SPECIES OF ESCHERICHIA-AEROBACTER ORGANISMS RESPONSIBLE FOR SOME DEFECTS IN DAIRY PRODUCTS[1]

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The bacteria of the Escherichia-Aerobacter group have long been recognized as responsible for various defects in dairy products, but comparatively little attention has been given to the identification, on a species basis, of those forms isolated. While there is no immediate prospect that such identification will supply information of importance in connection with the control of the defects, work along this line appears advisable in order to show whether few or many species are involved in the production of abnormalities in milk and its derivatives. The proposals to use the numbers of Escherichia-Aerobacter organisms in pasteurized milk as a means of controlling pasteurizing operations also suggest the advisability of knowing something of the species of this group that may be present in dairy products.

A number of defects in milk and cream, due to organisms of the Escherichia-Aerobacter group, have been studied recently at the Iowa Agricultural Experiment Station and the results of the identification studies are reported herein.

METHODS

Pure cultures of the organisms were isolated from defective milk or cream, or from normal milk from the supply in which the defect was being encountered, by one or more of several methods which may be classified as follows:

I. Direct plating of quantitative dilutions on
   a. Standard agar (for milk analysis).

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b. Eosin methylene-blue agar (Levine, 1921).
After incubation at 37°C., cultures were isolated and their purity checked.

II. Enrichment methods.
a. Quantitative dilutions of the sample were inoculated into Durham tubes of Kessler and Swenarton's (1927) gentian-violet lactose peptone bile medium (prepared from the dehydrated product) and incubated at 37°C.
1. After twenty-four hours incubation, material from the lowest and highest dilutions in which gas had been produced was streaked on eosin methylene-blue agar plates.
2. If, after forty-eight hours incubation, gas was present in a higher dilution than after twenty-four hours incubation, material from this tube was streaked on an eosin methylene-blue agar plate.
Cultures were isolated as under I.
b. Enrichment by growing in milk at various temperatures.
1. Several tubes of litmus milk were inoculated with a small quantity of the sample and the inoculated milk incubated at 7° to 10°, 15°, 21°, 32°, and 37°C. As soon as the inoculated milk showed the original defect it was plated in dilution on eosin methylene-blue agar and on standard agar.
2. If the sample was normal when received, it was distributed equally in several sterile, cotton-stoppered, six-ounce bottles and incubated at 7° to 10°, 15°, 21°, 32° and 37°C. As soon as any of the milk showed the defect it was plated in dilution on eosin methylene-blue agar and on standard agar.
Cultures were isolated as under I.

It should be emphasized that enrichment methods may not be equally favorable for all Escherichia-Aerobacter organisms and that, as a result, when more than one species is present their quantitative relationships may be changed.

The cultures isolated were identified according to a modification by Yale (1931) of Bergey's (1930) classification of the genus Escherichia and the genus Aerobacter.

DESCRIPTIONS OF SAMPLES STUDIED

Sample 1 was normal raw milk from a central Iowa supply in which ropiness was being encountered; the standard plate count was 130,000 per milliliter. The milk kept well at 7° to 10°C., but became ropy and developed an unclean, slightly acid odor on standing for a short time at temperatures of 15° to 30°C.

Sample 2 was sour raw cream that was ropy when shipped to

the laboratory from a creamery in northern Iowa, but which lost its ropiness during transit.

Sample 3a was ropy pasteurized milk from a plant in northwestern Iowa. The milk was normal when delivered to a consumer but became ropy on holding in a cellar for forty-eight hours.

Sample 3b was normal pasteurized milk from the plant supplying sample 3a.

Sample 4 was raw milk from a central Iowa supply. It was submitted because a few consumers of the supply complained that the milk seemed to be full of gas. Following its return by a consumer, the sample had been kept in a refrigerator for twenty-four hours before it was brought to the laboratory. When received, the sample appeared normal, but on shaking or warming it foamed markedly. The standard plate count was 590,000,000 per milliliter, and the count on eosin methylene-blue agar (forty-eight-hour incubation at 37°C.) was 510,000,000 per milliliter.

Cottage cheese is made almost daily at the Iowa State College dairy plant from skim milk that has been pasteurized at 145°F. for thirty minutes. As a rule, 1200 pounds of this milk are warmed to about 32°C. in a cheese vat and inoculated with 100 pounds of butter culture. The inoculated milk is kept at about 32°C. until sufficient acid has developed to allow completion of the cheesemaking process.

On October 8, 1931, milk set for cottage cheese developed considerable gas. Sample 5a was taken from the culture used and sample 5b from the gassy milk.

On October 15, 1931, gas formation in milk set for cottage cheese was again encountered. Sample 6a was taken from the culture used and sample 6b from the gassy milk.

On February 18, 1932, milk set for cottage cheese developed acid very slowly (about fourteen hours being required to reach an acidity of 0.4 per cent), and an unclean odor like that produced by some of the Escherichia-Aerobacter organisms appeared. Sample 7 was taken from the milk. The culture used seemed to be satisfactory.

On February 25, 1932, a slow acid development and an unclean

odor were again encountered in milk set for cottage cheese. Sample 8 was taken from the milk. The culture used seemed to be satisfactory.

RESULTS OBTAINED AND COMMENT

The species of Escherichia-Aerobacter organisms isolated from abnormal dairy products in which the defects seemed to be due to organisms of this group, are shown in table 1.

The ropiness which developed in sample 1 on holding in the laboratory was due to *E. neapolitana*, and no other Escherichia-Aerobacter species was found. The culture isolated produced a pronounced ropiness in inoculated milk. Sadler and Middlemass (1926) described a case of ropy milk caused by an atypical *E. neapolitana*.

Sample 2 yielded ropy *A. aerogenes* cultures but no other Escherichia-Aerobacter species. *A. aerogenes* appears to be rather commonly the cause of ropiness in dairy products as is evident from the studies of Buchanan and Hammer (1915), Hammer (1916), Sadler (1917), Mounce (1923) and Yale (1931).

Samples 3a and 3b both contained a variety of Escherichia-Aerobacter species, but only a portion of the isolated cultures produced ropiness in milk. The ropy cultures secured from sample 3a were either *A. aerogenes* or *A. cloacae* while those from sample 3b were *A. cloacae*. Both samples contained *E. pseudocoloides*, which Yale (1931) found to be the most commonly occurring species of the genus Escherichia in pasteurized milk. Yale (1931) also reported that *A. cloacae* was the only species of the genus Aerobacter that he found in pasteurized milk. The variety of species of the Escherichia-Aerobacter group found in the samples suggests rather extensive contamination of the milk.

All of the cultures isolated from sample 4 were *A. oxytocum*. This species appears to be rather common in dairy products. The producer who submitted the sample was told to clean and sterilize his milking machine and other utensils thoroughly. On the day following the clean-up the milk gave a standard plate count of 7000 per milliliter; no colonies were visible on eosin methylene-blue agar plates poured with 0.01 ml. of the milk.

TABLE 1

Species of Escherichia-Aerobacter organisms isolated from samples of defective dairy products

SAMPLE	PRODUCT	DATE RECEIVED	DEFECT	METHODS USED FOR ISOLATIONS	SPECIES ISOLATED
1	Raw milk	6/23/31	Normal when received; became ropy on standing	IIb1; IIb2	*E. neapolitana**
2	Raw cream	8/11/31	Ropy	Ia; IIb1	*A. aerogenes**
3a	Pasteurized milk	10/28/31	Ropy	Ib; IIa1; IIa2; IIb1	*A. aerogenes**
					*A. cloacae**
					A. oxytocum
					E. grünthali
					E. pseudocoloides
3b	Pasteurized milk	10/28/31	Normal when received; became ropy on standing	Ia; Ib; IIa1; IIa2; IIb1; IIb2	*A. cloacae**
					E. leporis
					E. anindolica
					E. gastrica
					E. astheniae
					E. pseudocoloides
4	Raw milk	9/22/31	Gassy	Ia; Ib; IIa1; IIa2	*A. oxytocum**
5a	Culture for cottage cheese	10/ 8/31	Gassy	Ia; Ib; IIa1; IIa2	*E. coli**
					*E. anindolica**
5b	Milk for cottage cheese	10/ 8/31	Gassy	Ia; Ib; IIa1; IIa2	*E. coli**
					*E. anindolica**
6a	Culture for cottage cheese	10/15/31	Gassy	Ia; Ib; IIa1; IIa2	*A. aerogenes*
					A. oxytocum
					E. coli
					E. grünthali
6b	Milk for cottage cheese	10/15/31	Gassy	Ia; Ib; IIa1; IIa2	*A. aerogenes*
					A. oxytocum
					E. coli
					E. grünthali

TABLE 1—*Concluded*

SAMPLE	PRODUCT	DATE RECEIVED	DEFECT	METHODS USED FOR ISOLATIONS	SPECIES ISOLATED
7	Milk for cottage cheese	2/18/32	Slow acid development; unclean odor	Ia; Ib	*A. aerogenes* *A. oxytocum* *E. paragrünthali*
8	Milk for cottage cheese	2/25/32	Slow acid development; unclean odor	Ia; Ib; IIa1; IIa2; IIb1	*A. aerogenes* *E. coli* *E. paragrünthali*

* Definitely responsible for original defect of sample.

Samples 5a and 5b each yielded two Escherichia species, *E. coli* and *E. anindolica*, and these organisms were presumably responsible for the gas formation; the *E. coli* cultures were especially active gas producers. Since the organisms found in the milk set for cottage cheese were also present in the culture used, the difficulty was presumably due to a contaminated culture rather than to the milk.

Samples 6a and 6b represent essentially the same situation as samples 5a and 5b, except that both Aerobacter species (*A. aerogenes* and *A. oxytocum*) and Escherichia species (*E. coli* and *E. grünthali*) were involved. The Aerobacter cultures were very active gas producers.

Samples 5a to 6b, inclusive, represent conditions rather similar to those reported by Hammer (1916).

The milk which developed acid slowly and showed an unclean odor when set for cottage cheese (samples 7 and 8) yielded two Aerobacter species (*A. aerogenes* and *A. oxytocum*) and two Escherichia species (*E. paragrünthali* and *E. coli*). All of the cultures isolated produced an unclean odor when inoculated into milk; the odor produced by the Aerobacter species was more pronounced and objectionable than that produced by the Escherichia species. Cultures of *E. paragrünthali* and *A. oxytocum* which had been isolated from the milk, were studied from the standpoint of their ability to inhibit the development of butter culture organisms. It was found that either of the species or a mixture of the two would inhibit the butter culture organisms if they

were permitted to grow for considerable periods before the latter organisms were inoculated, but would have little, if any, inhibiting influence with short or no preliminary incubation periods. From these observations it seems probable that the Escherichia-Aerobacter organisms were not responsible for the slow acid development in the milk set for cottage cheese.

CONCLUSIONS

A number of Escherichia-Aerobacter species was isolated from dairy products showing defects that were apparently due to organisms of this group. Ropiness was found to be due to *E. neapolitana*, to *A. aerogenes* and to *A. cloacae*. An unusual case of gassiness in market milk was caused by *A. oxytocum*. Gas in butter cultures and in milk set for cottage cheese was apparently due to a variety of Escherichia-Aerobacter species, and the same was true of an unclean odor in milk set for cottage cheese.

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