Coliform Organisms in Dairy Products and Their Control

E. L. Fouts and T. R. Freeman

Dairy Products Laboratory, Florida Agricultural Experiment Station
Gainesville, Florida

There is a group of bacteria scientifically known as Escherichia-Aerobacter group and more commonly spoken of as the coli-aerogenes group or the colon group and in more recent years as coliform organisms. With the advent of inspection of milk and other dairy products by military agencies this group of organisms has assumed a significance never before attained in connection with their presence in dairy products. Escherichia and Aerobacter are both genus names and under each are listed several species. In order to clarify this division, the type species of each of the above genus will be discussed.

An organism was isolated from feces in 1886 by Escherich and it was later named Escherichia coli to honor the discoverer and to give some information regarding the organism. The organism is quite commonly present in the intestinal tract of man and most animals. Whether or not it ever occurs in nature without fecal contamination is an unsettled question. It will grow to some extent outside of the body, but it does not seem to be common in unpolluted soil and water. Its presence in water is universally regarded as being indicative of sewage pollution. Such water is considered unfit for human consumption, not because Escherichia coli itself possesses marked pathogenic powers but because its presence indicates sewage pollution and the possible presence of disease-producing organisms, such as the typhoid bacillus.

Aerobacter aerogenes also was first described by Escherich in 1885. Like Escherichia coli it is frequently found to be present in the intestines of man and animals. It differs from Escherichia coli, however, in that it is not uncommon in nature outside the intestinal tract. It is commonly found in soils and grains. It is not known to cause disease. It is frequently confused with Escherichia coli and unfortunately so since it does not have the sanitary significance of Escherichia coli. At the present time it seems to be generally accepted that members of the genus Escherichia are significant in indicating fecal contamination while the presence of the Aerobacter is of doubtful significance in this respect since its presence may or may not indicate fecal contamination.

Acute infections of the udder caused by the colon organisms have been observed which cause the milk to become abnormal in appearance. The milk may contain millions of colon organisms per cubic centimeter. The coliform organisms grow well in milk. Milk pails, milking machines, and other equipment which have not been properly washed and sterilized provide excellent places for them to grow. There seems to be a close relationship between the number of coliform organisms in milk and its keeping quality. Milk of a relatively low bacterial count may show poor keeping quality due to a high proportion of the bacteria present being of the coliform group.

Ropiness in milk and cream frequently is the cause of considerable loss in the dairy industry. In many instances this condition has been traced to coliform organisms, particularly *Aerobacter aerogenes*. Feeds are known to be the source of the organisms causing ropiness, and organisms of the *Aerobacter* group are almost always present in feeds.

Gasiness in cheese is a problem particularly in raw milk cheese. Organisms of the coliform type have been found to be the cause of this trouble in many instances and are responsible for certain off-flavors.

Organisms of the genus *Aerobacter* are practically always present in cream used for butter making. While these organisms are destroyed by pasteurization, recontamination is responsible for much defective butter. Experiments have shown that organisms of the *Aerobacter* type usually can be isolated from off-flavored butter. These same organisms put back into the cream before churning will cause the off-flavor to be reproduced.

In the manufacture of ice cream, the cream, milk, and butter used often contain large numbers of coliform organisms. While some few strains of these organisms resist pasteurization in ice cream mixes at 145° F. for 30 minutes, few can withstand the higher temperatures of 155°-160° F. commonly used for ice cream mix.

Improperly sterilized equipment often is a source of recontamination of mix after pasteurization. Fruits, nuts, colors, and extracts frequently are serious sources of recontamination unless special precautions are used. While it frequently is not done, it is desirable to treat all materials added to the mix after pasteurization in such a manner as to eliminate all danger of contamination from this source.

All dairy products in the raw or unpasteurized condition may show the presence of these organisms. Their numbers, however, should be small because good sanitary practices will keep the total number of bacteria at a low point. That these organisms normally are present even in high quality raw milk is testified to by the fact that the regulations governing the production of certified milk permit up to 10 coliform organisms per cubic centimeter in the raw product.

The sanitary significance of coliforms in milk and other dairy products depends almost entirely on whether the milk is raw or pasteurized. Most workers on this subject are in agreement that the presence of coliform organisms in water indicates sewage pollution and that such water is unfit for drinking. Experience and research has shown that the presence of coliform organisms in raw milk in most cases simply means that there may have been a certain amount of contamination with animal feces.

Certain workers have shown that the growth of these organisms in milk and on utensils makes for an incorrect interpretation of the results of the test for these organisms and that more often than otherwise the high coliform counts in raw milk are due to growth on utensils and in the milk rather than from direct manurial contamination. While the coliform test is not an acceptable sanitary index for raw milk, the conditions which are responsible for the presence of these organisms in fresh raw milk are undesirable.

Organisms of this type in pasteurized milk present an entirely different view of the picture. There seems to be some disagreement in the results of various workers as to whether these organisms survive pasteurization. Some operators who are looking for excuses have welcomed the information that in certain experiments a small percent of the coliform organisms survived ordinary pasteurization exposures. In well managed and operated plants coliform organisms are seldom recovered from 1 c.c. quantities of freshly pasteurized milk.

Most workers on this subject agree that the presence of coliform organisms
The presence of the organisms in bottled samples may be due to heat-resistant strains, faulty pasteurization, recontamination, or growth in the bottled milk. When a positive test is obtained on bottled milk, a line test should be made in the plant where the milk was bottled. The number of these organisms may vary greatly in bottled milk. In case of contaminated equipment, the first milk bottled may show much greater counts than the milk bottled later in the run.

If tests on the bottled milk show the presence of the organisms then the next step is to find where the organisms are coming from in order to eliminate them from subsequent runs of milk. Samples should be taken from pasteurizer at the end of the holding period and at several other places after the milk has passed over or through the various pieces of equipment after pasteurization. With the results of these tests it usually is possible to detect the cause of the trouble and eliminate it. Naturally, the small plant without laboratory facilities cannot make these tests and such plants must rely on extra effort in washing and sanitizing their equipment when a sample of their bottled milk has been found to be positive to the coliform test.

From the public health standpoint it is assumed when freshly pasteurized milk is positive to the coliform test that it was either improperly pasteurized or that it may have been recontaminated by careless handling after pasteurization.

The washing and sterilizing processes are of utmost importance. Thorough washing with hot water containing a good washing powder, using suitable brushes, followed by a good rinse prepares the equipment for sterilization. The sterilization process may be accomplished by any approved method and should be done just before the milk is put into the system. Unfortunately, the most careful attention to sanitation and sterilization occasionally fails to produce milk which will react negatively to the coliform test. In some instances carelessness is the chief cause but more frequently a lack of knowledge by the operator or the use of faulty or worn equipment is responsible for the presence of the organisms in pasteurized milk. Pasteurizing vats, valves, pumps, coolers, pipe lines, and bottle-filler valves frequently are found to be the source of the contaminating organisms. In one instance a small pocket on the underside of a surface cooler due to faulty construction was found to be contributing large numbers of coliform organisms to otherwise properly pasteurized milk. A line test had traced the contamination to the cooler, and a careful examination of the cooler revealed the focal point of contamination. Repairs were made and the trouble disappeared.

Old style milk pumps often are sources of contamination and even the newer pumps must be properly cared for or they may cause trouble. Dust from a field where livestock is grazing may carry the organisms into the milk by way of an uncovered cooler. Draining the rinse water from the bottle valves with the hand just before bottling may contaminate an otherwise well sterilized bottle filler. A clean sterile bottle should be used for this purpose. When bottles are put on the machine by hand care should be taken that the hand does not touch the top of the bottle.

These are only a few of the many places to look when trouble occurs. Each plant will have slightly different problems depending on the equipment used. After every precaution has been taken for the proper handling of milk and trouble still persists, a thorough study should be made of the individual problem. A very small pocket of contamination can cause much trouble. Usually it can be located and remedied
if it is realized that so great a trouble can be caused by a small source of infection.

If the reader desires to learn more about the significance of this group of organisms or to learn the methods used to examine milk and other dairy products for their presence he is referred to the following book: Standard Methods for the Examination of Dairy Products, 8th Edition, published and sold by the American Public Health Association, 1790 Broadway, New York, New York.

The charts accompanying this article illustrate in a diagrammatic way the methods used in the laboratory to demonstrate the presence of coliform organisms in milk, cream, and certain other dairy products.

Figure 1 shows the steps necessary when the presumptive test is performed in such a way as to determine the probable number of organisms per 100 cubic centimeters of milk. In this test either of two methods may be used as illustrated. In one method a liquid medium (broth) is used and gas formation (10 percent or more) indicates a positive presumptive test. In the second method the milk is plated on a solid differential medium. The plates are incubated and if typical colonies are observed a positive presumptive coliform test is indicated.

Figure 2 shows the steps necessary to complete the test for coliform organisms in these dairy products. If the liquid medium was used in the presumptive test then the test is completed by following the steps shown on the upper part of the chart. If solid medium was used in the presumptive test then the steps shown on the lower part of the chart are followed.

Perhaps it should be explained that in routine control work in the milk plant usually only 1 to 3 tubes are used and rarely is it necessary to complete the test for coliform organisms as illustrated in Figure 2. The technic outlined however is one that should be understood by every worker in a milk control laboratory.
Figure 1.

PRESUMPTIVE TEST FOR COLIFORM ORGANISMS IN MILK, CREAM, ETC.

**Inoculation Procedure**

1. Sample
2. 1 cc inoculation
3. Incubate 18 hrs. at 37° C.
4. Brilliant green broth or Formate ricinoleate broth

**Positive Test**

1. Incubate 48 hrs. at 37° C.
2. 10 % or more of gas

**OR**

1. Incubate 20 - 24 hrs. at 37° C.
2. Violet red bile agar or Desoxycholate agar

**NOTE:** If no gas appears in fermentation tubes, or no dark red colonies are formed on agar plates, presumptive test is considered negative.

**Dark red colonies at least 0.5 mm. in diameter.**
Figure 2.

**COMPLETED TEST FOR COLIFORM ORGANISMS IN MILK, CREAM, ETC.**

**Inoculation Procedure.**

1. **Positive tube from presumptive test.**
   - Streak on eosin methylene blue or Endo's agar.
2. **Select discrete, typical colonies.**
   - Incubate 18-24 hrs. at 37°C.
   - Lactose broth.
3. **Nutrient agar slant.**
   - Incubate 28 hrs. at 37°C.
   - Examine growth under microscope.
   - Gram-negative, non-spore-forming, rod-shaped bacteria (so spore-forming bacilli).
4. **Fish one colony.**
   - Incubate at 37°C.
   - Lactose broth.
   - As soon as gas formation occurs, carry out entire procedure outlined above. If no gas forms in 48 hrs., colony is considered to contain no coliform organisms.

**Positive Completed Test**

**Production of gas**