganisms"). All processes needed for life occur within a single cell. Most bacteria are considered *prokaryotes*. Their basic cell structure differs from cells of plants and animals (*eukaryotes*); for example they lack a true nucleus and have a unique cell wall. Bacteria can be found wherever life exists; some are considered useful, such as those responsible for nutrient conversion (e.g., decomposition) and food fermentations (e.g., cheese), while others are harmful, such as those responsible for food spoilage and disease. Individual bacteria are named by *Genus* and *species* (e.g., *Bacillus cereus*, *Pseudomonas fluorescens*), as are all living organisms. They are classified according to their appearance and structure and by specific characteristics of their metabolism and growth, including nutrient requirements, growth temperatures, oxygen requirements, their ability to use specific substrates (e.g., certain sugars), and by specific by-products of their metabolism. Currently, genetic profiling techniques have become standard tools in the identification/classification of bacteria, often beyond species level (e.g., sub-species, sub-types, allelic types). There are literally thousands of species of bacteria, but only select groups are of concern to the dairy industry. The following will describe the general characteristics important for characterizing bacteria that are common in milk and dairy products. Although not specifically covered, comments pertaining to dairy fungi (yeast & molds) are also included.

GENERAL CHARACTERISTICS

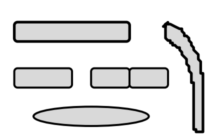
Appearance - Size and Shape:

To see bacteria, a microscope is required, generally one with a magnification of 1000X. Bacteria are measured in microns (1 micron = 1/1000 mm = 1/25,000 inch). When a standard light microscope is used, bacterial cells are normally stained to make them easier to see. Bacteria can be observed in milk by staining a dried milk smear on a microscope slide with a specific "milk-stain" (e.g., Levowitz-Weber Stain). Bacteria grown in a petri dish (e.g., on a semi-solid nutrient "agar" media) or in a nutrient broth can be smeared and dried on a slide and stained with a simple stain (e.g., methylene blue) or complex stain (see *gram-stain*, next page) for observation. Bacteria exist in a variety of shapes, sizes and arrangements, which can be defining characteristics. Typical of what might be seen in milk and dairy products are:

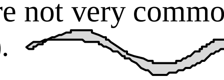
Cocci ------ Spherical cells, 0.4 - 1.5 microns. Occur as single cells, pairs, chains or clusters. (e.g., Genera - *Streptococcus*, *Staphylococcus*).



Bacilli ------ Rod shaped, 0.5 - 30 microns. Occur as single cells, pairs or chains (e.g., Genera - *Lactobacillus, Bacillus, Pseudomonas*).

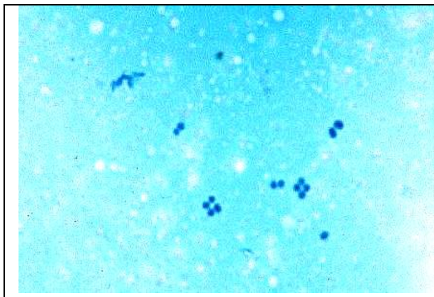


Other ------ Spiral, helical or club shaped rods of varied size. Generally are not very common in milk. (e.g., Genus – *Campylobacter* (Spiral); *Corynebacterium* (club)).

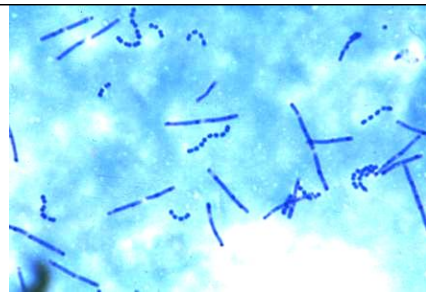


Provided with support from the NY State Dairy Promotion Order, dairy farmers dedicated to the production, manufacture & distribution of quality dairy products.

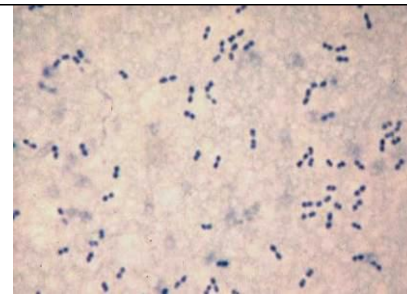
Milk smears under the microscope stained with Levowitz-Weber Stain:



a. Cocci in "tetrad" clusters. 1000× magnification.



b. A smear of yogurt culture - long rods and cocci in chains.



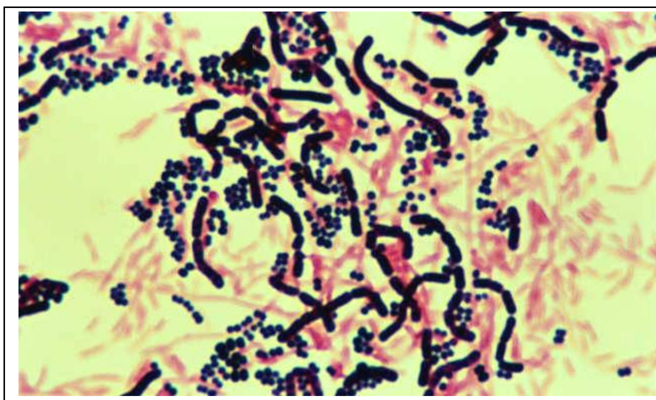
c. Cocci in pairs ("diplococci") and a few short chains.

Gram-Stain Reaction:

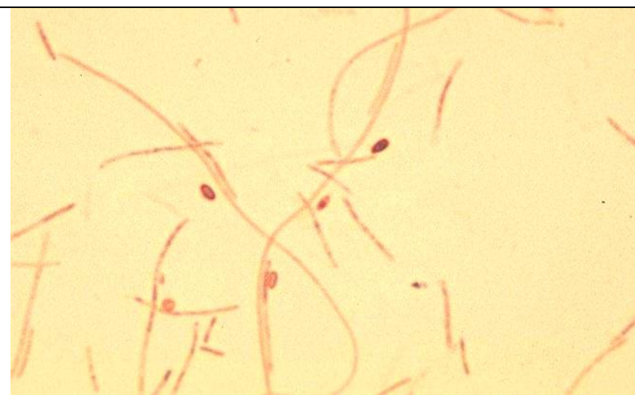
Most bacteria are classified as either "**gram-positive**" or "**gram-negative**." This is typically determined by the **gram-stain procedure**, which is used to view and differentiate bacteria under the microscope; it is one of the first steps used when classifying bacteria. The gram-stain is a four step procedure with Crystal Violet (blue) and Safranin (red) as the primary stains. Depending on the characteristics of the bacteria (i.e., different cell wall structures), they will stain either blue (gram-positive) or red (gram-negative). In some cases an organism classified as "gram-positive" may stain red or appear grainy with blue and red shades. These organisms are often referred to as "gram-variable":

Gram-positive (blue) … e.g., *Bacillus* (rod); *Streptococcus* (cocci); *Staphylococcus* (cocci)
Gram-negative (red) …. e.g., *Pseudomonas* (rods); *E. coli* & other coliform bacteria (rods)
Gram-variable ……….. Stain blue or red depending on conditions; most are truly Gram-pos.

There are a few generalizations based on the gram-stain reaction that can be made of microorganisms common to dairy products. For example, gram-negative bacteria do not survive pasteurization; bacteria that do survive are gram-positive (*but not all gram-positive bacteria survive*); certain gram-negative bacteria, if present, will spoil milk faster under refrigeration compared to gram-positive spoilage organisms; specific antibiotics are more effective against gram-positive than gram-negative bacteria.



Large gram-positive (blue) rods and cocci in chains and clusters and gram-negative rods (red).



Spore-stain of a *Bacillus* species. Vegetative cells stain red while spores stain green or appear clear.

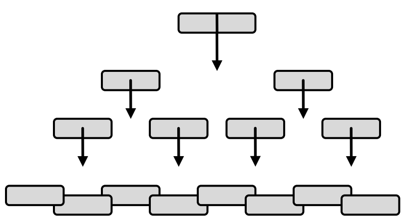
Endospore (Spore) Formation:

Endospores, or bacterial "spores," are protective, dormant structures that allow an organism to survive under adverse conditions. When conditions become unfavorable (e.g., lack of nutrients), vegetative growth ("multiplication") stops and "spores" begin to form within the cell. During sporulation a thick coating develops and encases the cell's genetic material. Spores forming inside a cell may be seen as swollen, possibly clear, areas or may not be apparent at all. Special spore stains facilitate seeing spores under the microscope (see page 2). Bacterial spores released from the cell have increased resistance to heat, drying, nutrient deprivation, chemicals, sanitizers, and other conditions that would normally kill the vegetative, actively growing cell. Spores can remain dormant for extended periods of time (e.g., for years). When conditions become favorable, a spore can "germinate" and return to an actively growing state. Spores may be "activated" into growth by heat or some other "trigger." Spores are produced by only a few select groups of bacteria. Bacteria in the genera *Bacillus*, *Paenibacillus*, and *Clostridium* are common gram-positive, spore-forming rods, which have some importance to dairy. Some strains of spore-formers stain gram-variable. Spores are commonly found in soil, manure and other environmental sources.

BACTERIAL REPRODUCTION (GROWTH)

Bacteria reproduce by a process known as **Binary Fission**; one cell divides into two cells, each of which divides into two more cells and so on. Bacterial **Growth** is defined as an increase in cell numbers or cell mass. **Growth Rate** is the change in cell numbers or mass per unit time. The time it takes for a bacterial population to double or go through one reproductive cycle is called the **Generation Time**. Generation times vary with each organism and are dependent on nutrient availability and environmental conditions (e.g., temperature). Under optimum conditions for growth, generation times may be as short as 10 to 20 minutes for some bacteria. When conditions are less favorable for growth, such as when temperatures are low, generation times will be longer (growth rate is slower), sometimes dramatically (e.g., it may take days for one cell division).

If One Bacterial Cell Reproduced Every Hour,
in 24 Hours There Would Be ~17,000,000 Cells

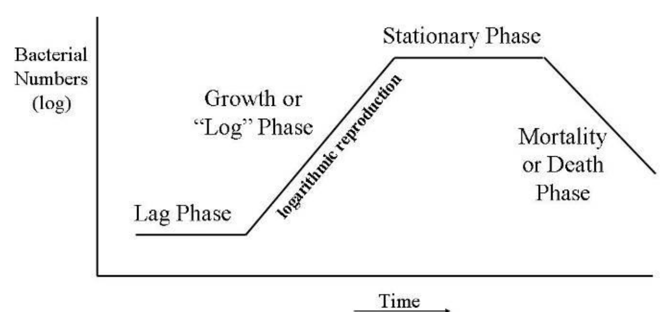


and so on .....

Hour	Count	Hour	Count
0	1	9	512
1	2	10	1,024
2	4	11	2,048
3	8	12	4,096
4	16	:	:
5	32	18	262,144
6	64	:	:
7	128	:	:
8	256	24	~17,000,000

During cell division, bacteria may not totally separate from each other. Some bacteria divide in one specific direction. With cocci (spherical bacteria), this type of cell division can result in pairs (diplococci) or chains (streptococci) that are characterizing features of certain bacteria. Other bacteria divide in several directions, resulting in tetrads or clumps. Rods generally divide in one direction resulting in pairs or chains connected end to end. Examples of cell arrangements are on page 1 & 2.

Bacterial Growth Curve: When bacteria are introduced into a new growth environment, they often first go through a **lag phase**, or adjustment period, where no growth is apparent. This is followed by the active **exponential** or **logarithmic growth phase**. As the environment changes (e.g., nutrients deplete, inhibitors develop), growth will level off to a **Stationary Phase**, after which cells will then eventually begin to die off in the **Death Phase**.



REQUIREMENTS AND CONDITIONS FOR GROWTH

Bacterial species vary widely as to what conditions are conducive for growth. Conditions that are optimum for one organism may be lethal for another. Nutrient availability, pH, moisture, the presence or absence of oxygen and other gases, the presence of inhibitors and temperature can all influence the growth of bacteria. In most cases these are not independent variables but are interactive.

Nutrient Requirements:

Most bacteria have similar basic nutrient requirements, although different organisms will vary in their specific needs. Some bacteria require defined nutrients or conditions that make them difficult to culture. Generally, all bacteria require the following, in one form or another:

- Energy Source – from carbohydrates, proteins, lipids
- Carbon Source – from carbohydrates, proteins, lipids, carbon dioxide
- Nitrogen Source – from proteins, peptides, amino acids, ammonia, nitrates
- Vitamins – primarily water soluble B-vitamins
- Minerals, Metal Ions & Salts – such as potassium, phosphorus, calcium, magnesium, iron

Milk provides sufficient nutrients needed to support the growth of a large selection of microorganisms. Bacteria are capable of utilizing the proteins, fats, carbohydrates and vitamins in milk for their growth and metabolism. Different bacterial species may differ in regard to the **enzymes** they have or need to break down milk components. Enzymes are biological catalysts, usually protein in nature, that facilitate a biochemical reaction, either breaking down or building a biological compound (e.g., "protease" enzymes break down proteins; "lipase" enzymes break down fats). Microbial growth and the resulting increase in microbial numbers and enzyme activity results in measurable changes in milk components and characteristics and the development of by-products that directly affect the product either in a beneficial (e.g., cheese fermentation) or harmful (e.g., milk spoilage) manner.

pH Requirements:

The measurement of acidity and alkalinity, or **pH**, is expressed on a scale of 0-14, which is the negative logarithm of hydrogen ion activity of a solution or food.

0 . . . . 7 14
Acidic — Neutral — Alkaline

Most bacteria grow best at a neutral (7.0) or slightly higher pH, although this varies with different organisms. Most bacteria will not grow below pH 4.0 or above pH 10.0. The pH of normal milk is around 6.7, which allows the growth of many types of bacteria. Cultured dairy products have lower pH values.

Dairy Starter Cultures or *Lactic Acid Bacteria* (LAB) produce lactic acid and are capable of growth at lower pH values than other bacteria (pH 4.0 – 5.0). These organisms are used to intentionally "ferment" milk to make products such as yogurt, buttermilk and cheese. Culturing milk prevents many spoilage or harmful bacteria from growing. LAB may produce sufficient acid to eventually limit their own growth if allowed to do so. LAB may also occur as wild contaminants that cause undesirable spoilage in certain products (e.g., excess acid and/or gas). Yeast and mold are capable of growth over a wider pH range, including very low pH values, which is why they are common causes of spoilage of cultured milk products.

Moisture or Available Water:

All bacteria require relatively high levels of available moisture, which can be expressed as **water activity, (a_w)**. Pure water has an a_w of 1.00, while fluid milk is extremely close to this value. Sugars and salts react with water in a manner that binds the water and lowers the level of available water. Some cheeses have water activities less than 0.90 due to concentration and water binding by salts and solutes. Drying processes lower the available water by removing it ($a_w \sim 0.20$). Most bacteria require a water activity of greater than 0.91 and generally will not grow in or spoil foods that are lacking available water. Fluid milk is a perishable food that easily spoils, while milk powder can be stored unrefrigerated for long periods of time. Yeast and molds generally require less water for growth than bacteria, which is why some cheese and foods such as jams and jellies are only spoiled by these types of microorganisms.

Oxygen Requirements:

Some bacteria require oxygen while other bacteria will not grow in its presence. Oxygen may be toxic to certain bacteria. Bacteria are classified based on requirements for the presence or absence of oxygen as:

Aerobic – aerobes require oxygen for growth

Anaerobic – anaerobes grow only in the absence of oxygen (oxygen may be lethal)

Facultative Anaerobic – facultative anaerobes can grow with or without oxygen

Milk contains dissolved oxygen; thus it supports the growth of aerobic and facultatively anaerobic microorganisms. Rarely do strict anaerobes grow in milk. Some bacteria such as certain starter cultures are considered "microaerophilic," meaning they grow best in lower levels of oxygen. Cheese may have a reduced oxygen environment due to the growth of dairy cultures and other bacteria. An oxygen-free environment may occur in the center of some cheeses, allowing the growth of certain anaerobic bacteria, some of which cause serious defects (e.g., late gas-blowing). *Clostridium botulinum* is an anaerobe that produces a deadly toxin that has been associated with dairy foods on rare occasions.

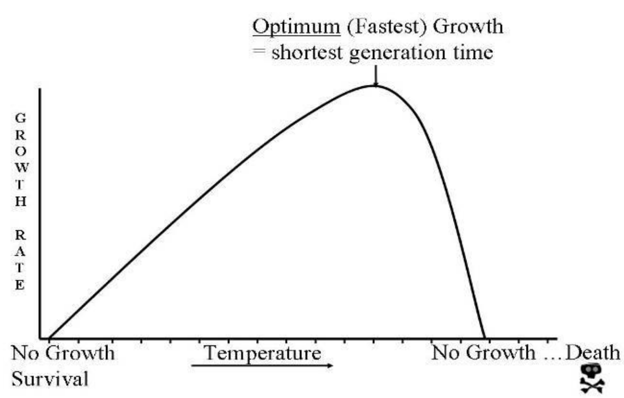
Influence of Inhibitors:

There are a number of chemical substances that can inhibit the growth of (*bacteriostatic*) or kill (*bactericidal*) bacteria. Some examples relevant to dairy microbiology are drugs or antibiotics, lactoferrin (natural in raw milk), carbon dioxide, lysozyme (an enzyme), sanitizers, organic acids, preservatives (e.g., potassium sorbate) and natural inhibitors formed by microorganisms (e.g., nisin).

Temperatures for Growth:

The optimum growth temperature for a bacterium is the temperature at which its generation time is shortest or it grows the fastest. Each bacterium has a minimum and maximum temperature for growth, which will vary between species and strains and with other environmental conditions. Outside of this range, growth does not occur. Bacteria are often grouped based on their optimum, minimum and maximum temperatures for growth. These are not rigid ranges, as some bacterial species may overlap into adjacent groups. General groupings of bacteria and approximate ranges are as follows:

Thermophilic ...	Min:	104°F (40°C)
"Heat Loving"	Max:	140°F (60°C)
	Opt:	122-131°F (50-55°C)
Mesophilic …...	Min:	41°F (5°C)
Medium Temps	Max:	122°F (50°C)
	Opt:	86-98°F (30-37°C)
Psychrophilic ...	Min:	32°F (0°C) or less,
"Cold Loving"	Max:	77°F (25°C)
	Opt:	≤69°F (≤20°C)



Psychrotrophs:

The types of bacteria that are of most significance to the dairy industry are those that can grow under refrigeration conditions. "*Cold Tolerant*" *organisms capable of growth at temperatures at or below 7°C (44.6°F), regardless of their optimum temperatures* are generally referred to as **Psychrotrophs** or **Psychrotolerant** (the term currently used by some microbiologists to describe this group). "Mesophilic" bacteria (medium optimum temp.) that grow under refrigeration would be considered *psychrotrophs*. Regardless of the range of temperatures for growth, temperatures colder than the optimum for an organism will generally increase the generation time (slow its growth). When temperatures approach the freezing point of water (32°F/0°C), growth of most microorganisms is prevented, although a few organisms will continue to grow very slowly at or even below freezing. Most microorganisms will survive freezing (without growth), depending on the medium in which they are frozen.

When temperatures exceed the maximum for an organism, growth stops. As temperatures are increased further, heat eventually become lethal. Heat is often used to kill microbes; the temperatures and holding times for milk pasteurization (e.g., 145°F/63°C for 30 minutes) are designed to kill the most heat resistant pathogens common to raw milk. Generally, higher temperatures result in greater kill. Bacterial spores may be activated by higher heat, but as temperatures increase they will eventually reach a point that will cause death. Complete sterilization (kills all microbes, including spores) requires steam under pressure (e.g., 248°F/121°C for 15 minutes) or high, dry heat for a long period of time (e.g., 338°F/170°C for 1 hr.).

Temperature versus Generation Time
(example of one organism studied)

Temperature	Generation Time
37°C (100°F)	20 Minutes
32°C (90°F)	25 Minutes
27°C (80°F)	40 Minutes
21°C (70°F)	60 Minutes
16°C (60°F)	150 Minutes
10°C (50°F)	12 Hours
4.4°C (40°F)	>24 Hours

BACTERIA OF CONCERN IN FLUID MILK

Pathogenic/Foodborne Illness Bacteria:

Pasteurization was originally designed to destroy pathogenic bacteria that caused tuberculosis, brucellosis, typhoid and Q-fever (most heat resistant target organism), illnesses that were often associated with the consumption of raw milk. Milk pasteurization, coupled with improved animal husbandry procedures, has virtually eliminated most of these types of illnesses. Raw milk may also harbor other organisms associated with foodborne illness, including *Salmonella*, *Listeria*, *Campylobacter*, *Yersinia* and certain strains of *E. coli*. These organisms are also killed by pasteurization. However, cross-contamination of processed dairy products with raw milk and/or the direct consumption of raw milk have resulted in relatively recent outbreaks of foodborne illnesses involving these organisms. Pasteurized milk products can also be contaminated by poor processing and handling conditions and/or poor worker hygiene.

Pathogens of Historical Significance (currently rare)

Coxiella burnetti……………..	Q-fever, flu-like
Mycobacterium spp. ………....	Tuberculosis
Brucella abortus	Brucellosis, abortions
Salmonella typhi ……………..	Typhoid fever
Streptococcus spp. …………...	Septic sore throat
Corynebacterium diphtheriae .	Diphtheria

Pathogens Associated with more Recent Outbreaks

Salmonella spp. ………...	GI illness*, secondary**
Campylobacter jejuni …..	GI illness*, secondary**
Yersinia enterocolitica …	GI, pseudo-appendicitis
E. coli (O157:H7) ……...	GI (hemorrhagic), HUS (kidney failure)
Listeria monocytogenes ..	GI illness*, meningitis, sepsis, stillbirths

* GI illness = gastro-intestinal illness. Symptoms may include nausea, vomiting, diarrhea, cramps & sometimes fever & chills.
** Secondary = non-GI symptoms may follow, e.g., arthritic rxn.

For more information on Foodborne Pathogens (The Bad Bug Book):
http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/default.htm

Mastitis Causing Bacteria:

Bacteria that can cause ***mastitis***, an infection of the mammary gland of dairy cattle, include contagious (e.g., *Staphylococcus aureus*, *Streptococcus agalactiae*) and environmental (e.g., coliforms) organisms. Mastitis can result in increased somatic cell counts (i.e., white blood cells) and in some cases, increased bacteria counts in the bulk milk, both of which result in decreased milk quality. For more information on mastitis, visit the NMC (formerly the National Mastitis Council) web site: http://www.nmconline.org.

Psychrotrophic (Psychrotolerant) Bacteria:

Psychrotrophic or psychrotolerant bacteria are capable of growth at 44.6^{o}F (7°C) or less. Psychrotrophs are of primary concern to the dairy industry since they grow and cause spoilage in raw or processed dairy products commonly held under refrigeration.

a) The most commonly occurring psychrotrophs in milk are gram-negative rods, many belonging to the genus *Pseudomonas*. Gram-negative psychrotrophs generally do not survive pasteurization, thus they occur in processed milk and dairy products as post-pasteurization contaminants (PPC). Some strains of thermoduric bacteria are capable of growth under refrigeration storage (*see below*).

b) Psychrotrophs are common in the dairy environment. Milk soils (e.g., on dirty equipment) can support the growth of psychrotrophs and other contaminants that can contaminate subsequent milk. Marginal cooling can result in relatively large numbers of these organisms in milk. Psychrotrophs might also be present in low numbers in untreated water supplies used for rinsing dairy equipment.

c) Psychrotrophic bacteria produce a variety of enzymes that cause chemical deterioration of milk resulting in off-flavors. Some of these enzymes are not inactivated by pasteurization or by other heat treatments and may continue to degrade milk products, even when the bacterium is destroyed. This has been shown to be a concern with shelf-stable (Ultra-High Temperature) milk, but there is limited information relative to conventionally pasteurized milk.

Thermoduric Bacteria:

Thermoduric bacteria are a miscellaneous group of bacteria that are capable of surviving pasteurization or other heat treatments. As a general rule, all thermoduric bacteria are gram-positive. Spore-forming bacteria (e.g., *Bacillus, Paenibacillus*) comprise some of the most heat-resistant bacteria.

a) Chief sources of thermoduric bacteria in milk are poorly cleaned equipment including old rubber parts, areas of milkstone build-up, separators and other difficult to clean or neglected areas (soil build-up). They may contaminate milk at the farm or at the plant. Poor pre-milking hygiene procedures (e.g., dirty cows) may also influence thermoduric levels in raw milk, especially of spore-formers.

b) Very high thermoduric counts in raw milk could result in counts in the pasteurized milk made from that milk that exceed the 20,000 cfu/ml legal limit. This is rare as counts are normally < 500 cfu/ml.

c) Most thermodurics are not psychrotrophic, but some are. In the absence of gram-negative psychrotrophs, certain thermoduric bacteria can grow and cause spoilage in pasteurized milk. Heat Resistant Spore-Forming Psychrotrophs (HRSP), such as strains of *Bacillus* and *Paenibacillus*, are considered common thermoduric psychrotrophs that have become limiting factors in milk shelf-life.

Coliform Bacteria:

Coliform bacteria are defined as "aerobic or facultatively anaerobic, gram-negative rods, that ferment lactose with the production of acid and gas." These characteristics allow selective detection and counting of these types of bacteria in milk and dairy products. They are considered "**indicator organisms**" because they are easy to detect and their presence in food & water indicate some form of contamination; e.g., the presence of "fecal" coliforms (*E. coli*) suggests the possibility of fecal contamination.

a) They are called *coliforms* because some members of the group (e.g., fecal coliforms) are found in the intestines (colon) of warm-blooded animals. However, many strains of coliform bacteria are common as environmental contaminants and/or are associated with other habitats (e.g., plant matter).

b) Coliforms are almost always found in raw milk, although with good production methods, the numbers can be kept very low. Sources of coliform contamination include dirty cows (manure, bedding, soil), dropped milking units, dirty equipment and, in some cases, cows with coliform mastitis.

c) Coliforms do not survive pasteurization. When detected in processed milk or dairy products, they *indicate* recontamination after pasteurization (Post-Pasteurization Contamination).

SELECT ORGANISMS COMMON TO MILK & DAIRY PRODUCTS:

Grouping/Organisms[1]	General Characteristics and Importance to Milk or Milk Products
Gram-Positive Cocci:	
Enterococcus spp.	Short chains or pairs of cells. "Fecal" streptococci (but are not coliform); common in fecal matter, but also in the dairy farm environment. Used as indicator organisms in some foods. Acid producers. Some strains have some heat resistance.
Lactococcus lactis	Short chains or pairs. "*Lactic*" streptococci; produce lactic acid. Some strains are used as "mesophilic" dairy starter cultures. High numbers may occur in poor cooling of raw milk. Some strains produce a "malty" defect in milk as well as acid defect.
Micrococcus spp.	Irregular clusters or tetrads, cells tend to be larger. Some are associated with udder skin. Some strains are thermoduric and are associated with milkstone on equipment.
Staphylococcus aureus	Single, pairs or irregular clusters. A cause of contagious mastitis. May cause food poisoning due to toxin development if present in high numbers in foods.
Streptococcus agalactiae	Chains, often very long. May appear as chains of pairs or with oval cocci stretched in the direction of the chain. Cause of contagious mastitis.
Streptococcus uberis	Pairs and chains of moderate length. Considered a cause of environmental mastitis, although some evidence suggests that it may be spread cow to cow.
Streptococcus thermophilus	Chains, moderate to long. Dairy "thermophilic" starter culture (incubation ~110°F) used for making yogurt and certain cheeses.
Gram-Positive Rods:	
Corynbacterium bovis	Irregular shaped rods, some "club" shaped. Cause of bovine mastitis, although some strains may be natural inhabitants of the skin and mucosal membranes.
Lactobacillus delbrueckii sub-sp. *bulgaricus*	Long rods, some chains. Dairy "thermophilic" starter culture (incubation ~110°F) used for making yogurt and certain cheese.
Microbacterium lacticum	Irregular rods, some "V-Forms." Thermoduric bacterium, some strains with relatively high heat resistance for a non-spore former.
Gram-Positive Rods, Spore-Forming:	
Bacillus cereus	Relatively large, thick rods. Some strains are psychrotrophic. Some strains cause foodborne illness if allowed to grow to sufficient levels (toxin mediated).
Bacillus spp. (other spore-formers)	Many different spore-forming *Bacillus spp.* in milk. Rods vary in size. Some are psychrotrophic, some are not. Some are gram-variable. Most are thermoduric in the spore state, but not as vegetative cells. Common in soil & dairy environment.
Clostridium tyrobutyricum	Anaerobic spore-former that causes "late gas blowing" defect in certain Swiss and Dutch style cheeses. Associated with poor silage and dirty cows.
Paenibacillus spp.	Spore-forming group with psychrotrophic strains important in their potential to survive pasteurization and limit milk shelf-life. Most were previously classified as *Bacillus spp*. Some strains may stain as "gram-variable."
Gram-Negative Rods:	
Pseudomonas fluorescens (also *P. putida, P. fragi*)	Rods, often in pairs, end-to-end. Psychrotrophic strains of bacteria that are a main cause of reduced shelf-life due to post-pasteurization contamination.
Escherichia coli (*E. coli*)	"Fecal Coliform" associated with manure/environmental contamination. Used as an indicator organism. Some pathogenic strains (e.g., O157:H7). May cause mastitis.
Coliform Bacteria	*Enterococcus, Citrobacter, Klebsiella, E. coli*. Associated with fecal/environmental contamination. Some strains are psychrotrophic. Some may cause mastitis. Coliforms are used as indicators of post-pasteurization contamination in milk and other products.
Other Gm-neg. Psychrotrophs	A number of other gram-negative psychrotrophs are reported in older literature.

[1] NOTE: Names of microorganisms frequently change. Most listed reflect current nomenclature, however, some may be from older literature. For listing of potential human pathogens, see table on page 6 and refer to *The Bad Bug Book*: **http://www.fda.gov/Food/FoodSafety/FoodborneIllness/FoodborneIllnessFoodbornePathogensNaturalToxins/BadBugBook/default.htm**

BACTERIA IN RAW AND PROCESSED MILK

Bacteriological Standards:	Raw Producer Milk ………..	100,000 cfu/milliter (ml) total count
(For Grade "A" Milk)	Commingled Raw Milk ……	300,000 cfu/ml total count
cfu = colony forming units	Pasteurized Milk ……….....	20,000 cfu/ml total count, 10 coliform

Raw Milk: Milk, when secreted in the udder of a healthy cow is virtually sterile. As milk passes through the teat cistern and teat canal, it may be contaminated with low levels of bacteria (<1000 cfu/ml), which are generally not significant to milk quality or safety. Milk from a cow with mastitis (infection of the mammary gland) however, may harbor large numbers of the infectious bacteria. After it leaves the cow, milk can be contaminated from the exterior of the cow (dirty cows), the environment and/or poorly cleaned equipment. Poor cooling allows faster growth rates and can result in rapid increases in bacterial numbers in raw milk before it is processed. While the legal limit for bacteria in raw milk is 100,000 cfu/ml, the production of milk with bacteria counts less than 10,000 cfu/ml should be easily achievable for most farms.

Pasteurized Milk: Pasteurization (i.e., 161°F for 15 sec.), while designed to destroy potential pathogens in raw milk, substantially reduces the total numbers of bacteria present, increasing the shelf-life potential of milk. Unless gross recontamination has occurred, bacterial numbers in freshly pasteurized milk generally reflect the organisms that survive pasteurization (thermoduric). The legal limit for bacterial numbers in pasteurized milk is 20,000 cfu/ml. However, bacteria counts for most freshly pasteurized milks are less than 500 cfu/ml. Under proper refrigeration, the bacteria that become significant in the shelf-life and spoilage of milk are psychrotrophic in nature. These types of organisms generally occur as post-pasteurization contaminants (PPC), although a few thermoduric bacteria may be psychrotrophs. Heat resistant psychrotrophic spore-formers (HRSP) are often the limiting factor in milk shelf-life when PPC is prevented. Regardless of measures that prevent microbial contamination, the shelf-life of conventionally pasteurized milk should not be expected to exceed 21 days and still be considered "fresh."

Sources of Bacteria in Processed Milk:

1) Survive pasteurization (thermoduric)
2) Post-Pasteurization Contamination:
    a) Insufficient cleaning/sanitizing - valves, pipelines, gaskets, pasteurized milk tanks, fillers
    b) Personnel - hands, clothing, shoes, sneezes, coughs, poor equipment handling practices
    c) Environmental - air, dust, standing water and milk residue, drains, condensate

Ultra-Pasteurized (UP) Milk: Ultra-Pasteurization milk is heated to 280°F (138°C) for a minimum of 2 seconds. This much higher heat treatment results in the destruction of virtually all spoilage organisms. Coupled with near sterile handling systems, UP processing results in milk with 60-90+ days of shelf-life.

CONTROLLING BACTERIAL CONTAMINATION AND DEFECTS IN DAIRY PRODUCTS

Preventing Contamination:

Bacteria are ever present in both the farm and dairy plant environments. Although total prevention of microbial contamination of milk at the farm is impossible, it can be minimized by milking clean, healthy cows in a clean environment and by assuring that the milking system and storage equipment are properly cleaned, sanitized and maintained. Once raw milk leaves the farm (i.e., tank truck to plant storage) it must be properly handled to prevent further contamination before it is processed. Keeping the microbial load of raw milk as low as possible will help ensure the quality of the products made. Minimizing raw milk contamination with heat resistant psychrotrophs (e.g., HRSPs) is important to extending fluid milk shelf-life, as even low levels can eventually lead to milk spoilage. At all stages of raw milk handling, milk must be rapidly and properly cooled with temperatures maintained below 40°F (4.4°C).

At the dairy plant, preventing contamination after pasteurization is critical for product shelf-life and safety. This requires that the processing equipment and the plant environment be thoroughly cleaned and sanitized such that the possibility of microbial growth and contamination is limited. Once cleaned and sanitized, recontamination of the system and the milk should be prevented. Proper employee training in personal hygiene and processing procedures should be an essential part of every plant's quality assurance program.

Preventing or Slowing Microbial Growth:

Microbial growth can be controlled by: 1) eliminating sources of "bacterial food" by thoroughly cleaning the milk handling equipment and the environment, thus eliminating milk residues and other sources of microbial nutrition, at the farm, during transit and at the plant; 2) holding raw milk and dairy foods well below the optimum growth temperature of bacterial contaminants, generally less than 40°F (4.4°C) without freezing; 3) lowering the pH such as in cultured dairy products; 4) reducing the moisture or water activity (a_w) such as in dry milk products; and 5) adding microbial inhibitors or preservatives such as potassium sorbate addition to cottage cheese.

Eliminating or Killing Contaminants - Sanitation Procedures:

Chemical sanitizers are routinely used to reduce the load of microbial contaminants that may be present on milk/food contact surfaces. Most dairy sanitizers, when used correctly, kill off a broad spectrum of microorganisms. They generally do not "sterilize" equipment. Sanitization procedures should be performed after washing and immediately before processing, although an additional sanitizing step after equipment washing procedures can be helpful (e.g., an acid sanitizer rinse). Most chemical sanitizers are inactivated by organic matter and are ineffective on poorly cleaned surfaces. Sanitizers commonly used in the dairy industry include chlorine, iodine, quaternary ammonium, acid anionics and peroxyacetic acid.

Hot water sanitization is commonly used in many dairy plants. Hot water sanitization involves circulating water of at least 170°F (77°C) as determined at the outlet, for at least 5 minutes. Higher temperatures (>185°F) for longer times (10-20 minutes) are recommended to allow heat penetration into areas that are hard to reach. Hot water treatments should be followed by a cooling chemical sanitizer rinse or with cooled pasteurized water. Hot water sanitization often provides greater microbial kill and longer milk shelf-life than achieved with chemical sanitizers alone, but must be used with caution for personnel and equipment.

Eliminating or Killing Contaminants - Pasteurization:

Pasteurization procedures generally kill a large percentage of the bacteria commonly found in raw milk, including pathogenic organisms and those that rapidly cause spoilage. The higher the temperature used, the less time is required for equivalent kill. The most commonly used defined minimum temperature/time combinations are:

	Minimum Temperature / Time
Batch Pasteurization:	63°C (145°F) for 30 minutes
High-Temperature/Short-Time:	72°C (161°F) for 15 seconds

These procedures stand as legal definitions of pasteurization and are outlined in the "*Pasteurized Milk Ordinance*" (PMO), the guidance document specifying requirements and standards for Grade "A" milk products. The majority of fluid milk plants use High-Temperature/Short-Time pasteurization, with temperature/time combinations often exceeding the stated minimum requirement (e.g., 175°F for 20 seconds). Most bacteria that survive pasteurization generally do not grow or else grow slowly at refrigeration temperatures, causing problems later in shelf-life (see HRSP). Contamination after pasteurization with psychrotrophic spoilage bacteria is not uncommon. When post-pasteurization contamination of a product occurs, both the quality and the safety of the product are jeopardized.

References:

Brock, T. D., & M. T. Madigan. 1986. Biology of Microorganisms. Prentice Hall, Englewood Cliffs, NJ; Jay, J.M. 1996. *Modern Food Microbiology*. Chapman & Hall, NY, NY; Robinson, R.K. ed. 2002. *Dairy Microbiology Handbook*. J. Wiley & Sons, NY, NY; FDA, *Pasteurized Milk Ordinance*, 2007; FDA-CFSAN. *The Bad Bug Book*, available at: http://www.fda.gov/Food/FoodSafety/default.htm

Prepared by S. C. Murphy (Sr. Extension Associate) as an update of a D. K. Bandler Extension Handout. Edited by N. R. Carey.

Provided with support from the NY State Dairy Promotion Order,
Dairy Farmers dedicated to the production, manufacture and distribution of quality products.