Bacteria of the Escherichia-Aerobacter Group in Dairy Products[1]

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INTRODUCTION

THE Escherichia-Aerobacter group of bacteria, more commonly known as the colon group, is widely distributed in nature. While ordinarily saprophytic, its members may under certain circumstances invade plant and animal tissues. The importance of these organisms from the standpoint of disease, their value as an indicator of polluted water and shellfish supplies and their importance in dairy and food products both as the cause of defective conditions and as a measure of sanitary quality have served to make the group the object of extensive research.

Methods of detecting the colon group in water have frequently been applied to dairy products without recognition of the fact that methods suitable for water analysis are not necessarily suitable for the analysis of dairy products. Also, many workers have placed the same meaning on the presence of the colon group in milk as in water. The significance of the colon group in dairy products is entirely different from that in water and, furthermore, varies according to the kind of product.

HISTORICAL

The literature on the colon group is undoubtedly larger than that on any other group of organisms. While there are a number of good summaries covering this group as a whole such as that by Wilson (1), reviews of the literature are inadequate; and workers are handicapped by lack of knowledge of previous work. This may be, in part, responsible for the present confusion of ideas in regard to the group.

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The best reviews in this country cover applications to water bacteriology, such as Levine (2) and Prescott and Winslow (3), and are valuable for general information. Some idea of the extensive amount of work that has been done may be gained from the fact that the latter authors cite over 500 references to the group.

While a complete review of the literature with respect to the group as found in dairy products is desirable, it is not possible in this article. Many important references must necessarily be omitted.

TAXONOMY

The Escherichia-Aerobacter group is commonly referred to either as the colon group or as the coli-aerogenes group except in England, where the term coliform is used. Since the term Escherichia-Aerobacter group is lengthy and awkward to use continually, the term colon group will be used as a synonym. The colon group is a member of the larger colon-typhoid group which Bergey (4) divides into nine genera based on fine distinctions in respect to the fermentation of carbohydrates. Between clearly defined members of these genera are borderline types which are difficult to classify satisfactorily according to Bergey's Manual.

Because of the close relation between all of the organisms in the colon-typhoid group, some workers—among whom may be mentioned Orla-Jensen (5) and Winslow et al. (6)—feel that all of the gram-negative, nonsporeforming, nonchromogenic rods which grow aerobically should be placed in a single genus. The generic term Bacterium has been widely used but is really invalid on the ground that the original definition of the group and of the type species was inadequate, Buchanan (7). Breed and Conn (8) have pointed out that "many difficulties in our present classifications would disappear if Bacterium is retained as a temporary genus without a designated type species other than the historic but non-recognizable *Bacterium triloculare* to include all species of nonsporeforming rods that cannot be placed at once in well recognized and reasonably well defined genera." This would give justification to those who wish to be conservative and retain such names as *Bacterium coli, Bacterium prodigiosum*, etc.

Several other genera made up of gram-negative nonsporeforming rods which are capable of growing aerobically are very closely related to the colon group and differ chiefly in respect to their chromogenesis (Serratia, Flavobacterium) or in respect to a mucoid encapsulated growth (Klebsiella). Some of the species placed by Bergey in the genus Achromobacter ferment lactose with gas production and correspond closely to descriptions of organisms in the colon group. A number of the plant pathogens listed in the genus Erwinia are probably strains of colon organisms.

In water analysis the value of generic differentiation (Escherichia from Aerobacter) is a much discussed point. At the present time it seems to be generally accepted that members of the genus Escherichia are sig-

nificant in indicating fecal pollution, while the presence of the Aerobacter section is of doubtful significance in this respect, as it indicates fecal pollution under certain conditions only.

In the case of dairy products, generic differentiation is of no practical value where the test is used as an index to sanitary conditions, since both sections are added to milk in about equal numbers at the time of production and since growth of either section may take place on utensils. In establishing the nature of organisms responsible for defects in milk such as ropiness, generic differentiation is important, since practically all defective conditions are due to the members of the genus Aerobacter.

Number of species. The number of species in the Escherichia-Aerobacter group is a much debated question and many different classifications have been proposed. The number of possible species based on a system of classification depends upon the number of key characters used and may be determined by the formula 2^n where "n" is the number of characters studied, Levine (2). Thus, the possible number of species in the genus Escherichia according to Bergey's scheme with 7 characters is 128 species in contrast with 16 species according to Levine's scheme (2) which would use only 4 key characters.

Bergey lists 22 species in the genus Escherichia and 7 in the genus Aerobacter, whereas Levine lists 5 species in the genus Escherichia and only 2 in the genus Aerobacter. Since there appears to be no practical value in drawing fine distinctions between closely related strains, the simple classification of Levine is more practicable and therefore more preferable to the complex scheme used by Bergey.

Species in dairy products. The species belonging to the genus Escherichia which are most common in dairy products according to Yale (9) are *Escherichia coli, E. pseudocoloides* and *E. communior,* based on Bergey's scheme of classification. If we accept Levine's classification, *E. pseudocoloides* might easily be regarded as an atypical strain of *E. communior* differing chiefly in its failure to ferment dulcitol. Other species have been reported by various workers but seem to be present in fewer numbers than the above.

In respect to the genus Aerobacter in dairy products, Yale (9) found *Aerobacter aerogenes, A. oxytocum* and *A. cloacae* to be the only species present.

The intermediates occupying a position between the genus Escherichia and the genus Aerobacter and comprising organisms with a positive methyl red test reaction but able to utilize citrate as the sole source of carbon are also found in dairy products, usually comprising between 10 and 35 percent of the total number of cultures in the entire colon group. While many workers, including the author, feel that these organisms are more closely related to the genus Escherichia than to the genus Aerobacter, Pont (10) found that the intermediate forms showed a marked simi-

larity to *A. aerogenes* in cream and brought about a marked and rapid deterioration in quality accompanied generally by ropiness.

METHODS OF EXAMINATION OF DAIRY PRODUCTS

The methods of detection and enumeration of colon organisms in dairy products which are used at the present time were first developed for water analysis and if suitable for dairy products are so by chance rather than by design.

Early in the history of water analysis, when water supplies were mostly untreated, it was found that the use of plain lactose broth sometimes gave spurious results primarily because of the presence of lactose-fermenting, sporeforming organisms. With the gradual development of chlorination, the use of lactose broth gave proportionately more false tests due to the presence in relatively greater numbers of lactose-fermenting sporeformers.

Water bacteriologists have devoted much attention to the development of selective liquid media which would restrain the growth of false test organisms. These media mostly contain various combinations of bile salts and dyes to inhibit the growth of gram-positive and sporeforming bacteria. As might be expected, certain strains of organisms in the colon group are sometimes inhibited. In a search for a perfect medium, one fluid medium after another has been developed. Whether or not much of this work is justifiable is becoming more and more questionable since there seems to be so little difference between many of these media that the differences in results obtained by various investigators appear to be due to experimental errors rather than to differences produced by the action of the media. Also, it is not always evident that it is worth-while to devote the time and energy necessary to establish the presence of a relatively small percentage of weaker colon organisms. As Prescott and Winslow (3) aptly state, "It is disheartening to the believer in human common sense to see investigator after investigator demonstrate the value of a differential procedure and then discard it because it inhibits 5 percent of the colon bacilli discovered by a lactose broth confirmed test." These remarks are just as applicable to media for examination of dairy products as for water analysis.

Standard Methods of Water Analysis (11) at the present time specify that plain lactose broth shall be used in water analysis for primary inoculation and in the event of gas production may be confirmed by the use of a number of different selective media. This procedure is unsatisfactory in milk analysis since the predominance of streptococci in a milk sample frequently results in the production of an amount of acid sufficient to inhibit the growth of organisms of the colon group before there is visible gas formation when plain lactose broth is used.

The selective liquid media which are used most frequently in this country for dairy products are gentian-violet-lactose-peptone-bile, Kessler and Swenarton (12); brilliant-green-lactose-peptone-bile, 2 percent recommended by Standard Methods of Milk Analysis (13); and the more recent formate-ricinoleate broth developed by Stark and England (14). McCrady and Archambault (15) found that brilliant-green-bile yielded a somewhat larger number of positive presumptives than did gentian-violet-lactose-bile. In another series using only brilliant-green-bile, 86 percent of the tubes which showed at least 10 percent gas in 48 hours completely confirmed in the case of pasteurized milk and 99 percent in the case of raw milk. Where less than 10 percent of gas was produced in 48 hours, 45 percent of the tubes confirmed in the case of pasteurized milk and 92 percent in the case of raw milk.

It is significant to note that the number of confirmations was increased by 11.5 percent by fishing a second colony from the eosin-methylene-blue plate. It is possible that failure to completely confirm all tests where gas was produced was caused by the failure to recover organisms of the colon group since they were overgrown by other types or died after gas was formed. It by no means follows that gas production is due to false test organisms where results cannot be compeltely confirmed. This is a point that many workers have overlooked.

On the other hand, Stark and Curtis (16) believe that when 0.1 cc. or 1.0 cc. quantities of milk are employed, false positive tests sometimes take place in the brilliant-green medium. Working with pure cultures of lactose-fermenting sporeformers, and also with organisms capable of producing spurious results due to "symbiosis," they found that the inhibiting effect of brilliant-green was reduced by the addition of milk, especially by 1 cc. quantities, to such an extent that growth of these false test organisms took place in many instances.

These objections to brilliant-green-bile, especially for the examination of pasteurized milk, are not strongly supported by work in the field and appear to be more theoretical than practical. This appears to be due to the fact that lactose-fermenting sporeformers are so infrequent in milk and that the conditions necessary for false tests due to symbiosis occur but rarely.

Stark and England (14) found that formate-ricinoleate broth inhibited the growth of false test organisms and that addition of protein material did not materially change the surface tension of the medium. Bacteria belonging to the Escherichia-Aerobacter and Salmonella groups were able to produce gas from formic acid in the absence of any other fermentable substance, and this markedly increased the total amount of gas produced.

Minkin and Burgwald (17) found that yeasts are sometimes present in 1 cc. quantities of pasteurized milk and may grow in fluid media used to

determine the presence of the colon group. Yeasts are easily destroyed by pasteurization and, in the case of growth on equipment, are probably outnumbered by members of the colon group. Even if false tests should result from the growth of lactose-fermenting yeasts, they would be just as significant in revealing recontamination of pasteurized milk as are members of the colon group. The same statement holds true for certain strains of glucose-fermenting bacteria which may produce false tests in the formate-ricinoleate medium due to fermentation of the formate or which may cause spurious results in brilliant-green due to symbiotic action between organisms.

To sum up the situation with respect to liquid media, it would seem that both brilliant-green-bile broth and formate-ricinoleate broth are valuable for the examination of milk if used with an understanding of their real value and of their limitations.

Solid media. In order to secure reasonably accurate quantitative results where the number of colon organisms present may vary over a considerable range it is necessary to use several tubes of liquid media in each of three dilutions so selected that all tubes are positive in the lowest dilution and negative in the highest dilution. From the results obtained the most probable number of organisms can be derived from tables as given in Standard Methods (13).

McCrady and Archambault (15), having compared the relative precision of results of tubes and plates, observe that "one plate count of say 8 colonies, is a result quite as reliable as that obtained from 10 fermentation tubes." The chief difficulty involved in the use of a solid medium is to find one sufficiently selective and, at the same time, one which will permit plating of at least 1 cc. quantities of milk. Inasmuch as a plating medium containing lactose normally measures acid-forming rather than gas-forming organisms, it is not anticipated that a plating medium will be found which will be a perfect index to gas-forming organisms (colon group).

Unpublished data collected during the past two years by the author have shown that several plating media developed by two different agencies are sufficiently selective to permit plating of 1 cc. quantities of pasteurized milk when a special technic is used. Under these conditions the majority of the large red colonies which develop in 24 hours belong to the colon group.

A plating medium has advantages and disadvantages as compared with a liquid medium. Conditions under which advantages more than offset disadvantages are being studied at the present time by the author. Under practical conditions the degree to which accurate results are desired is a major factor in the selection of the method to be used. Also, from a research standpoint, it is quite evident that plating methods offer a much more accurate means of studying the relative numbers of different types of lactose-fermenting organisms than any enrichment scheme.

SANITARY SIGNIFICANCE IN RAW MILK

For many years it has been the popular opinion that the presence of *Escherichia coli* in milk indicated pollution with cow manure. Conn (18), who pioneered in dairy bacteriology in this country and made many important contributions, states as follows: "Thus, it follows that, although the presence of *B. coli* may render water unsafe, because it suggests sewage contamination, its presence in milk does not render the milk unsafe, but merely indicates that there may have been a certain amount of contamination with animal feces."

Ayers and Clemmer (19) in a classic piece of work showed convincingly that growth of the colon group in milk or on the surface of utensils makes a correct interpretation of results impossible as to where growth may have taken place. More recently Sherman and Wing (20) and others have confirmed this viewpoint and added further information.

Yale and Eglinton (21) concluded that even in fresh raw milk which has not been held longer than three to four hours following production the colon test has only a slight value when used as a routine test because: (1) Much time and energy are required to make the observations necessary to determine the true significance of high colon counts; (2) the test is misleading, since similar sanitary conditions sometimes result in low colon counts and at other times in high colon counts; (3) while colon infections of the udder are uncommon, samples taken during the early stages of the infection may show very high colon counts; and (4) high colon counts are usually not due to manurial contamination but to growth on the utensils and even in milk.

While not a reliable sanitary index, the conditions which account for the presence of these organisms in fresh raw milk are all undesirable.

SANITARY SIGNIFICANCE IN PASTEURIZED MILK

The significance of the colon group in pasteurized milk is entirely different from that in raw milk. The question whether or not these organisms survive pasteurization has been the subject of much study. While opinions on this point seem to be conflicting, they are more in agreement than many workers realize. Thus, Ayers and Johnson (22) in a classical study on the ability of colon bacilli to survive pasteurization found in test tube experiments that at 60° C. (140° F.) 95 cultures out of 174, or 54.6 percent, survived, while at 62.8° C. (145° F.) 12 cultures, or 6.9 percent, survived. The time of heating was 30 minutes.

Under present commercial conditions, colon organisms are seldom recovered from 1 cc. quantities of freshly pasteurized milk. This does not conflict with the results obtained by Ayers and Johnson since they showed that colon bacilli survived pasteurization on account of the resistance of a few cells (low majority thermal death point). They worked with much larger numbers than occur in commercial supplies, where occasionally

colon organisms are absent from 1 cc. quantities of raw milk previous to pasteurization. The recent work of Henneberg and Wendt (23) in Germany supports this viewpoint. They found that heat-resistant strains (63° C. for 24-30 minutes) were not common, and they failed to recover them in 1 cc. quantities of commercially pasteurized milk. However, an examination of 50 cc. to 100 cc. quantities of milk showed the presence of heat-resistant strains in 2 out of 30 samples.

The presence of colon organisms in bottled samples normally indicates recontamination following pasteurization, McCrady and Langevin (24), Slack and Maddeford (39) and Sherman (25). McCrady and Langevin have shown that frequently recontamination may be detected by the colon test in cases where the standard agar plate count is not sufficiently sensitive. This was also observed to be the case by Chilson, Yale and Eglinton (26).

Process samples yield more information than do street samples of bottled milk in which growth may have taken place. It is desirable that positive results from street samples be followed by the taking of process samples. The presence of colon organisms in street samples may be due to any one or to a combination of the following conditions: (1) Heat-resistance; (2) faulty pasteurization; (3) recontamination; (4) growth in the bottled milk.

The number of colon organisms may vary greatly in the bottled milk. In the case of contaminated equipment the first milk bottled may show the presence of considerable numbers of colon organisms, since the organisms may be rinsed from the equipment to such an extent that they are absent from 1 cc. quantities of milk bottled later in the run. Thus, conclusions as to the reason for positive tests should not be formed from tests of street samples until a series of samples has been examined.

DEFECTS IN MILK AND CREAM

In many cases the presence of colon organisms in dairy products results in the production of defects which cause a great economic loss to the industry.

Mastitis. Acute colon infections of the udder are not as rare as many investigators suppose, and there are numerous references to the subjects in the literature. Hardenbergh and Schlotthauer (27) have reviewed this problem and also report observations of their own. Recently Smith and Henderson (28) have reported a case which has interest in that analyses of the milk were made before, during and following the infection.

Soon after an acute colon infection takes place, the milk becomes so clearly abnormal in appearance that it should be easily detected and kept out of the supply. Shortly prior to this, the milk may contain millions of colon organisms per cc., Yale and Eglington (21). The inclusion of this milk in a city supply may have no direct public health significance due to

the dilution factor; but, if used undiluted or diluted only slightly, it may conceivably produce intestinal upsets or other disorders and is objectionable on other grounds also.

Flavor and keeping quality. Organisms of the colon group may produce a flavor in milk and cream termed "cowy," Pont (10), or "feedy," Sadler and Irvin (29). The English workers, Barkworth (30) and Hoy and Newland (31), feel that there is a correlation between the number of colon organisms and keeping quality. Hoy and Newland showed that milk of a low bacterial count may have a very poor keeping quality, which is connected with the presence of a high proportion of organisms of the colon group.

Ropiness. Considerable economic loss is caused in the industry each year through outbreaks of ropy milk and cream. In many cases this ropy condition is due to members of the Aerobacter section, principally *Aerobacter aerogenes* and *A. oxytocum,* as shown by Yale (9). More rarely, ropiness may be due to *Aerobacter cloacae* or to *Escherichia neapolitana,* as found by Sarles and Hammer (33). Stark and Foter (34) have shown the importance of feeds as a source of ropy milk organisms.

In the case of pasteurized milk and cream ropiness is usually caused by contamination rather than by survival of these organisms during the heat treatment. Kelly (35) suggests ways for preventing and controlling these outbreaks.

Cheese. Gassiness in cheese is a major problem which is as old as the cheese industry and was studied prior to 1900 by a number of investigators. Organisms of the colon group are the offenders in many instances. In addition to delaying the action of starters, gas and other by-products are produced which result in poor quality cheese.

Cheesemakers have had limited success in overcoming gassiness by control with "starters" or by the addition of chemicals such as potassium nitrate. In this instance an ounce of prevention is worth a pound of cure. Harrison (36) found that the addition of potassium nitrate (saltpeter) to colored cheese produced discoloration and recommended that the use of nitrates be discontinued.

Ice cream. Whether a routine test for organisms of the colon group in ice cream would be worthwhile in giving information concerning sanitary quality not afforded by the total count is uncertain. Cream and other materials used in the preparation of the mix may contain colon organisms in large numbers. Fabian and Coulter (37) found that in ice cream mix many strains survive a temperature of 62.8° C. for 30 minutes, while a few strains survived 65.5° C. Although ice cream has a greater protective action than skim milk, under commercial conditions this may have little significance since ice cream mixes are usually pasteurized at higher temperatures than milk. Recontamination with colon organisms may also result from addition of contaminated flavor extracts and fruits or

from contact with contaminated equipment such as pipe lines, freezers and containers.

Butter. For many years it has been thought that organisms of the colon group were sometimes responsible, in part at least, for defective butter. Very little has been published on the subject. At the Iowa Agricultural Experiment Station organisms belonging to the colon group have frequently been isolated from butter showing flavor defects. Hammer and Yale (38) identified 25 cultures from 17 samples of off-flavored butter and found that 60 percent were *Aerobacter aerogenes,* 12 percent *A. cloacae* and 16 percent *A. oxytocum,* while 12 percent belonged to the intermediate group. This indicated that organisms belonging to the genus Aerobacter were more important than organisms of the genus Escherichia in causing flavor defects. This opinion was confirmed when portions of cream were inoculated with pure cultures representing several species in each group and then churned. When the butter was held for 10 days at 7° C. and also at 18° C., species belonging to the genus Escherichia did not develop off flavors. On the other hand, members of the genus Aerobacter grew more rapidly than those of the genus Escherichia and regularly developed unclean odors and flavors in the salted and unsalted butter held at 18° C. No defects occurred in the case of the salted butter held at 7° C., but off flavors sometimes were apparent with the unsalted butter.

It should be pointed out that organisms belonging to the genus Aerobacter are practically always present in cream supplies used for buttermaking. When present in large numbers they are deleterious to cream quality. These organisms are practically always destroyed by pasteurization. Recontamination is undoubtedly responsible in many cases for the presence of colon organisms in butter.

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