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BACTERIA, YEASTS AND MOLDS AND THEIR RELATION TO MILK AND MILK PRODUCTS

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Bacteria, yeasts and molds, also known as microorganisms, belong to the plant kingdom and are members of the plant group known as Thallophyta. (Thallo = young shoot; phyte = plant.) They do not have leaves, stems or roots. This group is divided into several sections. One of these is the Fungi, which includes the three classes: bacteria, yeasts and molds.

BACTERIA

The bacterial content of milk and cream should always be kept as low as possible. If present in considerable numbers in milk or cream and the temperature for their growth satisfactory, the bacteria may seriously affect the flavor of the product and may produce enough acid to coagulate the casein. The market value of the product may be considerably lowered. Large quantities of milk and cream have been returned by the diary plants, especially during the warmer months, to the producers on account of low quality caused by bacteria.

It is fundamental to know where the bacteria occurring in milk or cream come from in order that they may be controlled. It is more important to have a program of keeping the bacteria out of milk and cream than it is to devise means for destroying them when present in these products. There is no known process that may be used for removing the flavor compounds formed by bacterial action in milk and cream and restoring the lost quality.

The following is a brief discussion of what bacteria are, where they come from, what they do, and how they may be controlled.

WHAT ARE BACTERIA?

Bacteria are minute one-cell organisms. Each cell is surrounded by a wall which controls the shape of the bacterium. The cell may be round (coccus), or oval, in shape and may occur as pairs or chains; others are rod-shaped (bacillus) of varying length and width; and a third form is spiral (spirillum) in shape. Two trillion average-sized bacteria weigh only about one gram (1 pound = 454 grams). Bacteria are generally colorless. None produce chlorophyll, the green coloring matter of the higher plants necessary for the production of carbohydrates from water vapor and carbon dioxide in the presence of light. Bacteria are not able to manufacture carbohydrates. The organisms are saprophytes or parasites and obtain their food from nonliving or living matter. The mechanism of obtaining food is by the simple process of osmosis. The nutrients in solution pass through a thin membrane located inside the cell wall. This membrane regulates, through osmotic pressure, the materials which may enter the contents of the cell, known as the protoplasm, and those which may leave. If the pressure inside the membrane is higher than on the outside, the substances in solution pass into the cell, and when the pressure is smaller the reverse takes place.

As a rule, bacteria reproduce by the simple process of division or fission. With rapidly growing bacteria the cell becomes longer, a division wall in the middle is formed, and the two halves separate to become two separate cells. Less frequent methods of reproduction are by means of buds and by the breaking apart of the cell and formation of reproductive cells.

Some types of bacteria are able to move around in the fluid containing them. The motion is accomplished by means of the active movement of minute hair-like threads, known as flagella attached to the cell.

Under certain conditions, usually stimulated by those unfavorable to growth, the cells of some species of bacteria (usually the rod-shaped) produce one or two spores. The spores are resistant to unfavorable conditions, such as low temperature, high temperature, drying, and the action of chemical agents. Under favorable conditions, germination of the spores occurs and bacterial cells are formed.

CLASSIFICATION OF BACTERIA

The bacteria are placed in the general botanical CLASS of Schizomycetes. (Schizo means to cleave or divide; mycetes means fungus.) Bacteriologists have divided the general class of Schizomycetes into seven ORDERS, each of which includes FAMILIES of bacteria. The families are again subdivided into GENERA. Each GENUS is further divided into SPECIES and the species are again subdivided into subspecies or VARIETIES.

The common lactic acid-producing bacterium (bacterium = singular, bacteria = plural), Streptococcus lactis, is classed in the order of Eubacteriales. As the individual bacterial cell of this organism is round (spherical), it belongs to the family Coccaceae or coccus organisms. Streptococcus (or chain-forming coccus) is the genus name and lactis is the species name. There may be a number of different varieties or types of Streptococcus lactis.

Not all of the organisms belonging to the genus Streptococcus are lactic acid producers. Another is Streptococcus citrovorus which ferments citric acid and produces desirable flavor in starter. Some of the disease-producing bacteria belong to the genus Streptococcus. Varieties of Streptococcus pyogenes cause blood poisoning, arthritis, tonsillitis, and other diseases. None of the lactic acid-producing or citric acid-fermenting streptococci associated with dairy products are pathogenic.

Several thousand different species of bacteria have been identified and classified. New species are continually being isolated and classified.

SIZE AND SHAPE OF BACTERIA

Bacteria are so small that the individual cells cannot be seen except by means of a microscope. The typical Streptococcus lactis bacterium is only 0.5 to 1.0 micron in diameter. (1 micron equals $\frac{1}{25,000}$ inch.) The cells are slightly oval. They appear in pairs or short chains. Lactobacillus acidophilus is rod-shaped; it has a diameter ranging from 0.5 to 1.0 micron and a length of about 2 microns. Some bacteria may be considerably longer.

Sour milk may contain 1 billion or more bacteria in one milliliter. A drop of starter may contain 50 million bacteria.

The bacteria commonly associated with dairy products are either spherical or rod-shaped in form. A spherical organism is called coccus (plural = cocci) and a rod-shaped organism is called bacillus (plural = bacilli). Examples are Streptococcus lactis and Lactobacillus casei. Some variation in the form of the bacteria is not uncommon. A good example is the ropy-milk organism, which is surrounded by a slimy coating. An example of a spore-forming bacterium is Bacillus subtilis. This organism is common in soil. If this bacterium is present in milk, it may cause curdling without acid production.

GROWTH OF BACTERIA

The growth of bacteria may be influenced by a number of factors:

1. The presence or absence of air. Some bacteria need oxygen for their growth. They are termed "aerobic." Others grow only in the absence of oxygen. They are called "anaerobic." Some bacteria may grow satisfactorily under either condition. They are then termed "facultative." Most of the facultative bacteria encountered in dairy products grow well in air or in a restricted air supply. The common lactic acid-producing bacterium Streptococcus lactis is facultative. The gas-forming bacterium Clostridium welchii is anaerobic. The bacterium causing tuberculosis, known as Mycobacterium tuberculosis, is aerobic.

2. The availability of food. When food in the proper form is present multiplication of bacteria is possible. The organisms generally need protein, carbohydrate and minerals. They also require water. Vitamins are required for the growth of some species of bacteria. Milk is a good food for many types of bacteria. The organisms may develop rapidly in fresh milk. Streptococcus lactis when growing in sterilized milk produces lactic acid from milk sugar. Under favorable growth conditions, the number of lactic acid bacteria in curdled milk may exceed one billion per cubic centimeter. Coagulation usually takes place when from 0.5 to 0.6 percent acid has developed. High acidity retards growth.

3. Moisture. Growth of bacteria takes place most rapidly when the product in which they are present contains a high percentage of moisture. Milk, which contains from 86 to 88 percent water, may be used as an example of an excellent medium for many bacteria. When the moisture content is quite low, as in dry milk, which contains only 1 to 3 percent water, growth is very slow and may not even take place.

4. Exposure to light. In general, bacteria are sensitive to light, particularly sunlight. They are usually quickly destroyed if exposed to sunlight or to ultraviolet light artificially produced. There are some exceptions. Certain sulfur bacteria, growing in mud and water containing hydrogen sulfide, require sunlight. They produce pigments of various colors.

5. Acidity or alkalinity. Most bacteria grow best when the product or medium in which they grow is nearly neutral in reaction. Some bacteria produce acid; others produce alkali. Different species of bacteria may be acid or alkali tolerant, or they may be acid or alkali sensitive. For example, *Streptococcus lactis* is acid tolerant or aciduric, whereas *Eberthella typhosa*, causing typhoid fever, cannot survive in a high acid medium. The bacteria growing in Limburger cheese are also acid sensitive.

   Recent published data (by Wilson and Tanner in Food Research, 1945) indicate that disease-producing bacteria may survive in sour milk such as cultured buttermilk for a considerable time. The pathogenic organisms survived in milk having an acidity ranging from 0.95 to 1.15 percent for from one day to seven weeks, and in milk which had an acidity ranging from 0.50 to 0.86 percent they survived for periods that ranged from two to nine weeks.

   An outbreak of gastroenteritis that was traced to *Straphylococcus aureus* in buttermilk which had been placed in insanitary containers occurred in 1943.

6. Temperature. Growth of most bacteria is rapid between a temperature of 60° and 100° F. Some species of bacteria grow well at a temperature of from 120° to 130° F. or even at pasteurizing temperature. They are called "Thermophilic," which means "heat loving." Others grow well at the lower temperatures, from the freezing point to 50° or 60° F. They are termed "Psychrophilic," which means "cold loving." These organisms are present in soil and in water such as lake or ocean, and even in cold brine. Bacteria may grow over a wide range of temperature. For example, *Streptococcus lactis* grows at a temperature ranging from 50° F. to slightly above 100° F. It will usually grow more rapidly than other bacteria present in milk when the temperature is from 60° to 80° F. By "optimum" temperature is understood the temperature at which the bacteria grow best. This is influenced to some extent by the concentration and availability of the food of the material in which they are present and by other factors. For example, the optimum temperature of *Lactobacillus thermophilus* ranges from 122° to 144.5° F. The optimum temperature of *Mycobacterium tuberculosis* is 98.6° F., and of *Lactobacillus casei* (present in Cheddar cheese), 86° F. The optimum temperature of the organism *Pseudomonas putrefaciens*, which causes a putrid flavor in butter, is 70° F., and that of *Achromobacter phosphoreum*, which is present in sea water, varies from 59° to 68° F.

   If the material containing the bacteria is brought to such a temperature that the bacterial cell freezes, growth is stopped. The organisms may not be killed by the cold. They will start growing again as soon as the temperature is raised. The freezing point of some fluids, as for example ice cream mix, which contains sugar, may be lower than the freezing point of water. Slow growth of the bacteria may take place in the mix even below 32° F.

7. Pressure (osmotic pressure). The pressure must be satisfactory for the movement of nutrients through the cell wall. If sugar or salt is added to a fluid in which bacteria are present to increase the pressure on the outside of the cell wall above that of the inside, there will be a movement of water from the cell protoplasm through the bacterial cell wall into the surrounding fluid. This results in a shrinkage of the cell and causes what is known as "plasmolysis." If enough sugar or salt is added to greatly increase the difference in pressure between the inside and outside of the cell wall, enough water may be extracted from the bacterial cell (osmosis) so that the bacteria die because of a lack of water in the cell. A reverse action may take place when bacteria are transferred from the medium having a high osmotic pressure to one which has a low osmotic pressure—as into pure water. The bacterial cell will swell and may break open. This is termed "plasmoptysis." When the osmotic pressure on the inside of the bacterial cell wall is the same as on the outside, the cell is "isotonic" with the solution surrounding it.

When growth is rapid the number of bacteria in the material containing the organism may double every 20 or 30 minutes. Each bacterial cell simply divides so that two single cells which are capable of further division are formed. Thus from this single cell, over 1,000,000 cells could be produced in 10 hours, 1,000,000,000 in 15 hours, and over 1,000,000,000,000 (13 figures) in 20 hours. Such growth probably never takes place. Products which are toxic to the bacteria are continuously being produced, as a result of the bacterial activity, causing many organisms to die. An initial seeding of only a few lactic acid-producing bacteria in milk may result in enough bacteria in the milk to cause souring after a few hours. If 1 percent lactic acid is formed (1 pound acid in 100 pounds milk), this acid has a retarding effect on the growth of the organisms. If exposed for some time to the acid milk, the bacteria gradually die.

If sterilized milk is inoculated with living bacteria and kept at a temperature favorable for the growth of the organisms, growth will begin. It will be slow at first—the lag period—unless the bacteria were actively growing when introduced into the milk. This initial period will be followed by a period of active multiplication, which continues until the supply of food becomes less available or certain by-products of the bacterial activity retard the growth. The growth finally ceases and the cells die. If the temperature of the bacterial culture is lowered from the optimum temperature during the latter stage of the active growth period, the life of the culture can be prolonged.

SOURCES OF BACTERIA

Bacteria are everywhere—on land, in the air, in water. Most bacteria are nonpathogenic and many types are beneficial. Bacteria are active in soil in connection with the breaking down of organic matter and making this available as plant food. Certain bacteria fix nitrogen from the air for use by plants. Bacteria are numerous in decaying vegetable and animal matter. They aid in the decomposition of various forms of organic matter. The bacterial content of the intestinal tract of humans and animals is high. Bacteria are active in breaking down refuse from the human and animal body. Insoluble organic compounds in the refuse are made soluble by the bacterial action.

Even the purest water usually contains some bacteria. River water or shallow well water often contains numerous bacteria. The numbers are particularly large if the water has been polluted with sewage. Sea water usually contains bacteria.

The air over cities carries many bacteria. The organisms are transported by dust and moisture particles in the air. The air over cultivated areas and over land covered with vegetation also contains bacteria, but few or no bacteria are present over desert land, over oceans or arctic areas. The upper regions of the air are undoubtedly free from bacteria. Disease-producing bacteria may be carried by moisture droplets in the air (sneezing and coughing).

Bacteria are common on the surface of, or in, various food products. Milk, for example—if raw or pasteurized—practically always contains some bacteria. Even if the milk is obtained from healthy cows under aseptic conditions (without outside contamination) it always contains some bacteria. When obtained under reasonably sanitary conditions, the milk may contain a few thousand bacteria per cubic centimeter. Bacteria are not desired in market milk, market cream or ice cream. Certain types of bacteria are very desirable in products such as buttermilk, cheese and acidophilus milk.

Bacteria may be present in considerable numbers on the skin and clothes of humans. They are common on the hands and arms and may be especially numerous in dirt lodged under the fingernails. Disease-producing bacteria may be present on the hands of a person who is attending another person who is diseased. The exudate from boils and skin disorders may contain an abundance of bacteria. Coughing or sneezing into the hands deposits many bacteria on them. Because of the danger of contaminating dairy products with bacteria from the hands, the dairy worker should be especially careful to avoid soiling the hands. He should wash them thoroughly, using soap free from a strong odor, after using the toilet and after handling soiled articles. After washing, he should wipe the hands on a single service towel. As the hair of a dairy worker is a potential source of bacteria, it should be washed often and a clean cap should be worn while he is working in the dairy plant.

Other common sources of bacteria in a dairy plant are:

(a) Flies, cockroaches and rodents: Flies are filthy; they feed and breed in excreta and refuse. They may carry thousands of bacteria on them from decomposing food, filth and sewage. They are a source of undesirable bacteria, including those pathogenic to man. Cockroaches and rodents likewise may carry bacteria on their bodies.

(b) Clothes worn by the diary worker: The outer garments when soiled may be a source of numerous bacteria. The use of a freshly laundered white suit and cap daily greatly reduces this source of bacteria,

(c) Cans, pails, bottles, hoses, butter moulds, cheese hoops, cracked table tops, cracked linings of milk and cream vats, stuffing boxes: Poorly cleaned, or clean but improperly sterilized, containers for milk, cream, butter, cheese and other dairy products may be an important source of bacteria. A deposit of milk solids on the inside surface of the containers offers a good medium for bacteria to grow in. The outside surface, especially the bottom part of the containers may, if soiled, be a source of very large numbers of bacteria. If butter moulds are placed on the floor, or slightly above the floor during packing and storing, the butter may become seriously contaminated with bacteria present in moisture from the floor.

ACTIVITY OF BACTERIA

Fortunately only a relatively small percentage of the known bacteria cause disease in plants, animals and humans. Many species of bacteria are of great economic value. Bacteria fix nitrogen from the air. The nitrogen is used in building plant protein. Bacteria break down organic matter, which can then be used as plant food. Bacteria are necessary for the breaking down of organic matter in sewage disposal plants.

Bacteria are used in certain industries. Species of the genus *Acetobacter* are used in vinegar manufacture. Species of the genus *Lactobacillus* may be used when manufacturing lactic acid which may be converted into polymethylacrylate plastic. Species of the genus *Streptococcus* are used in cheese making. Several species belonging to the genera *Lactobacillus* and *Leuconostoc* are responsible for acid production in the fermentation of sauerkraut.

Bacteria ferment starch and sugar with the formation of acid, alcohol and carbon dioxide. They break down protein to more soluble products such as peptones, peptids and amino acids. Fat is broken down into glycerol and fatty acid.

Bacteria may produce enzymes which bring about changes in food products. An enzyme is an organic substance which in very small amounts is able to induce significant chemical changes in relatively large amounts of material, yet it is not itself affected and remains unchanged.

Examples of bacteria-produced enzymes are: "Rennin," which transforms the calcium caseinate in milk into less soluble calcium paracasein; "Diastase," which breaks down, or hydrolyzes, the starch to dextrin and maltose; "Maltase," which hydrolyzes maltose to glucose; "Lipase," which breaks down fat into glycerol and fatty acid (rancid flavor); "Lactase," which changes milk sugar (lactose) into galactose and dextrose (glucose); "Pepsin," which breaks down protein to proteose and peptone, and "Trypsin," which breaks down protein to peptids and amino acids.

Associated with these changes generally is the production of certain flavoring substances. A change may be brought about in the physical condition of certain foods as a result of the bacterial activity. For example, when the lactic acid-producing bacteria have formed enough acid a physical change takes place in the casein present in the milk. The casein precipitates and a coagulum is formed. Curdling without acid production may be caused by some species of rennin-producing bacteria. In the ripening of cheese, the casein is gradually broken down into miscellaneous compounds resulting in a softer, waxier body of the cheese.

Sometimes undesirable pink or yellow colors in the food products are produced by the bacteria. Poisonous compounds and putrid, undesirable flavors may also occasionally be formed.

Many different species of bacteria cause spoilage of food. They may cause an objectionable flavor in meat, fish, eggs, and dairy products even to the extent of making the food inedible.

Specific types of bacteria cause disease in humans, animals and plants.

The bacterium Eberthella typhosa, causing typhoid fever in humans, is eliminated from the body of diseased persons or carriers in the excretions: feces and urine. Milk may be contaminated with typhoid organisms by (a) water containing the organisms, (b) the hands of a person having the disease or is a carrier of the organisms, (c) flies which have come in contact with the excreta of diseased persons or carriers.

The organism causing septic sore throat belongs to the species known as Streptococcus pyogenes. Although the organism is of human origin, it may gain entrance to the cow's udder and multiply there. The milk obtained from an infected udder may contain the disease-producing organism. Milk may become contaminated by coming in contact with the hands of a person who has the disease, and from moisture droplets bearing the organisms that fall into milk when a diseased person coughs or sneezes over a vessel containing milk.

The organism Mycobacterium tuberculosis may be present in the body of either human or cow. Different parts of the body may become infected, but the lesions commonly develop in the lungs, the abdominal organs, and in the bones. The lesions may be present in a cow's udder. Transmission to milk is by manure of infected cows and directly to the milk in the udder if this is infected. There is the possibility of contaminating milk with the organisms if a person who has tuberculosis of throat or lungs coughs or sneezes over a container of milk, or who handles milk in a dairy or milk plant.

Brucellosis is caused by species of bacteria which belong to the genus Brucella. The human form of the disease is frequently called undulant fever. Brucella abortus infects cattle. The disease in cattle is commonly called Bang's disease. Brucella melitensis infects goats and sheep. The disease in goats is commonly called Malta fever. Brucella suis infects hogs. All three species may affect humans. There are four ways in which humans may become infected with the Brucella organisms: (1) by direct contact with infected animals, (2) by contact with the meat and organs of the carcasses of infected animals, (3) by consuming milk or dairy products containing living Brucella bacteria; the organisms may be present in the milk from a cow which has Brucellosis, (4) by exposure to living Brucella organisms.

In addition to the above-mentioned milk-borne diseases: Typhoid fever, septic sore throat, tuberculosis, and brucellosis, there are several others that can be transmitted by milk and milk products.

COMMON TYPES OF BACTERIA IN MILK AND MILK PRODUCTS

The following is a list of some of the types of bacteria that occur in dairy products. Some of them are harmless or desirable; others are definitely undesirable.

1. Streptococcus lactis. Produces lactic acid in milk and cream. It is important in the manufacture of cheese and for the preparation of cultured buttermilk. Hammer and Baker identified varieties of the typical organism.

   (a) Var. maltigenes - produces a malt-like odor in milk and cream.

   (b) Var. hollandicus - produces a ropy condition in milk.

   (c) Var. anoxyphilus - reduces the pigment "litmus" slowly.

   (d) Var. tardus - coagulates milk slowly.

   (e) Streptococcus thermophilus - This is a related species and is heat resistant. It is killed at 162° to 165° F. Its optimum growth temperature ranges from 104° to 122° F.

2. Streptococcus citrovorus (Hammer). Is associated with Streptococcus lactis in sour milk and cream and in starter. It produces desirable aromatic compounds.

3. Streptococcus paracitrovorus (Hammer). Is associated with Streptococcus lactis in sour milk and cream and in starter. It produces desirable aromatic compounds.

4. Lactobacillus acidophilus - Produces lactic acid. Is used for the preparation of acidophilus milk. The organisms inhabit the intestinal tract. Maximum acid production in milk approximately 1.25 to 1.50 percent calculated as lactic. Optimum growth temperature 98.6° F.

5. Lactobacillus casei. Is present in ripened Cheddar cheese. If grown alone, it produces a maximum of 1.5 percent lactic acid in milk. Optimum growth temperature 86° F. The organisms cause a breakdown of the casein in cheese and aid in ripening.

6. Lactobacillus bulgaricus. Present in "Yoghurt" milk and in Swiss cheese. The organism produces from 2.7 to 3.7 percent lactic acid when grown in milk.

7. Propionibacterium shermanii. This organism is also present in Swiss cheese.

8. Bacterium linens together with nonsporeforming rod-shaped bacteria inhabits the surface of Limburger cheese. Yale found the surface of 10-to 14-day-old Limburger cheese to contain from 41 billion to 360

billion bacteria per gram of cheese in addition to numerous yeasts.

A number of different species of bacteria cause an undesirable condition in dairy products.

9. Escherichia coli (commonly referred to as the colon, or coliform organism). This species of organism inhabits the intestinal tract of man and animal. Its optimum growth temperature is 98.6° F. The bacteria form acid and gas in milk and in cheese but do not break down casein. Milk produced under dirty conditions and contaminated with manure contains numerous colon organisms. Hammer found that gas-forming colon organisms were able to produce from 0.56 to 0.79 percent lactic acid in milk held at 98.6° F. for one week. Cultures of this organism may be heat resistant. They may survive a pasteurization temperature of 143° F. for 30 to 40 minutes (Long, Hedrick and Hammer).

10. Aerobacter aerogenes also produces acid and gas from lactose in milk. Both the genus Aerobacter and the genus Escherichia belong to the family Enterobacteriaceae, The organisms are widely distributed. They occur on grain, on feed, in soil and in water. The organisms may also be present in the intestinal tract of animals. The gases produced by the bacteria are carbon dioxide and hydrogen.

11. Aerobacter cloacae (Aerobacter liquefaciens). This organism is found in soil, water and feces. If introduced in water used for washing butter, it may cause a change in butter during storage.

The coliform organisms of the Escherichia and Aerobacter groups are often present in manufactured dairy products, Two research workers (Fournelle and Macy) in a study ascertained the prevalence of these organisms in ice cream and determined the species and varieties of the organisms. The coliform organisms in 69 samples of commercial factory-packed ice cream and sherbet ranged in numbers from 0 to 9,180 per ml. In 30 samples of scoop ice cream the numbers ranged from 0 to 101,000 per ml. Of the factory-packed frozen product, 11.6 percent of the samples contained over 100 colon organisms per ml. while 43.3 percent of the scoop samples contained over 100 colon organisms per ml. The following table shows the species of the organisms in the factory-packed and in the scoop samples:

Organism	Factory-packed samples (227 cultures)	Scoop samples (89 cultures)
	percent	percent
Escherichia coli	5.3	1.1
E. coli var. acidilactici	1.3	1.1
E. coli var. neapolitana	3.1	5.6
E. coli var. communior	0.0	1.1
Escherichia freundii	27.3	28.1
Aerobacter aerogenes	23.8	18.0
Aerobacter cloacae	29.5	38.2
Non-coliform	9.7	6.8

12. *Pseudomonas putrefaciens*. This bacterium is commonly responsible for the putrid flavor which sometimes develops in butter. Hammer and Long found that the organism is widely distributed—in water (streams, lakes, roadside pools), on dairy plant floors, in dairy plant sewers and on dairy plant equipment. It is easily destroyed by heat, acid and salt. It does not survive ordinary methods of pasteurization. It produces phosphatase in milk and other media. It grows at temperatures ranging from 37.4° to 86° F.

13. *Pseudomonas fragi*. This is a lipase-producing species of bacteria. It was found by Hussong to cause rancid flavor in butter. In unsalted butter it may cause rancidity in a few days. The organism is generally found in raw milk and cream and in other dairy products. Poorly-cleaned churns are a good source.

14. *Proteus ichthyosmius* was found by Hammer to cause a fishy odor in evaporated milk.

15. *Pseudomonas graveolens* and *Pseudomonas mucidolens* were found by Olson and Hammer to cause a pronounced potato odor in milk.

16. *Bacillus coagulans*. Hammer found that this species of organism caused spoilage of evaporated milk. The cans had bulged and an undesirable flavor had developed. Cordes found cultures of this organism grown in milk to be destroyed at a temperature of from 233° to 235° F. when held for 15 minutes.

17. *Streptococcus liquefaciens*. This organism was found by Long and Hammer to be widely distributed and is present in dairy products. It forms lactic acid and breaks down protein. Some of the decomposition products have a bitter flavor. A temperature of 150° F. for 40 minutes will destroy the organism.

CONTROL OF BACTERIA

The following methods may be used;

1. Exposure to a high temperature over a sufficient time interval.
2. Exposure to a low temperature over a sufficient time interval.
3. Removal of constituents necessary for bacterial cell maintenance and growth.
4. Exposure to chemical agents.
5. Exposure to light rays.
6. Exposure to a current of electricity.

1. High Temperature

Pasteurization temperatures of from 142° to 150° F. maintained for 30 minutes; of 160° to 165° F. for 15 seconds; and of 190° to 200° F. momentarily are effective in destroying a high percentage of the bacteria present in milk and cream. All common pathogenic (disease-producing) bacteria are killed by efficient and approved pasteurization. Certain bacteria and spores are heat resistant and are not killed even at boiling water temperature at 212° F. For the destruction of all bacteria and spores, it is necessary to apply a temperature, in the presence of moisture, of 250° F. This can be obtained by applying steam at a pressure of 15 pounds per square inch above the sea level atmospheric pressure.

Although pasteurization is effective in killing any disease-producing bacteria that may be present in milk or cream and also a large percentage of other organisms present, the process should not be used as a substitute for sanitation and proper care of milk and cream either on the farm or in the milk products plant. When once contaminated with bacteria, damage has been done to the quality of the milk or cream. Frequently heat resistant bacteria (thermoduric) are present in the milk or cream in considerable numbers and may survive the pasteurization process. In a commercial plant it was found that when 24,816 samples of milk were individually pasteurized at 143° F. for 30 minutes, 22 percent of the samples contained over 5,000 bacteria per cubic centimeter after pasteurization. The surviving bacteria were thermoduric. Under commercial conditions where market milk was pasteurized with a plate pasteurizer at 161° F. for 16 seconds, the counts of the pasteurized milk in 15 runs ranged from 14,000 to 56,000 per cubic centimeter and averaged 30,200. (Hileman, Leber, Spreck, New York.) The majority of the bacteria that survived the pasteurization process belonged to the genus Micrococcus. The most common species were: Micrococcus candidus, Micrococcus epidermidis, Micrococcus luteus and Micrococcus varians. They found that the micrococci made up the predominant heat resistant bacterial flora of dirty milking machines, strainers and pails on farms, and about one-half of the thermoduric organisms isolated from milk cans. The micrococci originate in the udder of the cows, in the soil, in the air, and on the skin of the cow. They grow in improperly-cleaned and not-sterilized utensils.

Certain heat resistant strains of coliform organisms may also be encountered. Long, Hedrick, and Hammer have reported their observations with reference to these organisms. Coliform organisms of the genus Escherichia isolated from cheese survived pasteurization at 143° F. for 30 minutes. Coliform organisms isolated from milk pasteurized in a milk plant at 144° F. for 30 minutes were kept at 37° F. for 13 months. One of these cultures after storage survived 143° F. for 30 minutes. Of 116 Escherichia cultures obtained from butter 63.8 percent survived 143° F. for 20 minutes, 41.4 percent survived 143° F. for 30 minutes, and 24.1 percent survived 143° F. for 40 minutes. Of 17 Aerobacter cultures, 15 did not survive 143° F. for less than 20 minutes. The two that survived 143° F. for 20 minutes did not survive 143° F. for 30 minutes. The suggestion was made in the report that "*Escherichia* cultures isolated from pasteurized dairy products should be tested for heat resistance before assuming that pasteurization was inadequate or that contamination had occurred."

Sterilization of equipment for bacteriological work is accomplished by subjecting the equipment to hot air maintained at a temperature of from 320° to 350° F. for at least one hour in an oven heated by gas or electricity.

Sterilization of dairy utensils and equipment can be accomplished by:

a. Exposure to steam, for at least 15 minutes at 170° F. or over, in a cabinet.

b. Exposure to steam for at least 5 minutes at 200° F. or over, in a cabinet.

c. Exposure to steam from a jet for at least 1 minute (inverted cans and pails only).

d. Immersion of the equipment in, or exposure to, a chlorine solution for at least 2 minutes. The solution should be made up to a strength of 100 parts per million of active chlorine. After sterilization the used solution must contain not less than 50 parts per million of active chlorine.

e. Immersion in hot water, at 170° F. or over, for at least 2 minutes.

f. Exposure to a flow of hot water, at 170° F. or more at the outlet, for at least 5 minutes.

g. Exposure to air at a temperature of not less than 180° F. for not less than 20 minutes in a hot air cabinet.

The methods listed for dairy utensils and equipment may not be effective in the destruction of every bacterial cell present. The term "bactericidal treatment" would be more correct than the term "sterilization," as sterilization means the destruction of all living organisms.

2. Low Temperature

If the food product, containing bacteria, is held at a temperature below freezing, there will be a gradual lowering in the viable organisms present. For example, if ice cream is kept at a temperature of from -10° to 0° F., the number of bacteria will gradually decrease. Bacteria in ice from polluted water may survive for a considerable period. Even disease-producing bacteria may survive for some time.

3. Absence of Food

Remove the food and the bacteria cannot grow. A 10-gallon milk can may appear to be clean inside, but a fine film of dry milk, invisible to the eye, may be present on the surface. In the presence of moist air, bacterial growth will take place in this milk film. This film may be caused by allowing the milk remnant left after emptying the can of milk to dry on the surface of the can before the can is washed. It may also be formed by rinsing the can with hot water soon after the milk was poured out. This causes a precipitation of some milk solids.

If the milk remnant is removed by rinsing the can with cold or lukewarm water and the can thoroughly washed, steamed and dried, bacteria will have no food and therefore cannot multiply.

4. Exposure to Chemical Agents

Chemical agents such as salt, acid, alkali, chlorine, and chlorine compounds can be used to destroy bacteria. Other chemical agents are being developed.

A weak brine may have a retarding effect on the growth of bacteria, but a strong brine (20 to 30 percent) generally stops growth. The bacterial cells become plasmolyzed.

Acid in milk, cream, or cheese may have a retarding effect on the growth of some types of bacteria. Others are influenced only slightly. Strong acid, such as sulphuric and hydrochloric, destroys bacteria.

The germicidal action of alkalies is taken advantage of in the washing of milk bottles. An alkalinity of the solution in which the bottles are soaked of not less than 2.4 percent, of which 1.6 percent is caustic soda, is effective in destroying the bacteria present. Keeping the cleaned milking machine, teat cup liners and tubes in a solution containing 0.4 to 0.5 percent caustic soda, between milkings, is a satisfactory bactericidal treatment. Lime is effective in killing bacteria in small crevices in wooden equipment such as churns, moulds, and wooden cheese rakes. The common commercial dairy cleaning compounds, when used in the proper proportion, also have some germicidal action.

Chlorine or chlorine compounds may be used for destruction of bacteria on the surface of dairy equipment and in water. These compounds are nonpoisonous. Remnants of milk, cream, whey, etc. must be thoroughly removed before sterilization; otherwise, the efficiency of the method is greatly reduced. After sterilization the chlorine rinse which drains from the equipment should contain not less than 50 parts per million (ppm) of active chlorine. Water, if chlorine-sterilized, must not contain an excessive amount of organic matter. In chlorine sterilization the bacteria are apparently destroyed as a result of the change in the protein of the bacterial cell by the chlorine. Hypochlorite will oxidize protein.

5. Light

Most types of bacteria are destroyed by sunlight. The ultraviolet rays have a germicidal effect on the bacterial cell. Artificially-produced ultraviolet rays can be used. The application of this method of bacterial destruction has limited application at present in dairy plants. If used, precaution should be taken to avoid exposure of milk or dairy products to the light, as it may cause oxidized flavor.

6. Electricity

Many experiments have been made with the view of "electrocuting" bacteria by means of electricity. As early as 1882, a patent for electric sterilization was granted. Electrodes were applied to many foods, including whole carcasses of animals and chunks of butter. In tests with the electric pasteurization of the milk, the destruction of the bacteria has been due to the high temperature produced.

In recent work, Sandorf, electrical engineer at the Nevada Engineering Experiment Station, studied the effects of high voltage on the bacteria in milk. He applied a moderately high voltage to suitable electrodes immersed in the milk in such a manner as to minimize the effects of high temperature and contamination.

When 30 shots—voltage 7,500, the container being shaken between shots—were applied to milk which contained 22,000 bacteria per cc., the count was reduced to 4,000 per cc.

When 300 shots—voltage 8,000, the container being shaken between shots—were applied to milk which contained 162,400 bacteria per cc., the count was reduced to 100,800 per cc.

The temperature rise of milk by 200 shots was 0.33° F. per shot.

It was concluded that: "High voltage, per se (by itself) is not sufficiently destructive of bacteria in milk to justify optimistic claims for its use in sterilization." And further that: "The magnitude of the reduction did not attain values ordinarily achieved by the usual pasteurizing processes."

MOLDS

It was mentioned at the beginning of this paper that the molds belong to the division of the Thallophyta plant group known as the Fungi. The molds are grouped into classes, orders, families and genera. Many different species of each genus have been isolated and classified.

A few molds are of importance in the dairy industry for the successful ripening of cheese (Roquefort, Gorgonzola, Stilton and Camembert are examples), but in general they are objectionable in dairy plants and in dairy products. Molds cause spoilage of cream, butter and cottage cheese. They discolor the surface of the products and cause a rancid, moldy flavor to develop. Molds also damage walls, ceilings, paper containers, clothes and shoes. They have caused tremendous losses in the dairy industry.

The whole plant structure of the mold fungus is known as the mycelium. The molds may be divided into two types on the basis of the structure of the mycelium (mycelium = singular, mycelia = plural). One type has its thread-like branches, known as hyphae, divided into small cells by means of cross walls termed septa. The other type has no cross walls in the branches. Most molds reproduce by means of spores. These are minute seed-like bodies produced in large numbers.

COMMON MOLDS ASSOCIATED WITH MILK AND MILK PRODUCTS

1. Genus Oospora. Species of this mold cause either a dry or slimy growth on the surface of sour milk, cream or cottage cheese. Oospora lactis grows best at a temperature near 80° F.

2. Genus Penicillium. A large number of species of this genus have been isolated. It is one of the common molds. It is the greenish-colored form that grows on cheese, on the surface of old cream, on fruit and on other food products. Common types are Penicillium roqueforti, Pencillium camemberti, Penicillium expansum, and Penicillium notatum. The last-named mold is used for the preparation of penicillin.

3. Genus Aspergillus. There are many species of this mold. They may be found growing on grain, silage, fruit, vegetables. The color produced by the different species may be yellow, yellow-green, orange, brown or black. Common species are Aspergillus niger (black), Aspergillus candidus (white-cream), Aspergillus flavus (yellow-green), Aspergillus glaucus (green). Species of Aspergillus glaucus and Aspergillus repens have been responsible for the production of "buttons" on the surface of sweetened condensed milk.

4. Genus Rhizopus nigricans. This mold has a root-like structure called rhizoids. From the rhizoids develop sporangiophores which hold the sporangia at their tips. The spores are contained in these sporangia. The mold which is gray-black grows on grain, feed, bread, vegetables and other foods.

5. Genus Cladosporium. To this genus belong species which produce grayish-black colored spots on vegetables, paper, wood, and on other products.

6. Genus Alternaria. This mold grows well on many different materials such as hay, leaves, vegetables.

SOURCES OF MOLD

1. On the farm. On feed such as hay and silage, manure, the cow's hair, flies, on moist utensils. Also in soil, water, the air.

2. Cheese factory surroundings. On driveways, receiving platforms including wall below, whey tanks, whey tank supports, and drains below whey tanks.

3. Inside the cheese factory. Receiving room--sample bottles and racks, walls and ceiling; manufacturing room--walls and ceiling, wooden vat frames, wooden presses, cloths, etc.; curing room--shelves, poorly-paraffined-cheese, old cut cheese, old bandage, damp starched circles.

Molds grow well on milk, cream, butter and cheese. They also grow well on foods that contain starch and sugar. The plants require air and moisture for their growth. Their optimum temperature ranges from 85° to 100° F., but they grow well at lower temperatures. Acid does not retard their growth.

CONTROL OF MOLDS

Ordinary pasteurization temperatures and holding periods will result in killing molds in milk and cream. It should be emphasized, however, that all particles of the milk and cream must be subjected to the pasteurization temperature during the required holding period. This includes the foam and also the milk or cream present in the outlet valve if this is not of the modern flush type. Keeping vat covers open during the holding period or allowing milk or cream to pass through a flash pasteurizer at several degrees below the required temperature may result in the survival of mold spores.

Dry heat is not as effective as moist heat in killing mold spores. If submitted to dry heat at 170° to 180° F., mold spores may not be destroyed even after several hours of exposure. A temperature of from 250° to 300° F. may be required for completely destroying mold spores by means of hot air.

Whereas mold growth is slow at low moisture contents and low temperatures, the spores are very resistant and may remain viable (capable of living under proper conditions) for long periods of time.

It can be stated that mold growth is retarded or stopped by:

1. Exposure to low temperature.

2. Light, particularly sunlight.

3. Absence of oxygen.

4. Low moisture content.

5. High salt concentration.

6. Chemical agents.

To control the growth of molds in a refrigerator room and in a storage room, maintain a low temperature and a low humidity. Ozone is not recommended. It causes surface oxidation resulting in a breakdown of the fat. (Jour. Dairy Science, Vol. XXVI, p. A225, 1943.) Also prevent moist air pockets in the corners of the room. In the cheese-curing room, turn the fresh cheese daily. Remove all moldy cheese from the curing room. Keep the shelves clean. Do not store supplies in the curing room. Mold-resistant paint may be used on walls and ceiling.

YEASTS

The third group of microorganisms is known as the yeasts. They are the Blastomycetes, a class of the Fungi which produce buds. When seen under the microscope, yeasts are single-cell plants that are either oval or cylindrical in shape. They reproduce by budding and by cell division. Spores or seed-like bodies are produced by the true yeasts. The yeasts that produce no spores are termed false yeasts, Torulae, pseudo yeasts and Mycoderma. Most species of yeast are considerably larger than bacteria.

COMMON SOURCES OF YEASTS

1. On the farm. On feed, such as on silage and other fermented feeds. On insects, fruit nectar and flowers, leaves of plants, in soil and manure, on utensils, on unclean strainer cloths, in milk and cream remnants.

2. Cheese factory surroundings. The milk receiving platform, the whey tank and whey tank surroundings.

3. In the cheese factory. On walls and floor, particularly where milk, cream or whey have remained for several days, in milk, cream or whey remnants, on wooden vat frames, on wooden cheese presses, particularly the lower part of the legs, on improperly-washed cloths, in pump bearings, in gutters and in drains.

Although yeasts have great value in certain industries, they are very undesirable in the dairy industry. (An exception is the Mycoderma which can be used with Lactobacillus bulgaricus to produce about 2 percent or over lactic acid in skim milk for concentrated sour skim milk.)

Species of the true yeast genus Saccharomyces, which includes the yeasts used in bread, wine and beer industries, ferment several sugars. Only one of the groups of these yeasts is able to ferment lactose.

Yeasts grow well at temperatures between 70° and 100° F. In sour cream kept at a temperature favorable for the growth of the yeasts, the lactose fermenters produce considerable carbon dioxide gas, often sufficient to cause foaming.

COMMON YEASTS IN MILK PRODUCTS

A lactose-fermenting yeast present in spoiled cans of sweetened condensed milk was isolated by Hammer. He named it Torula lactis-condensi. The yeast was oval in shape. Later Olson and Hammer isolated a spherical-shaped yeast from barreled sweetened condensed milk. They named it Torula globosa.

Hammer isolated two white-colored, lactose-fermenting yeasts from cream. He named them Torula cremoris and Torula sphaerica. The former is elliptical and the latter spherical-shaped.

Pink-colored yeasts are also present in milk, cream and whey, and in young cheese.

CONTROL OF YEASTS

The usual methods of pasteurization are effective in killing the common yeasts present in milk and cream, provided it is correctly done. As the common yeasts present in milk or cream form no spores, they are undoubtedly more easy to kill than species of sporeforming yeasts. It has been found that yeasts may remain viable in butter stored at a low temperature for a considerable period. Spores of true yeasts may survive in soil from one season to the next. Methods that are effective in destroying nonsporeforming bacteria are effective in destroying lactose-fermenting yeasts. Heat treatment, particularly, is effective.