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Some studies on the Escherichia-Aerobacter group of bacteria in dairy products

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SOME STUDIES ON THE ESCHERICHIA-AEROBACTER GROUP OF BACTERIA IN DAIRY PRODUCTS

By

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INTRODUCTION

The Escherichia-Aerobacter group of bacteria comprises one of the important groups found in dairy products. Milk even under careful conditions of production practically always contains some of these organisms. The fact that the Escherichia type represents organisms coming from the intestinal tract of man and animals while the Aerobacter type represents organisms coming from soils and grains makes a distinction between the two imperative.

Possibility of growth together with the differences between the two types makes any attempt at attaching sanitary significance to the presence of these organisms in milk difficult. For this reason, it has not been looked upon with favor in this country. The ability of certain strains to survive pasteurization temperatures has been reported by a number of investigators so that their presence in pasteurized milk may not always be due to faulty pasteurization.

The presence of Escherichia-Aerobacter organisms in dairy products is always undesirable for in addition to forming acid and gas from lactose, they produce undesirable flavors and aromas. Among the defects reported as due to this group of organisms are ropiness in milk and cream, gas in cottage and cheddar cheese and in evaporated and sweetened condensed milk.

In most of the previous work done on this group in dairy products, identification of organisms has not been carried to a species basis. Accordingly, there is little information available on the relative numbers of the various species in dairy products and on those species commonly responsible for abnormal conditions.

This study has been undertaken with the object of studying on a species basis, the Escherichia-Aerobacter group of organisms occurring in dairy products with special reference to the numbers present under different conditions, their survival during commercial pasteurization, the defects caused by them and their biochemical action on the constituents of milk.

CLASSIFICATION

Various schemes for the classification of bacteria have been proposed from time to time. In this study, the scheme outlined in Bergey's (6) Manual of Determinative Bacteriology has been followed. Although not formally approved by the Society of American Bacteriologists and in no sense official or standard, it represents the classification most widely used by American bacteriologists at the present time.

The Escherichia-Aerobacter or colon-aerogenes group of bacteria may be considered to include non-spore-forming Gram-negative rods which ferment lactose with the production of acid and gas and which are capable of growing aerobically.

A survey of the species listed in Bergey's Manual in the genus Escherichia and the genus Aerobacter shows a number of species which were originally described as being unable to ferment lactose with the formation of gas. These species with the original references are as follows:

E. foetida (Perez, 1899(33)); E. noctuarii (White, 1923(48)); E. sphingidis (White,1923(48)); E. ichthyosmia(Hammer,1917(16)); E. iliaca (Ford, 1903(13)); and A. bombycis (Glaser,1924(14)).

The first indication that species had been wrongly included in the genus Escherichia and the genus Aerobacter was gained from E. ichthyosmia (Bac. ich Thyosmius) originally described by Hammer (16) as fermenting glucose and sucrose but not lactose with the production of acid and gas. This species should have been placed in the genus Proteus instead of the genus Escherichia. As the above six species do not belong in the Escherichia-Aerobacter group according to Bergey's key to the genera of the tribe Bacterieae, they have been omitted from the revised classification.

Bergey's Key to the genera of tribe Bacterieae

A. Ferment dextrose with production of acid or acid and gas.

1. Gas formed from dextrose.

a. Gas formed from lactose.

b. Acetyl-methyl-carbinol not formed from dextrose.

Genus XVII. Escherichia.

bb. Acetyl-methyl-carbinol formed.

Genus XVIII. Aerobacter.

aa. Gas not formed from lactose.

b. Gas formed from sucrose.

Genus XIX. Proteus.

E. schaefferi has been omitted from the revised key as it has been considered synonymous with E.coli in accordance with the suggestion of Weldin (45). Bergey et al differentiate E. schaefferi by its failure to

coagulate milk. Weldin has pointed out that coagulation of milk is a questionable character for specific differentiation and that the original description was so incomplete it did not sufficiently differentiate the organism from E. coli. He states that later descriptions have added little of value.

Errors in Bergey's Classification of the Genus Escherichia with Corrections

Revision and correction of Bergey's classification represents an attempt to perfect the present scheme. That this assistance is welcomed by the Committee on Manual is shown by their statement in the Preface to the First Edition.

"The assistance of all bacteriologists is earnestly solicited in the correction of possible errors in the text; in the collection of descriptions of all bacteria that may have been omitted from the text; in supplying more detailed descriptions of such organisms as are described incompletely; and in furnishing complete descriptions of new organisms that may be discovered, or in directing the attention of the committee to publications of such newly described bacteria."

The description of each species has been compared with the description in the original article except where Weldin has described the species in question.

Inasmuch as Weldin (45) in 1927 made a complete review of the literature, his descriptions when agreeing with Bergey's have been considered correct without referring to the original.

In a number of cases, the key and specific descriptions are not in agreement.

Errors with corrections are given in table I.

Revision of Bergey's Key to Species of the Genus Escherichia

Bergey's key has been revised to enable the user to trace unknown species more rapidly. The changes made are based upon the fact that the key cannot be used to complete identification but merely to serve as a rapid means of determining the species which must be confirmed by a careful check with the complete description.

The present key in some cases lists two species under the same final character, for example:

1. No acid or gas in sucrose.
    A. Gelatin not liquefied.
        a. Motile.
        b. Acid and gas in salicin.
        c. Nitrates reduced.
        d. Milk acid; coagulated.
            1. Escherichia coli.
            2. Escherichia paragrünthali.

TABLE I

Corrections to Bergey's (1930) Key to Species and Descriptions of Species Belonging to the Genus Escherichia.

Species	Character	Bergey's statement in Key to species	Bergey's statement in Description of species	Statement in original description	Reference
E.grünthali	litmus milk	slightly acid; becoming alkaline	slightly acid; becoming alkaline	acid; coagulation	Castellani and Chalmers (10)
E.vekanda	salicin	acid,gas	acid,gas	-	"
	raffinose	acid,gas	acid,gas	-	"
E.neapolitana	gelatin liquefaction	+	-	-	Weldin (45)
E.pseudocoscorobae	gelatin liquefaction	+	-	-	"
	salicin	acid,gas	-	-	"
E.astheniae	gelatin liquefaction	+	-	-	"
E.plebeia	nitrate reduction	-	+	+	Ford (13)
E.gastrica	indol formation	no statement	not formed	usually produced	"
E.alba	nitrate reduction	+	+	-	Schrire (38)

This necessitates reading through two specific descriptions before finding a differentiating character. The present key has been revised by adding an additional character for separating the two species in the above case the fermentation of dulcitol. It is now possible to identify the species with reading but one specific description.

1. No acid or gas in sucrose.

A. Gelatin not liquefied.

a. Motile.

b. Acid and gas in salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

e. Acid and gas in dulcitol.

1. Escherichia coli.

ee. No acid or gas in dulcitol.

2. Escherichia paragrünthali.

A needless repitition of characters appears in Bergey's key to species of the genus Escherichia where they are the same for species listed under them, for example:

1. No acid or gas in sucrose.

A. Gelatin not liquefied.

a. Motile.

b. Acid and gas in salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

1. Escherichia coli.

2. Escherichia paragrünthali.

dd. Milk acid; not coagulated.

3. Escherichia schaefferi.

ddd. Milk slightly acid; becoming alkaline.

4. Escherichia vekanda.

bb. No action on salicin.

In the above key, "c. Nitrates reduced" is superfluous as all the species fermenting salicin with acid and gas production reduce nitrates so that it is not a differentiating character. In order to simplify the key and hasten identification, non-differentiating characters have been omitted from the revised key.

A further improvement in the key has been brought about by indentation of characters, for example:

1. No acid or gas in sucrose.

A. Gelatin not liquefied.

a. Motile.

b. Acid and gas in salicin.

c. Milk acid; coagulated.

d. Acid and gas in dulcitol.

1. Escherichia coli.

dd. No acid or gas in dulcitol.

2. Escherichia paragrünthali.

cc. Milk acid; not coagulated.

While indentation of characters adds to the publishing cost, it should be insisted upon as it makes a much easier key to follow.

Bergey's key and the revised key to the species of the genus Escherichia follow. In order to facilitate comparison of the two, the number by which a species is listed in Bergey's key is included in parenthesis in the revised key.

Revisions of Bergey's Specific Descriptions of Species Belonging to the Genus Escherichia

The following changes have been made in accordance with the specific descriptions found in the original references.

E. grünthali "Litmus milk; Slightly acid, coagulated, becoming alkaline" to "Litmus milk; Acid, coagulated."

E. vekanda "Acid and gas in salicin and raffinose" to "No acid or gas in salicin and raffinose."

E. alba "Nitrates reduced" to "Nitrates not reduced".

E. gastrica "Indol not formed" to "Indol usually produced".

Much of the difficulty in recognizing species described in the literature is due to incomplete descriptions. In the review of original descriptions, a number of characters were found which have not been included in Bergey's specific descriptions. It is suggested that these be added in order to facilitate recognition of species. While in certain cases these characters have been already listed in the key, it is advisable that they be also repeated in the specific descriptions.

E. coli	"No acid or gas in sucrose."
E. paragrünthali	"No acid or gas in sucrose, dulcitol or adonitol."
E. formica	"No acid or gas in sucrose or salicin".
E. alba	"No acid or gas in dulcitol, saccharose, adonitol, inulin, sorbitol, dextrin, salicin, raffinose or inositol."
E. alcalescens	"No action on salicin".
E. ellingeri	"No action on salicin."

Bergey's Key to the Species of the Genus Escherichia

1. No acid or gas in sucrose.

aa. Gelatin not liquefied.

a. Motile.

b. Acid and gas in salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

1. Escherichia coli.

2. Escherichia paragrünthali.

dd. Milk acid; not coagulated.

3. Escherichia schaefferi.

ddd. Milk slightly acid; becoming alkaline.

4. Escherichia vekanda.

bb. No action on salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

5. Escherichia formica.

dd. Milk slightly acid; becoming alkaline.

6. Escherichia pseudodysenterieae.

7. Escherichia grünthali.

aa. Non-motile.

b. Acid in salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

8. Escherichia anaerogenes.

bb. Acid and gas in salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

9. Escherichia enterica.

bbb. No action on salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

10. Escherichia vesiculiformans.

11. Escherichia foetida.

cc. Nitrates not reduced.

d. Milk acid; coagulated.

12. Escherichia acidilactici.

AA. Gelatin liquefied.

a. Motile.

b. No action on salicin.

c. Nitrates reduced.

d. Milk acid.

13. Escherichia alba.

2. Acid and gas in sucrose.

A. Gelatin not liquefied.

a. Motile.

b. Acid and gas in salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

14. Escherichia communior.

15. Escherichia pseudocoloides.

bb. No action on salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

16. Escherichia anindolica.

dd. Milk slightly acid; becoming alkaline.

17. Escherichia alcalescens.

AA. Gelatin liquefied.

a. Motile.

b. Acid and gas in salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

18. Escherichia leporis.

cc. Nitrates not reduced.

d. Milk slightly acid; becoming alkaline.

19. Escherichia noctuarii.

20. Escherichia sphingidis.

bb. No action on salicin.

c. Nitrates reduced.

d. Milk acid; litmus reduced.

21. Escherichia ichthyosmia.

dd. Milk acid; coagulated.

22. Escherichia gastrica.

ddd. Milk acid; coagulated; peptonized.

23. Escherichia iliaca.

cc. Nitrates not reduced.

d. Milk slightly acid; becoming alkaline.

24. Escherichia plebeia.

aa. Non-motile.

b. Acid and gas in salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

25. Escherichia neopolitana.

26. Escherichia pseudocoscorobae.

bb. No action on salicin.

c. Nitrates reduced.

d. Milk acid; coagulated.

27. Escherichia astheniae.

28. Escherichia ellingeri.

dd. Milk slightly acid, becoming alkaline.

29. Escherichia galactophila.

Revised Key to Species of the Genus Escherichia

1. No acid or gas in sucrose.

A. Gelatin not liquefied.

a. Motile.

b. Acid and gas in salicin.

c. Acid and gas in dulcitol.

1. Escherichia coli (1)*.

cc. No acid or gas in dulcitol.

2. Escherichia paragrünthali (2).

bb. No action on salicin.

c. Milk acid; coagulated.

d. Acid and gas in dulcitol.

3. Escherichia formica. (5).

dd. No acid or gas in dulcitol.

4. Escherichia grünthali (7).

cc. Milk slightly acid; becoming alkaline.

d. Nitrates reduced.

5. Escherichia volkanda (4).

dd. Nitrates not reduced.

6. Escherichia pseudodysenteriae (6).

aa. Non-motile.

b. Acid in salicin.

*Number by which the species is listed in Bergey's key.

7. Escherichia anacrogenes (8).

bb. Acid and gas in salicin.

8. Escherichia enterica (9).

bbb. No action on salicin.

c. Nitrates reduced.

9. Escherichia vesiculiformans (10).

cc. Nitrates not reduced.

10. Escherichia acidilactici (12).

AA. Gelatin liquefied.

a. Motile.

b. No action on salicin.

11. Escherichia alba (13).

2. Acid and gas in sucrose.

A. Gelatin not liquefied.

a. Motile.

b. Acid and gas in salicin.

c. Acid and gas in dulcitol.

12. Escherichia communior (14).

cc. No acid or gas in dulcitol.

13. Escherichia pseudocoloides (15).

bb. No action on salicin.

c. Milk acid; coagulated.

14. Escherichia anindolica (16).

cc. Milk slightly acid; becoming alkaline.

15. Escherichia alcalescens (17).

aa. Non-motile.

b. Acid and gas in salicin.

16. Escherichia neapolitana (25).

bb. No action on salicin.

c. Milk acid; coagulated.

d. Indol formed.

17. Escherichia pseudocoscorobae (26).

dd. Indol not formed.

18. Escherichia astheniae (27).

cc. Milk slightly acid; becoming alkaline.

19. Escherichia galactophila (29).

AA. Gelatin liquefied.

a. Motile.

b. Acid and gas in salicin.

20. Escherichia leporis (18).

bb. No action on salicin.

c. Milk acid; coagulated.

21. Escherichia gastrica (22).

cc. Milk acid; becoming alkaline.

22. Escherichia plebeia (24).

aa. Non-motile.

b. No action on salicin.

23. Escherichia ellingeri (28).

Bergey's key to the species of the genus Aerobacter has been changed by omitting A. bombycis as according to Glaser's (14) original description, A. bombycis does not ferment lactose with gas production. Bergey's key and the revised key follow.

Bergey's Key to Species of the Genus Aerobacter

A. Non-motile.

1. Acid and gas formed in sucrose.

a. No acid or gas in dulcitol.

1. Aerobacter aerogenes.

aa. Acid and gas in dulcitol.

2. Aerobacter oxytocum.

2. No acid or gas in sucrose.

3. Aerobacter chinense.

AA. Motile.

1. Acid and gas formed in sucrose.

4. Aerobacter cloacae.

5. Aerobacter bombycis.

2. No acid or gas in sucrose.

6. Aerobacter levans.

Revised Key to Species of the Genus Aerobacter

A. Non-motile.

1. Acid and gas formed in sucrose.

a. No acid or gas in dulcitol.

1. Aerobacter aerogenes.

aa. Acid and gas in dulcitol.

2. Aerobacter oxytocum.

2. No acid or gas in sucrose.

3. Aerobacter chinense.

AA. Motile.

1. Acid and gas formed in sucrose.

4. Aerobacter cloacae.

2. No acid or gas in sucrose.

5. Aerobacter levans.

In view of the large number of atypical cultures found in this study to compare with E. formica / A. aerogenes and A. oxytocum in all respects except that of indol production and in view of the constancy of this character as reported later in this article, it is suggested that the specific descriptions of these organisms be changed to read, "Indol may or may not be formed". In the present study, these cultures have been identified as atypical but it would be preferable to recognize them as typical strains.

Recognition of the Genus Citrobacter

During the course of study, twenty-five cultures were found which upon repeated purification gave a positive methyl red test, a negative Voges-Proskauer reaction and which were able to utilize citrate as the sole source of carbon. These cultures could not be identified according to Bergey's scheme of classification and corresponded to the intermediate section described by Koser (25) and (26). Koser found organisms of this type to be widely distributed in unpolluted soil. Of 72 cultures from unpolluted soil, 31.9 per cent belonged to this group.

Werkman and Gillen (47) propose to give this group generic recognition under the genus name Citrobacter. In view of the fact that this seems to be a fairly well defined group it has been recognized in this study. Their generic diagnosis is as follows:

"Gram negative non-sporulating short rods; produce trimethylene glycol from glycerol; citrates serve as the sole source of carbon and urates as the sole source of nitrogen; fail to produce acetoin from glycerol or dextrose; methyl red positive (or weakly so); attack many of the carbohydrates with the production of acid and gas; nitrates reduced."

IDENTIFICATION OF CULTURES ISOLATED FROM DAIRY PRODUCTS

In most of the previous work done on the identification of Escherichia-Aerobacter organisms occurring in dairy products, the Escherichia type has merely been separated from the Aerobacter with no study of individual species. Many of the results reported may also be criticized in view of the fact that cultures were isolated by an enrichment method which afforded the opportunity for one type to outgrow another.

The little work that has been done towards identification on a species basis is hard to interpret in the light of our present schemes of classification so that the species described are quite problematical.

In view of the above, the study reported herein was planned with the object of determining (1) the Escherichia-Aerobacter species present in dairy products according to a revised scheme of Bergey's classification previously described and (2) the relative occurrence of each type and species.

Review of Literature

Levine (27) summarized the incidence of aerogenes types among colon bacilli isolated from milk by six different investigators, as follows:

Investigators	Number of strains studied	Per cent aerogenes section (V.P.+; M.R.-)
Orr* (32)	850	39.0
MacConkey (28)	26	57.8
Rogers, Clark & Davis (35)	124	47.5
Hulton (21)	18	72.3
Wood (49)	93	17.5
Stokes (42)	271	59.8
	1382	43.1

*Glucose fermenters, lactose reaction not recorded.

Hunter (22) reported that of 590 cultures isolated from milk B. coli communis represented 0.5 per cent of the total cultures isolated, B. coli communior 50 per cent, B. acidi lactici 2.2 per cent and B. lactis aerogenes 47.2 per cent. However, a study of Hunter's work shows that he has reported the above species as giving both acid and alkaline reactions to the methyl red test so that it is impossible to recognize these species in the light of our present system of classification which uses the methyl red test to differentiate the Escherichia from the Aerobacter type.

Klimmer, Hauft and Borchers (24) in a study of the coli-aerogenes bacteria in milk concluded that a

separation of the coli bacteria from the aerogenes bacteria was uncertain as different methods employed with the same bacterial strains gave different results.

Maulhardt (29) studied fifty samples of milk, cow feces and stools. His investigation of the milk samples showed B. coli in 88 per cent of the samples, B. lactis aerogenes in 48 per cent and B. acidi lactici in 84 per cent.

Methods Used

Isolation of Cultures

Cultures were isolated by both an enrichment method using gentian violet lactose peptone bile broth and a direct plating method using eosin methylene blue agar.

Enrichment Method

The gentian violet lactose peptone bile broth medium devised by Kessler and Swenarton (23) was used for the presumptive test. These workers compared plain lactose broth, gentian violet lactose broth and gentian violet lactose peptone bile broth as to their reliability for detecting coli-aerogenes organisms in milk. They found that the gentian violet lactose peptone bile was the most reliable of these media, and that formation of gas in this medium when inoculated with milk or diluted milk was a

positive indication of the presence of B. coli which for practical purposes needed no confirmation. The gentian violet inhibited the growth of gram positive organisms while the bile decreased the inhibitory action of the gentian violet on the coli-aerogenes group. It was found impossible to use plain lactose broth due to the frequent occurrence of positive presumptive tests in the higher dilutions while the low dilutions were negative. This was explained as due to the fact that the S. lactis organisms in milk outnumber the Escherichia-Aerobacter and develop sufficient acid in the low dilutions to inhibit their growth.

The Bacto Gentian Violet Lactose Bile (dehydrated) used had the following composition:

Bacto-Oxgall ..... 10 parts
Bacto-Peptone..... 10 parts
Bacto-Lactose..... 10 parts
Gentian Violet..... 0.04 parts

Thirty grams were dissolved in 1000 cc. of distilled water, tubed in fermentation tubes and sterilized for 20 minutes at 15 pounds pressure. During sterilization, steam was allowed to escape from the autoclave so that the air would be completely driven from the fermentation tubes.

The dilutions used for inoculation were multiples of ten. Two tubes were used for each dilution. Gas

positive tubes were recorded after 24 and 48 hours incubation at 37.5° C. The comparative results at 24 and 48 hours were so nearly alike that no further mention of them has been made in this study. In a few instances gas was present in a higher dilution after 48 hours than after 24 hours, especially during the latter part of the study when some of the results were based on incubation at 30° C.

The highest and lowest dilutions showing the presence of gas after 24 hours incubation were streaked on eosin methylene blue agar using a technic such that well isolated colonies would develop. The colonies were identified as to Escherichia or Aerobacter type from their appearance on the eosin methylene blue agar according to Levine's (27) descriptions as follows:

Bact. coli (Escherichia)

"Well isolated colonies are 2-3 m.m. in diameter, neighboring colonies show little tendency to run together; colonies slightly raised; surface flat or slightly concave, rarely convex; dark almost black centers which extend more than 3/4 across the diameter of colony; internal structure of central dark portion difficult to discern; colonies dark, button-like, often concentrically ringed with a greenish metallic sheen by reflected light."

Bact. aerogenes (Aerobacter)

"Well isolated colonies are larger than coli; usually 4-6 m.m. in diameter or more; neighboring colonies run together quickly; colonies considerably raised and markedly convex; occasionally the center drops precipitately; centers deep brown, not as dark as Bact. coli and smaller in proportion to the rest of the colony. Striated internal structure often observed in young colonies. Much lighter than Bact. coli. Metallic sheen not observed except occasionally in depressed center when such is present."

In cases where direct plating was unsuccessful, cultures were isolated from the streak made from the highest dilution as it seemed likely that the predominant type would be secured in this manner, the types present in fewer numbers being lost through dilution. One culture was isolated from each distinct type of colony and the relative frequency, based on a total value of ten, noted. For example, where a culture represented a type approximately 90 per cent predominant, it was given a relative frequency value of 9.

Isolations were not made using the enrichment scheme unless the eosin methylene blue plates were poor as where several types are present, one type may outgrow the other and erroneous results be secured. In order to

eliminate this factor as much as possible, isolations were made from tubes incubated for 24 hours instead of 48 hours. The possibility of drawing erroneous conclusions from eosin methylene blue agar streaks from the presumptive test has been well shown by the work of Ruchhoft, Kallas, Chinn and Coulter (36) with the Chicago Sanitary Board. They present data to show that it is possible for either Bact. coli or Bact. aerogenes to overgrow the other in lactose enrichment when they are both initially present in equal numbers and show that this may produce distorted views of the initial condition as determined by eosin methylene blue streaked isolation plates. They also found that isolated colonies could not be relied upon to be pure as typical Bact. coli colonies contained both Bact. coli and Bact. aerogenes and vice versa.

In order to obtain information on the overgrowing of one type by another, the streaks from the lowest dilution were compared with those from the highest.

Direct Plating Method

Direct plating of the raw milk and cream samples for determination of the number of Escherichia-Aerobacter organisms per unit volume and for isolation of cultures was desirable for a number of reasons. In the first place, it served as a check on the counts secured by the presumptive test. In the second place, it eliminated the objection

present with the isolation of cultures by the enrichment scheme as there was no possibility of one type overgrowing another where plates contained between 30 and 300 colonies. A more exact idea could be gained of the relative frequency of the different types. Lastly, there was an advantage in that the medium did not contain gentian violet which is known to have an inhibitory action on certain strains of Escherichia-Aerobacter.

Eosin methylene blue agar was selected as the plating medium as preliminary study showed that it was possible to detect Escherichia-Aerobacter colonies from other types with good success where dilutions of 0.1 cc. or higher were used. In lower dilutions than this, the large quantity of milk present made it impossible to distinguish the desired types so that the method proved impractical for high quality raw milk produced under winter conditions where the number of Eschericha-Aerobacter organisms was ordinarily less than 10 per cc.

Colonies of the S. lactis type were not confusing on the eosin methylene blue agar plates due to their small size. Staphylococci were easily differentiated by their colony appearance, having a deep blue color in most cases. Certain colonies of spore-forming-rods most nearly approached the Escherichia-Aerobacter colonies in appearance but were generally much lighter in color.

Klimmer, Haupt and Borchers (24) used eosin methylene blue gentian violet agar in the direct plating of milk. They compared lactose bouillon with eosin methylene blue gentian violet agar and brom-thymol-blue lactose trypaflavin agar. The results obtained were consistent and they concluded that the three methods were equally good for the determination of the coli-aerogenes titre of milk.

In view of the above results, eosin methylene blue gentian violet agar was tried out in this study but discarded in favor of the plain eosin methylene blue agar, as the addition of the gentian violet made it more difficult to identify types and as it was easy to recognize the Escherichia-Aerobacter from the gram-positive types on plain eosin methylene blue agar plates.

The eosin methylene blue agar was made up according to Levine's (27) modification, as follows:

Distilled water	910 cc.
Peptone (Difco)	10 grams
Dipotassium phosphate (K_2HPO_4).....	2 grams
Agar	15 grams
Lactose, 20 per cent solution	50 cc.
Eosin, 2 per cent aqueous sol.	20 cc.
Methylene blue, 0.5 per cent aqueous sol. ...	20 cc.

The agar, peptone and dipotassium phosphate were first dissolved over the open flame and then filtered through cotton. The filtered solutions of the dyes were next added, the medium tubed in ten cc. quantities and sterilized in the autoclave for 20 minutes at 15 pounds pressure.

With direct plating of the diluted milk or cream it was difficult to identify the Aerobacter and Escherichia types due to the large number of sub-surface colonies which had the same appearance. Two methods were developed to overcome this difficulty.

In the first method, the diluted milk or cream was placed in the bottom of the petri dish and the plates poured in the usual manner. Several dilutions were plated in duplicate, so that plates would result which contained between 25 and 250 typical Escherichia-Aerobacter colonies. Ten of these colonies were then picked from a representative portion of the plate to lactose broth. Gas positive tubes were streaked on eosin methylene blue agar plates and cultures isolated from the different types noted. The percentage of each type present was noted and a relative frequency value assigned to each as previously outlined.

In the second method, plates were first poured with eosin methylene blue agar and predried by placing in the 37.5° C. incubator over night. The diluted milk or cream, 0.1 cc. or higher, was then placed upon the

surface and distributed with a glass rod bent at a right angle. After the diluted material had been absorbed upon the surface of the agar, the plates were inverted and incubated for 48 hours at 37.5° C. As all of the resulting colonies were surface colonies, Escherichia-Aerobacter types were recorded directly, a culture of each type isolated, and relative frequency values ascertained. As part of the inoculum clung to the distributing rod, this method did not give as accurate a total count as the other method but the relative proportion of the various types was maintained.

The entire scheme for the isolation of Escherichia-Aerobacter cultures from milk and cream is outlined in diagram I.

Identification of Cultures.

Cultures we e identified according to the revision of Bergey's classification as previously outlined in this report.

As the cultures isolated could not be relied upon to be pure, they were first purified by replating on eosin methylene blue agar and picking single isolated colonies, to plain agar slopes. They were next checked for formation of gas from lactose. Examination for presence

DIAG

Scheme for Isolation of Escherichia-Aeroba

Milk or Cre

Standard Plate Count per cc.
250,000

Gentian violet lactose peptone bile broth						Probable E-A count per cc. in 48 hours
hours at 37.5°C.	dilution in cc.					
	1.0	0.1	0.01	0.001	0.0001	
24	++	++	+-	--	--	
48	++	++	++	--	--	250

Lowest dilution showing gas in 24 hours streaked on eosin methylene blue agar and the colony types noted as compared with the highest positive dilution. Cultures not isolated except in cases where types present were not found with highest dilution and where direct plating method was unsuccessful.

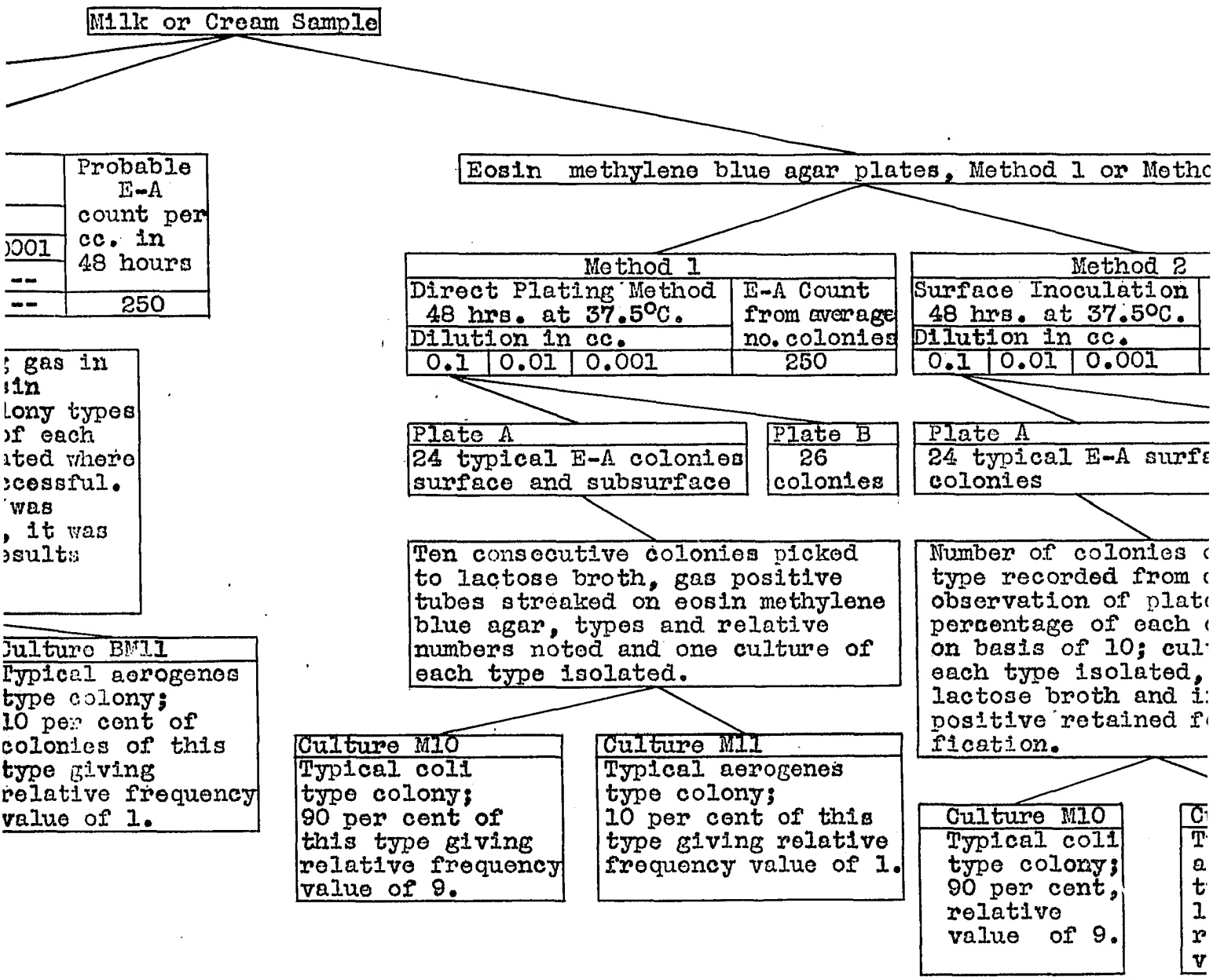
Highest dilution showing gas in 24 hours streaked on eosin methylene blue agar, colony types and relative frequency of each noted and cultures isolated where direct plating was unsuccessful. Where a higher dilution was positive after 48 hours, it was streaked, and 24 hour results discarded.

Culture BM10
Typical coli type colony; 90 per cent of colonies of this type giving relative frequency value of 9.

Culture BM11
Typical aerogenes type colony; 10 per cent of colonies of this type giving relative frequency value of 1.

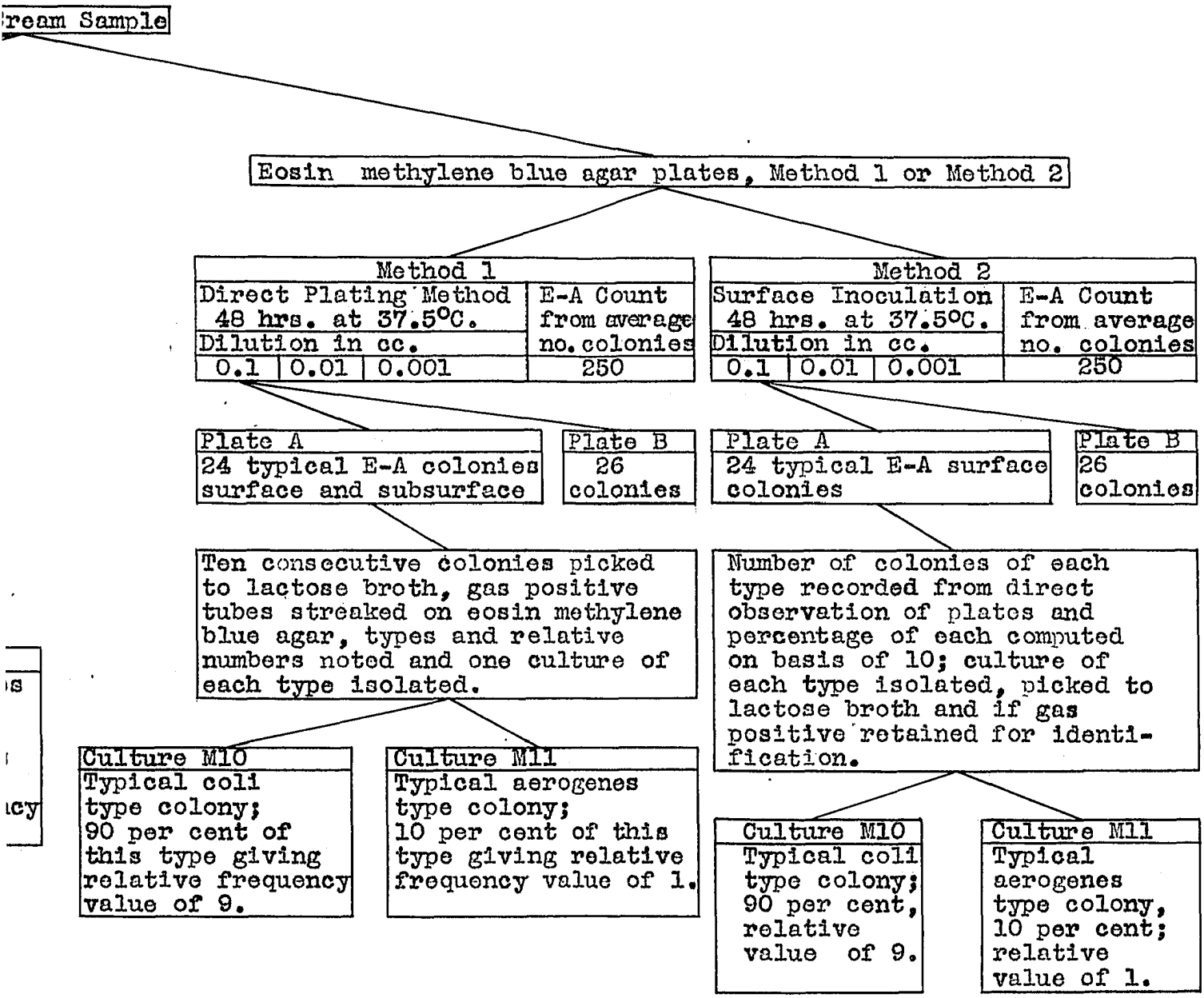
DIAGRAM I

scherichia-Aerobacter Cultures from Milk and Cream Samples.



AGRAM I

bacter Cultures from Milk and Cream Samples.



of spores was made from agar slope cultures left for five days at room temperature. All those cultures which proved to be non-spore-forming gram-negative lactose-fermenters were retained for identification.

The characters studied for identification were the methyl red and Voges-Proskauer tests, utilization of citrate as the sole source of carbon, motility, liquefaction of gelatin, reduction of nitrates, formation of indol, litmus milk action and the fermentation of glucose, lactose, sucrose, dulcitol and salicin.

The methyl red and Voges-Proskauer reactions were carried out by growing cultures for five days at 30° C. in ten cc. of medium of the following composition:

Glucose.....	0.5 per cent
Proteose-Peptone(Difco).....	0.5 per cent
Dibasic potassium phosphate....	0.5 per cent

For the methyl red test, five drops of methyl red solution were added to 5 cc. of the cultured medium. A positive reaction was indicated by a distinct red color and a negative reaction by a yellow color. The indicator solution was prepared by dissolving 0.1 gram of methyl red in 300 cc. of 95 per cent alcohol and diluting to 500 cc. with distilled water.

The Voges-Proskauer reaction was carried out according to Werkman's (46) modification. Two drops of a two per cent solution of $FeCl_3$ were added to five cc.

of the medium and followed by 5 cc. of a 10 per cent solution of NaOH. The mixture was shaken and allowed to stand for a short time when a permanent copper color appeared in the case of a positive reaction.

Utilization of citrate as the sole source of carbon was determined by growing cultures for three days at 37.5° C. in citrate medium of the following composition devised by Koser.

Sodium ammonium phosphate.....	1.5 parts
Monobasic potassium phosphate.....	1.0 parts
Magnesium sulphate.....	0.2 parts
Sodium citrate.....	3.0 parts

A positive result was indicated by the presence of growth.

Motility was determined by growing cultures at room temperature in glucose broth for 18 to 20 hours and immediately examining by use of a hanging drop slide.

Gelatin liquefaction was determined by growing cultures for 14 days at 37.5° C. in a gelatin medium of the following composition:

Beef extract.....	0.3 parts
Peptone.....	0.5 parts
Gelatin.....	12.0 parts

After the incubation period, the cultured medium was cooled in ice water. A positive result was indicated by failure of the gelatin to solidify.

Reduction of nitrates was determined after four days incubation at 37.5° C. in a medium of the following composition:

Beef extract..... 0.3 parts

Peptone..... 0.5 parts

Potassium nitrate..... 0.1 parts

The presence of nitrite was determined by putting a few drops of suphanilic acid and alpha-napthylamine reagents in each broth culture. A positive test was indicated by a distinct pink or red in the broth. The reagents were made up according to directions in the Manual of Methods for the Pure Culture Study of Bacteria (41) as follows: "Prepare sulphanilic acid reagent by dissolving 8 grams of sulphanilic acid in 1 liter of dilute sulphuric acid (1 part concentrated acid to 20 parts water). Prepare alpha-napthylamine reagent by dissolving 5 grams of a naphthylamine in 1 liter of very dilute sulphuric acid (1 part concentrated acid to 125 parts water)."

Formation of indol was determined after three days incubation at 37.5° C. in a one per cent solution of Bacto Tryptophane Broth (dehydrated). The Gôre technic was followed, using the following solutions as outlined in the Manual of Methods for the Pure Culture Study of Bacteria (41).

Solution 1

Para-dimethyl-amino-benzaldehyde..... 1 gram

Ethyl alcohol (95 per cent) ..... 95 cc.

Hydrochloric acid, concentrated ..... 20 cc.

Solution 2

Saturated aqueous solution of potassium persulfate ($K_2S_2O_8$).....

The plug of the culture tube (of white absorbent cotton) was removed, moistened first with five drops of solution No. 2 then with the same amount of solution No. 1. The plug was replaced and pushed down until within an inch of the surface of the culture. A positive test was indicated by the appearance of a red color on the plug due to the volatile indol.

Action in litmus milk was determined after growth for 14 days at 37.5° C.

Fermentation of carbohydrates with acid and gas production was determined after incubation for three days at 37.5° C. The medium was composed of one part peptone and one part of the test carbohydrate. A five per cent alcoholic solution of brom-cresol-purple was added as an indicator at the rate of 0.5 cc. per liter of medium. Acid production was indicated by the appearance of a yellow color and gas production was noted in fermentation tubes. These carbohydrates were sterilized for 20 minutes at 15 pounds

pressure with the exception of lactose broth which was sterilized for 12 minutes at 12 pounds pressure in order to lessen the chances of inversion. The lactose broth tubes were then held in the incubator over night to detect unsterile tubes.

Results Obtained

Identification of Cultures Isolated from Dairy Products

Raw Milk

One hundred and two samples of raw milk were examined for the numbers and species of Escherichia-Aerobacter organisms present. These samples represented the raw milk supply of the College Dairy and 13 pasteurizing plants located in 7 Iowa cities. None were taken during July and August and the larger part between January and June.

Comparative Escherichia-Aerobacter counts and identity of the cultures isolated from raw milk are reported in table II. A comparison of the Escherichia-Aerobacter counts on 50 samples by the enrichment and plating methods shows that they check well when it is considered that the probable number of organisms per cc. with two tubes per dilution is only an approximation. Buchanan and Fulmer make the statement that for accurate results five samples per dilution should be used. This was

TABLE II

Comparative Escherichia-Aerobacter Counts and Identity of Cultures Isolated from Raw Milk.

Sample Number	E-A Count: EMB Plate	E-A Count: Broth Tube	Method of Isolation	Cult. No.	EMB Type	Identified Species	Relative Frequency
1			AP	M1		E. grünthali	10.0
5			AP	M2		E. communior	10.0
9		<1	BT	BM3		E. coli	10.0
11		6	BT	BM4		E. paragrünthali	6.7
			BT	BM5		E. vesiculiformans	3.3
14		3	BT	BM7		E. communior	5.0
			BT	BM10		E. coli	5.0
15		250	BT	BM8		E. formica*	5.0
			BT	BM11		" " *	5.0
23	700	600	AP	M14		" " *	5.0
		—	BT	S10		E. coli	5.0
24	40	60	AP	M20	A	A. aerogenes	5.0
			BT	S12	E	E. coli	5.0
25	30	25	AP	M22	E	E. communior	6.7
			AP	M23	A	A. oxytocum	3.3
33	130	250	AP	M24	E	E. enterica	10.0
39	1,400	2,500	AP	M25	E	E. grünthali	2.5
			AP	M26	I	A. aerogenes*	7.5
44	no growth	6	BT	BM27	E	E. anaerogenes	10.0
59	8,000	25,000	AP	M36	I	A. oxytocum	10.0
64	90	250	AP	M39	E	E. vesiculiformans	10.0
67	60	60	AP	M42	E	E. vesiculiformans	6.7
			AP	M43	A	A. cloacae	3.3
72	440	2,500	AP	M49	I	E. anaerogenes	10.0
73	20	25	AP	M51	E	E. pseudocoloides	5.0
			BT	BM50	E	E. pseudocoloides	5.0
75	no growth	13	BT	BM53		E. paragrünthali	10.0
76	no growth	6	BT	BM54		E. pseudocoloides	10.0
77	unsat.	60	BT	BM55	A	A. aerogenes	10.0
82	no growth	<1	BT	BM59	E	Citrobacter	10.0
83	15	60	BT	BM60	E	Citrobacter	10.0
84	30	250	BT	BM65	E	E. pseudocoloides	5.0
			BT	BM66	E	E. paragrünthali	5.0
86	no growth	<1	BT	BM68	A	A. aerogenes	10.0
87	20	6	BT	BM69	E	E. pseudocoloides	10.0

77	unsat.	60	BT	BM55	A	A. aerogenes	10.0
82	no growth	<1	BT	BM59	E	Citrobacter	10.0
83	15	60	BT	BM60	E	Citrobacter	10.0
84	30	250	BT	BM65	E	E. pseudocoloides	5.0
			BT	BM66	E	E. paragrünthali	5.0
86	no growth	<1	BT	BM68	A	A. aerogenes	10.0
87	20	6	BT	BM69	E	E. pseudocoloides	10.0
90	unsat.	6	BT	BM71	I	A. aerogenes	10.0
94	unsat.	250	BT	BM74	E	E. coli	10.0
96	unsat.	6	BT	BM75	A	A. aerogenes*	10.0
100	no growth	50	BT	BM78		A. aerogenes*	10.0
101	no growth	<1	BT	BM79		E. pseudocoloides	10.0
102	400	250	AP	M80	A	A. oxytocum	8.7
			AP	M81	wine	E. paragrünthali	1.3
104	170	2,500	AP	M82	E	Citrobacter	6.5
			AP	M83	E	E. enterica	3.5
105	unsat.	25,000	BT	BM85	wine	E. communior	2.0
			BT	BM86	E	E. enterica	8.0
106	unsat.	2,500	AP	M84	E	E. formica*	10.0
107	unsat.	2,500	BT	BM87	E	E. neapolitana	10.0
108	unsat.	250,000	BT	BM88	E	E. vesiculiformans	10.0
110	50	60	AP	M91	E	E. pseudocoloides*	5.0
			BT	M96	E	E. pseudocoloides*	5.0
111	10	13	AP	M92	I	Citrobacter	10.0
113	130	250	AP	M93	I	E. anaerogenes	5.0
			BT	M97	I	E. vesiculiformans*	5.0
114	500	60	AP	M94	E	Citrobacter	10.0
116	no growth	25	BT	M98	A	A. oxytocum*	10.0
118	no growth	60	BT	M99	I	A. aerogenes*	10.0
119	unsat.	600	BT	M100	E	E. coli	10.0

* Atypical

Key

<1	Either gas entirely absent from 1 cc. quantities of milk or one tube positive, the other negative.
AP	Culture isolated from eosin-methylene-blue agar plates.
BT	Culture isolated by enrichment method from broth tubes.
A	Aerobacter type colony.
E	Escherichia type colony.
I	Intermediate type colony.
unsat.	Plate count not determined due to overcrowded plates or spreaders.
Relative Frequency)	The number of organisms based on a total of ten organisms of Escherichia-Aerobacter types per sample.

TABLE II (continued)

Sample Number	E-A Count: EMB Plate	E-A Count: Broth Tube	Description of Cultures Isolated: Method of Isolation	Cult. No.	EMB Type	Identified Species	Relative Frequency
121	4,400	25,000	AP	M102	E	Citrobacter	9.6
			AP	M103	A	A. cloacae	0.4
125	unsat.	600	AP	M104	E	E. coli	10.0
126	unsat.	2,500	BT	M108	E	E. paragrünthali	10.0
127	no growth	3	BT	M109	E	E. paragrünthali	10.0
128	no growth	3	BT	M110	E	E. communior	10.0
130	no growth	3	BT	M111	E	E. communior	10.0
134	400	250	AP	M113	E	E. coli	10.0
135	25	60	AP	M116	E	E. paragrünthali	10.0
136	no growth	6	BT	M119	A	A. oxytocum*	5.0
			BT	M120	E	E. coli	5.0
137	550	2,500	AP	M117	E	E. pseudocoloides*	10.0
138		2,500	AP	M118	E	E. vesiculiformans	10.0
139	16,000	25,000	AP	M122	E	A. aerogenes	5.0
			AP	M123	A	A. cloacae	5.0
141	400,000	>25,000	AP	M121	E	E. enterica	10.0
143		60	BT	R1	A	E. coli*	5.0
			BT	R2	A	E. paragrünthali	5.0
146		25	BT	R4	A	E. coli	10.0
149		600	BT	R3	A	Citrobacter	10.0
152		600	BT	R5	A	A. cloacae	2.5
			BT	R6	I	Citrobacter	7.5
156		3	BT	R7	A	Citrobacter	10.0
159		600	BT	R8	A	A. cloacae	10.0
163		>1	BT	R9	A	E. coli*	10.0
167		250	BT	R10	A	A. cloacae*	10.0
170		250	BT	R11	A	A. cloacae*	5.0
			BT	R12	E	Citrobacter	5.0
174		250	BT	R13	A	A. cloacae*	10.0
177		250	BT	R14	A	A. cloacae*	10.0
181		60	BT	R15	A	A. cloacae*	10.0
189		60	BT	R16	E	E. coli	10.0
193		250	BT	R17	A	E. communior	10.0

not possible in this study as it would have seriously limited the number of samples which could have been handled. Discussion of the counts obtained appears later in the section dealing with number of organisms present in raw milk. During the early part of the study, winter samples regularly gave a low Escherichia-Aerobacter count by the dilution method, gas often being absent from 1 cc. quantities of milk. As these results were contrary to those reported by a number of other investigators, it was thought that the technic used might be at fault until a comparison of the two methods showed that Escherichia-Aerobacter colonies were not developing on eosin methylene blue agar plates prepared from a 0.1 cc. dilution, the lowest dilution that could be used for satisfactory plates.

Ninety-one cultures were isolated from seventy samples of milk. Thirty-four of the cultures were isolated from eosin methylene blue agar plates poured by the direct plating method while fifty-seven cultures were isolated by the enrichment method. Cultures were isolated by the direct plating method whenever possible due to the possibility of one type overgrowing another during enrichment, as has already been discussed. Where the Escherichia-Aerobacter count was below 25 per cc., cultures were not ordinarily isolated from plates as too few colonies were present (two or three with 0.1 cc. dilution) to accurately

determine the relative proportion of the different types. Plates containing between 10 and 250 colonies were preferable for isolation purposes.

The comparative percentages of the species isolated from raw milk are summarized in table III. The percentages of organisms are based on relative frequency values calculated on a basis of ten organisms of Escherichia-Aerobacter types per sample. When compared with percentages as calculated on the basis of number of cultures isolated, as most workers have done, the results are practically the same. This was due principally to the fact that in the majority of cases (48 samples) but one species was detected.

The data show that the genus Escherichia comprised 61.7 per cent of the Escherichia-Aerobacter organisms, the genus Aerobacter 25.9 per cent and the genus Citrobacter 12.4 per cent. The genus Escherichia contained 10 species of which E. coli was most numerous (14.3 per cent of all Escherichia-Aerobacter organisms) followed by E. pseudocoloides, E. communior, E. paragrünthali, E. vesiculiformans, E. enterica, E. formica, E. anaerogenes, E. grünthali and E. neapolitana. The genus Aerobacter contained three species of which A. aerogenes was most numerous (11.1 per cent of all Escherichia-Aerobacter organisms) followed by A. cloacae and A. oxytocum.

TABLE III

Comparative Percentages of Species Isolated from Raw Milk.

Species	Escherichia-Aerobacter Cultures		Organisms
	Number	Per cent	Per cent
E. coli	12	13.2	14.3
pseudocoloides	9	9.9	9.3
communior	8	8.8	8.4
paragrünthali	8	8.8	8.4
vesiculiformans	6	6.6	6.4
enterica	4	4.4	4.5
formica	4	4.4	3.6
anaerogenes	3	3.3	3.6
grünthali	2	2.2	1.8
neapolitana	1	1.1	1.4
Total	57	62.7	61.7
A. aerogenes	9	9.9	11.1
cloacae	10	11.0	9.5
oxytocum	5	5.4	5.3
Total	24	26.3	25.9
Citrobacter	10	11.0	12.4
Grand Total	91	100.0	100.0

Pasteurized Milk

Sixty-four samples of pastuerized milk were examined for the numbers and species of Escherichia-Aerobacter organisms present. These samples were from the same source of supply as the raw milk samples reported in tables II and III so that results are comparable.

The number of Escherichia-Aerobacter organisms according to the enrichment method and identity of the cultures isolated from pasteurized milk are reported in table IV. Discussion of the Escherichia-Aerobacter counts appears later in this article in the section dealing with the numbers of Escherichia-Aerobacter organisms occurring in milk.

Due to the small numbers of Escherichia-Aerobacter organisms present, the samples could not be plated successfully. This made necessary the isolation of cultures by the enrichment method. In order to detect small numbers of organisms, 10 cc. quantities of pasteurized milk were used as inoculum in some cases.

Twenty-one cultures were isolated from nineteen samples of pasteurized milk. Eleven of these samples were taken at the College Dairy, ten from bottled milk and one from the pasteurizing vat following pasteurization. The remaining eight samples were collected by extension workers

TABLE IV

Number of Escherichia-Aerobacter Organisms and Identity of Cultures Isolated from Pasteurized Milk.

Sample Number	E-A Count	Description of Cultures Isolated			
	Broth Tube	Culture Number	EMB* Type	Identified Species	Relative Frequency*
60	>250	BM37	E	E. pseudocoloides**	10.0
62	25	M38	E	E. paragrünthali	10.0
65	<1*	BM40	E	E. vesiculiformans**	10.0
70	<1	BM41	E	E. grünthali	10.0
80	25	BM56	A	A. cloacae**	10.0
85	6	BM67	A	A. cloacae	10.0
93	>250	BM73	I	E. paragrünthali	10.0
109	6	BM89	E	E. pseudocoloides	5.0
		BM90	A	A. cloacae	5.0
120	<1	M101	E	E. pseudocoloides	10.0
145	<1	PB1	A	Citrobacter	10.0
154	<1	PB3	A	E. coli	10.0
158	<1	PB4	E	Citrobacter	5.0
		PB5	A	Citrobacter	5.0
166	<1	PB9	A	E. coli	10.0
172	>25	PB12	E	Citrobacter	10.0
176	6	PB13	E	Citrobacter	10.0
183	<1	PB14	A	Citrobacter	10.0
184	<1	PB15	A	Citrobacter	10.0
194	<1	PV1	A	E. pseudocoloides**	10.0
195	3	PB16	I	E. communior	10.0

* See key for table II

** Atypical

at six Iowa pasteurizing plants. Three of these samples were from bottled milk and five were taken from the pasteurizing vat following pasteurization.

The percentages of the species isolated from pasteurized milk on the basis of numbers of cultures are summarized in table V. The data show that the genus Escherichia comprised 57.2 per cent of the Escherichia-Aerobacter organisms, the genus Aerobacter 9.5 per cent and the genus Citrobacter 33.3 per cent. The genus Escherichia contained six species of which E. pseudocoloides was most common (23.8 per cent of all cultures) followed by E. coli, E. paragrünthali, E. communior, E. grünthali and E. vesiculiformans. A. cloacae (9.5 per cent of all cultures) was the only species found belonging to the genus Aerobacter.

A comparison of the predominant species of the raw milk samples reported in table III and of the pasteurized milk samples reported in table V shows significant differences. While the total percentage of species belonging to the genus Escherichia is about the same in each case (61.7 and 57.2 per cent), E. pseudocoloides constitutes 9.3 per cent of all organisms of the genus Escherichia in the case of the raw and 23.8 per cent in the case of the pasteurized milk. Species belonging to the genus Citrogacter also constitute a larger percentage of

TABLE V

Percentages of Species Isolated from Pasteurized Milk.

Species	Cultures	
	Number	Per cent
E. pseudocoloides	5	23.8
coli	2	9.5
paragrünthali	2	9.5
communior	1	4.8
grünthali	1	4.8
vesiculiformans	1	4.8
Total	12	57.2
A. cloacae	2	9.5
Citrobacter	7	33.3
Grand Total	21	100.0

the flora in the pasteurized milk, 33.3 per cent as against 12.4 per cent in the case of the raw milk. These results would indicate either that E. pseudocoloides and the species belonging to the genus Citrobacter are more heat resistant than the other species in this group or that they are more likely to occur as contaminants following pasteurization.

Heat resistance studies on two cultures, BM73 (E. paragrünthali) isolated from a bottle of pasteurized milk and BM90 (A. cloacae) isolated from a sample taken directly following pasteurization were made. The detailed results of this study are discussed later in the section dealing with the number of organisms occurring in pasteurized milk. Ten minutes at 62° C. (143.6° F.) was the longest survival time noted which indicates that the presence of these organisms in the pasteurized samples was due either to faulty pasteurization or to contamination following pasteurization.

Unfortunately, the importance of E. pseudocoloides and the species belonging to the genus Citrobacter was not realized at the time and no heat resistance studies were made of these species.

Raw Cream

Thirteen samples of sweet and eleven of sour raw cream were examined for the numbers and species of

Escherichia-Aerobacter organisms present. These samples represented part of the cream supplied the College Dairy for buttermaking between February and April, 1930. In most instances, they were composite samples representing a number of patrons. Cream deliveries were made three times a week during the period of sampling so that the cream examined was approximately two days old.

Comparative Escherichia-Aerobacter counts and identity of the cultures isolated from raw cream are reported in table VI. Due to the small number of samples, no attempt was made to classify the data on the basis of sweet and sour cream.

A comparison of the Escherichia-Aerobacter counts on 23 samples by the enrichment and direct plating methods shows that they are in general agreement. Eighteen of the samples were plated successfully by the direct plating method which was a higher percentage of success than in the case of the raw milk due to the larger number of Escherichia-Aerobacter organisms present. This made it possible to use higher dilutions in plating and less trouble was encountered with confusing types of colonies.

Forty-two cultures were isolated from the twenty-three samples of raw cream, thirty-six by the direct plating method and six by the enrichment method.

TABLE VI

Comparative Escherichia-Aerobacter Counts and Identity of Cultures Isolated from Raw Cream.

Sample Number	E-A Count: Agar Plate	E-A Count: Broth Tube	Method of Isolation*	Cult. No.	EMB* Type	Identified Species	Relative Frequency*
28	unsat.*	>2,500	AP	C1	E	E. coli	10.0
29	unsat.	25,000	AP	C2	E	Citrobacter	10.0
30	unsat.	25	BT	BC3	E	E. communior	9.0
			BT	BC4	A	A. cloacae	1.0
31	110	60	BT	BC5		E. formica**	3.3
			BT	BC6		E. formica**	6.7
32	75,000	25,000	AP	C7	A	A. aerogenes	8.0
			AP	C8	E	E. coli	2.0
35	unsat.	>25,000	AP	C10	A	A. oxytocum	10.0
37	53,000	60,000	AP	C11	A	A. cloacae**	0.6
			AP	C12	E	Citrobacter	5.7
			AP	C13	A	A. cloacae**	3.7
38	4,300	3,000	AP	C9	E	A. aerogenes**	10.0
41	34,000	60,000	AP	C14	E	A. aerogenes**	3.0
			AP	C15	A	A. cloacae**	6.0
			AP	C16	wine	A. aerogenes**	1.0
42	90,000	25,000	AP	C17	E	A. aerogenes**	10.0
45	unsat.	250	BT	BC19	E	A. aerogenes**	7.0
			BT	BC20	A	A. aerogenes**	3.0
46	1,000	600	AP	C21	I	E. neapolitana	6.7
			AP	C22	A	A. oxytocum	3.3
47	12,000	6,000	AP	C23	A	A. oxytocum	10.0
48	700,000	250,000	AP	C24	E	E. communior	10.0
49	25,000	25,000	AP	C25	A	A. cloacae**	10.0
51	230,000	250,000	AP	C26	E	E. communior	10.0
55	1,000	600	AP	C34	E	E. enterica	9.0
			AP	C35	I	E. coli	1.0
56	100,000	250,000	AP	C32	E	E. paragrünthali	1.0
			AP	C33	E	E. anaerogenes	9.0
57	39,000	60,000	AP	C31	I	A. aerogenes**	2.0
			AP	C29	E	Citrobacter	4.0
			AP	C30	I	A. aerogenes**	4.0

30	unsat.	25	BT	BC3	E	E. communior	9.0
			BT	BC4	A	A. cloacae	1.0
31	110	60	BT	BC5		E. formica**	3.3
			BT	BC6		E. formica**	6.7
32	75,000	25,000	AP	C7	A	A. aerogenes	8.0
			AP	C8	E	E. coli	2.0
35	unsat.	>25,000	AP	C10	A	A. oxytocum	10.0
37	53,000	60,000	AP	C11	A	A. cloacae**	0.6
			AP	C12	E	Citrobacter	5.7
			AP	C13	A	A. cloacae**	3.7
38	4,300	3,000	AP	C9	E	A. aerogenes**	10.0
41	34,000	60,000	AP	C14	E	A. aerogenes**	3.0
			AP	C15	A	A. cloacae**	6.0
			AP	C16	wine	A. aerogenes**	1.0
42	90,000	25,000	AP	C17	E	A. aerogenes**	10.0
45	unsat.	250	BT	BC19	E	A. aerogenes**	7.0
			BT	BC20	A	A. aerogenes**	3.0
46	1,000	600	AP	C21	I	E. neapolitana	6.7
			AP	C22	A	A. oxytocum	3.3
47	12,000	6,000	AP	C23	A	A. oxytocum	10.0
48	700,000	250,000	AP	C24	E	E. communior	10.0
49	25,000	25,000	AP	C25	A	A. cloacae**	10.0
51	230,000	250,000	AP	C26	E	E. communior	10.0
55	1,000	600	AP	C34	E	E. enterica	9.0
			AP	C35	I	E. coli	1.0
56	100,000	250,000	AP	C32	E	E. paragrünthali	1.0
			AP	C33	E	E. anaerogenes	9.0
57	39,000	60,000	AP	C31	I	A. aerogenes**	2.0
			AP	C29	E	Citrobacter	4.0
			AP	C30	I	A. aerogenes**	4.0
58	28,000	6,000	AP	C27	E	A. aerogenes**	3.0
			AP	C28	E	A. aerogenes**	7.0
71	200,000	60,000	AP	C45	wine	A. cloacae	0.5
			AP	C46	I	A. oxytocum	0.6
			AP	C47	wine	A. oxytocum	1.3
			AP	C48	wine	Citrobacter	7.6
88	89,000	250,000	AP	C61	A	A. aerogenes	0.1
			AP	C62	E	E. coli	9.9
89	160,000	600,000	AP	C63	A	A. aerogenes	0.4
			AP	C64	E	E. paragrünthali	9.6

* see key for table II

** atypical

The comparative percentages of the species isolated from raw cream are summarized in table VII. The data show that the genus Escherichia comprised 42.2 per cent of the Escherichia-Aerobacter organisms and 33.4 per cent of the cultures while the genus Aerobacter comprised 45.9 per cent of the organisms and 57.1 per cent of the cultures. It is evident from these differences that it would have been erroneous to determine the proportion of species of the Escherichia and Aerobacter genera on the basis of the number of cultures isolated.

The genus Escherichia contained seven species of which E. communior was present in largest numbers (12.6 per cent of all Escherichia-Aerobacter organisms) followed by E. coli, E. paragrünthali, E. formica, E. enterica, E. anaerogenes and E. neapolitana.

The genus Aerobacter contained three species of which A. aerogenes was present in largest numbers (25.4 per cent of all Escherichia-Aerobacter organisms) followed by A. oxytocum and A. cloacae.

The results indicate that the Escherichia and Aerobacter genera are present in about equal numbers in raw cream and that A. aerogenes is the species most commonly present, making up approximately 25 per cent of all organisms of the Escherichia-Aerobacter group.

TABLE VII

Comparative Percentages of Species Isolated from Raw Cream.

Species	Escherichia-Aerobacter Cultures: Number	Cultures: Per cent	Organisms: Per cent
E. communior	3	7.1	12.6
coli	4	9.5	10.0
paragrünthali	2	4.8	4.6
formica	2	4.8	4.3
enterica	1	2.4	3.9
anaerogenes	1	2.4	3.9
neapolitana	1	2.4	2.9
Total	14	33.4	42.2
A. aerogenes	13	30.9	25.4
cloacae	6	14.3	9.5
oxytocum	5	11.9	11.0
Total	24	57.1	45.9
Citrobacter	4	9.5	11.9
Grand Total	42	100.0	100.0

Ice Cream

Twenty samples of commercial ice cream from twelve plants were examined for the numbers and species of Escherichia-Aerobacter organisms present. The Escherichia-Aerobacter counts were determined by Mr. E. N. Fabricius by the enrichment method already described; since ice cream is normally held at low temperatures and since certain Aerobacter strains grow better at 30° C. than at 37.5° C., comparative counts were made at the two incubation temperatures. The Escherichia-Aerobacter counts and types of Escherichia-Aerobacter organisms were essentially the same at both temperatures so that comparative results are not reported.

The Escherichia-Aerobacter counts by the enrichment method and the identity of the cultures isolated at 30° C. are reported in table VIII.

Sixteen cultures were isolated from sixteen samples of ice cream. The percentages of species present based on the number of cultures belonging to each, are summarized in table IX. The data show that 31.3 per cent of the Escherichia-Aerobacter cultures belonged to the genus Escherichia while 56.2 per cent belonged to the genus Aerobacter. The remaining 12.5 per cent was made up of species belonging to the genus Citrobacter. The Escherichia organisms belonged to three species (E. coli, E. communior and E. pseudocoloides) while the Aerobacter

TABLE VIII

Number of Escherichia-Aerobacter Organisms and Identity of Cultures Isolated from Ice Cream.

Sample Number	E-A Count	Description of Cultures Isolated		
	Broth Tube	Culture Number	EMB* Type	Identified Species
197	25	F1	A	A. aerogenes**
198	25	F3	A	A. cloacae
200	3	F5	A	E. coli
201	25	F6	A	A. cloacae
202	25	F7	A	E. communior
203	250	F8	A	A. aerogenes**
204	25	F9	A	A. cloacae
205	250	F10	A	A. cloacae
206	2500	F11	E	Citrobacter
207	25	F13	I	E. coli
210	2500	F15	A	A. aerogenes
211	25	F16	I	A. cloacae
212	250	F17	A	E. communior
213	3	F18	wine	Citrobacter
215	25	F21	A	E. pseudocoloides**
216	250	F22	A	A. cloacae**

* see key for table II

** atypical

TABLE IX

Percentages of Species Isolated from Ice Cream.

Species	Cultures	
	Number	Per cent
E. coli	2	12.5
communior	2	12.5
pseudocoloides	1	6.3
Total	5	31.3
A. cloacae	6	37.5
aerogenes	3	18.7
Total	9	56.2
Citrobacter	2	12.5
Grand Total	16	100.0

organisms belonged to two species (A. cloacae and A. aerogenes). A. cloacae was the predominant species comprising 37.5 per cent of all the Escherichia-Aerobacter cultures studied.

As the history of the samples was not available, the significance of the presence of these species could not be determined. The limited number of cultures studied also makes the drawing of definite conclusions illogical.

Defective Butter

Twenty-five cultures, belonging to the Escherichia-Aerobacter group, which were isolated by Dr. B. W. Hammer and Mr. H. A. Derby from samples of defective butter were identified. These cultures were isolated by an enrichment method and were secured in a search for other organisms so that their significance is uncertain.

The identity of the Escherichia-Aerobacter cultures isolated is reported in table X. A. aerogenes was predominant, comprising 60 per cent of the 25 cultures. The remaining cultures were identified as A. oxytocum (16 per cent), A. cloacae (12 per cent) and species belonging to the genus Citrobacter (12 per cent). The absence of the Escherichia section indicates that conditions in butter are not favorable for growth or survival of these

TABLE X

Identity of Escherichia-Aerobacter Cultures Isolated from Defective Butter.

Sample Number	Culture Number	Species	Source of Sample
B1	3	A. aerogenes	Control butter from past. cream
B2	4	A. oxytocum*	Butter 1c
B3	8	A. aerogenes*	Defective butter
"	9	A. aerogenes	Defective butter
B4	10	A. aerogenes	Defective butter
B5	11	A. oxytocum	Defective butter, predominant type
"	12	A. oxytocum	Defective butter, predominant type
B6	14	A. cloacae	Defective contest butter from British Columbia, from surface
"	15	A. cloacae	From interior of above butter
B7	17	Citrobacter	Defective Iowa butter produced under unsanitary conditions.
B8	24	A. aerogenes*	Surface taint sample
"	25	A. aerogenes*	" " "
B9	26	A. aerogenes*	" " "
"	27	A. aerogenes	" " "
B10	28	A. aerogenes	" " "
"	29	A. aerogenes	" " "
B11	30	Citrobacter	" " "
B12	32	A. aerogenes	" " "
"	33	A. aerogenes	" " "
B13	34	A. aerogenes	" " "
B14	H1	A. cloacae	Defective butter
"	H6	A. cloacae	" "
B15	D1	Citrobacter	" "
B16	D2	A. aerogenes	One year old Canadian butter
B17	H70	A. aerogenes	Butter 16, direct isolation

* Atypical cultures

organisms at the temperatures at which butter is ordinarily held and suggests that the presence of the Aerobacter section in defective butter may be correlated with certain defects.

Ropy Milk and Cream

Organisms of the Escherichia-Aerobacter group have frequently been reported as the cause of ropy milk and cream outbreaks which yearly cause considerable economic loss.

The identity of Escherichia-Aerobacter cultures isolated from ropy milk and cream by workers at the Iowa Agricultural Experiment Station is reported in table XI. The data show that six of the nine cultures were A. aerogenes and that three were A. oxytocum. Further study of 63 A. aerogenes and A. oxytocum cultures isolated from non-ropy dairy products showed that a majority produced a ropy condition in litmus milk held at 30° C. in from one to three days. The ropy condition could be predicted, in most cases, by the appearance of a gummy layer at the surface. This condition was not noted with cultures belonging to the genus Escherichia. These results indicate that organisms of the Aerobacter type, particularly A. aerogenes and A.oxytocum, are commonly responsible for outbreaks of ropy milk and cream.

While the Aerobacter species are most commonly concerned, at least one outbreak has been reported as due

TABLE XI

Identification of Escherichia-Aerobacter Cultures Isolated from Ropy Milk and Cream.

Sample Number	Culture Number	Species	Source of Sample
1	19	A. aerogenes	Plymouth cream, ropy stock culture.
2	22	A. aerogenes	Cream, ropy stock culture
3	23	A. oxytocum*	Milk, ropy stock culture
4	RP1	A. aerogenes	Ropy raw milk outbreak
5	RP2	A. aerogenes	" " " "
6	RP3	A. aerogenes	" " " "
7	NR1	A. aerogenes	" " " "
8	B125	A. oxytocum	Slightly ropy sour cream
9	B126	A. oxytocum*	Ropy raw milk outbreak

* Atypical.

to the Escherichia type. Sadler and Middlemass (37) classed a number of cultures isolated from an outbreak of ropy milk as atypical E. neapolitana. They were atypical in that they failed to produce indol and grew better at 21° C. than at 37° C.

Atypical Cultures

Forty-eight Escherichia-Aerobacter cultures could not be identified according to the scheme of classification used. These cultures were repurified and all tests confirmed before making further study. As descriptions of these cultures differed from those of defined species by single characters not of sufficient importance to warrant the establishment of new species, they have been considered atypical.

The comparative numbers of typical and atypical cultures of each species are listed in table XII. The key to this table shows that the characters considered atypical were action on salicin and dulcitol, formation of indol and the liquefaction of gelatin.

The data show that 11 cultures belonging to the genus Escherichia (14.3 per cent of all cultures belonging to the genus Escherichia) were atypical. Three cultures of E. coli (15 per cent of all E. coli cultures), six of E. pseudocoloides (40 per cent) and one of E. vesiculiformans (14.3 per cent) were atypical in that

TABLE XII

Comparative Number of Typical and Atypical Cultures.

Species	Escherichia-Aerobacter Cultures		
	Typical	Atypical	Total
E. coli	17	3	20
communior	14	0	14
paragrünthali	12	0	12
pseudocoloides	9	6	15
vesiculiformans	5	2	7
formica	0	6	6
enterica	5	0	5
anaerogenes	4	0	4
grünthali	3	0	3
neapolitana	2	0	2
Total	77	11	88
A. aerogenes	25	21	46
cloacae	16	11	27
oxytocum	12	5	17
Total	53	37	90
Grand Total	130	48	178

Key to Atypical Cultures

E. coli	no action on salicin
" pseudocoloides	no action on salicin
" vesiculiformans	no action on salicin (1 culture)
"	no action on dulcitol (1 culture)
" formica	formation of indol
A. aerogenes	formation of indol
" cloacae	no action on salicin (1 culture)
	no noticeable liquefaction of gelatin (10 cultures)
" oxytocum	indol not formed

they had no action on salicin. One culture of E. vesiculiformans (14.3 per cent) was atypical in that it showed no action on dulcitol. Six cultures of E. formica (100 per cent) were atypical in that they formed indol.

Of cultures belonging to the genus Aerobacter, 37 (41.1 per cent of all cultures belonging to the genus Aerobacter) were atypical. Twenty-one cultures of A. aerogenes (45.7 per cent of all A. aerogenes cultures) were atypical in that they formed indol. Ten cultures of A. cloacae were atypical (37 per cent) in that gelatin liquefaction could not be observed. One culture was atypical in that no action on salicin could be detected. Five cultures of A. oxytocum (29.4 per cent) were atypical in that indol formation was not observed.

In view of the large number of cultures found to be atypical with respect to indol formation, it was decided to determine the constancy of this character by a study of 136 cultures of the Escherichia-Aerobacter group containing approximately equal numbers of indol-forming and non-indol-forming strains. The test for formation of indol was repeated on these cultures after they had been held for three months on agar slopes at 7.2° C. A perfect correlation was found which indicates that indol formation was a reliable and constant character for the particular species concerned in this study. In view of

the fact that indol formation is a character not used in the key for separation of species, it is suggested that the specific descriptions of the species E. formica, A. aerogenes and A. oxytocum be changed to read, "Indol may or may not be formed".

Ten cultures could not be identified because they were motile and did not liquefy gelatin. None of the species in the scheme of classification used would fit this description. These cultures might have been classified either as A. cloacae, atypical in that gelatin liquefaction did not take place or A. aerogenes, atypical in that motility was observed. As gelatin liquefaction is frequently slow and relatively difficult to recognize (Weldin (45)), it is probably a less reliable character than motility. For this reason, the cultures were classified as atypical A. cloacae, atypical in that they caused no noticeable liquefaction of gelatin.

Identification of Escherichia and Aerobacter Types from Appearance of Colonies on Eosin Methylene Blue Agar

The identification of Escherichia and Aerobacter types from the appearnce of colonies on eosin methylene blue agar is reported in tables II, IV, VI and VIII. The data show that the Escherichia and Aerobacter types were correctly identified in 70.5 per cent of the 129

cultures studied. Twenty-three cultures belonging to the genus Citrobacter were omitted from the comparison, being most commonly mistaken for the Escherichia type. In the case of the remaining 38 cultures wrongly identified, each type was mistaken for the other in about an equal number of cases.

The accuracy of the type identification noted here did not equal that reported by Levine (27) who found that in water analysis work in Iowa, 96.9 per cent of 102 colonies fished as probable Bact. coli proved to be such and 82.4 per cent of 122 colonies fished as Bact. aerogenes proved to be such. It is probable that an accuracy approximating Levine's could have been attained with more experience.

Discussion

The percentage of Escherichia-Aerobacter organisms in the raw milk which belonged to the genus Aerobacter (25.9 per cent) was low compared to the percentages found by six other workers which have been summarized by Levine (27). Levine states that 43.1 per cent of 1382 strains studied belonged to the aerogenes section. If additional samples had been obtained in this study during the summer months of July and August, it is possible that a higher proportion of organisms belonging to the genus

Aerobacter would have been found due to the ability of the Aerobacter species to grow better than the Escherichia at temperatures at which milk is ordinarily held. This belief is supported by the fact that 45.9 per cent of the Escherichia-Aerobacter organisms present in the raw cream belonged to the genus Aerobacter. As the cream was held approximately two days before delivery, more opportunity for growth was afforded than in the milk delivered daily.

Correct identification of the species concerned and establishment of the relative proportion of these species in various dairy products is a difficult problem. Enrichment methods must be relied upon for isolation purposes where small numbers are present. Direct plating methods, such as the one used in this study, offer good possibilities, especially with raw milk and cream, when the numbers of Escherichia-Aerobacter organisms are large enough to give agar plates suitable for isolation purposes.

Summary

Two hundred and four cultures belonging to the Escherichia-Aerobacter group which were isolated from two hundred thirty-six samples of raw and pasteurized milk and cream, butter and ice cream were identified on a species basis. Of the total number of cultures studied, 91 were from raw milk, 21 from pasteurized milk, 42 from

raw cream, 9 from ropy milk and cream, 16 from ice cream and 25 from defective butter.

A general summary of the percentages of species isolated from dairy products is given in table XIII. The data show the following:

(1) The genus Escherichia comprised 61.7 per cent of the Escherichia-Aerobacter group in raw milk; 57.2 per cent in pasteurized milk; 42.2 per cent in raw cream; 31.3 per cent in ice cream while none were found in samples of defective butter.

(2) The genus Aerobacter comprised 88 per cent of the Escherichia-Aerobacter group in defective butter; 56.2 per cent in ice cream; 45.9 per cent in raw cream; 25.9 per cent in raw milk and 9.5 per cent in pasteurized milk.

(3) The genus Citrobacter comprised 33.3 per cent of the Escherichia-Aerobacter group in pasteurized milk; 12.5 per cent in ice cream; 12.4 per cent in raw milk; 12 per cent in defective butter and 11.9 per cent in raw cream.

(4) E. coli was the predominant species in raw milk (14.3 per cent of all Escherichia-Aerobacter organisms); E. pseudocoloides in pasteurized milk (23.8 per cent); A. aerogenes in raw cream (25.4 per cent) and in defective butter (60 per cent); and A. cloacae in ice cream (37.5 per cent).

TABLE XIII

Summary of Percentages of Species Isolated from Dairy Products.

Species	Dairy Product Source				
	Milk		Raw Cream	Ice Cream	Butter
	Raw	Past.			
E. coli	14.3	9.5	10.0	12.5	
pseudocoloides	9.3	23.8	0.0	6.3	
communior	8.4	4.8	12.6	12.5	
paragrünthali	8.4	9.5	4.6		
vesiculiformans	6.4	4.8	0.0		
formica	3.6	0.0	4.3		
enterica	4.5	0.0	3.9		
anaerogenes	3.6	0.0	3.9		
grünthali	1.8	4.8	0.0		
neapolitana	1.4	0.0	2.9		
Total	61.7	57.2	42.2	31.3	0.0
A. aerogenes	11.1	0.0	25.4	18.7	60.0
cloacae	9.5	9.5	9.5	37.5	12.0
oxytocum	5.3	0.0	11.0	0.0	16.0
Total	25.9	9.5	45.9	56.2	88.0
Citrobacter	12.4	33.3	11.9	12.5	12.0
Grand Total	100.0	100.0	100.0	100.0	100.0

(5) The genus Escherichia contained ten species of which (considering all dairy products) E. coli was present in largest numbers followed by E. pseudocoloides, E. communior, E. paragrünthali, E. vesiculiformans, E. formica, E. enterica, E. anaerogenes, E. grünthali and E. neapolitana.

(6) The genus Aerobacter contained three species of which (considering all dairy products) A. aerogenes occurred in largest numbers followed by A. cloacae and A. oxytocum.

A. aerogenes and A. oxytocum were the species responsible for ropiness in nine samples of ropy milk and cream.

Forty-eight of the cultures could not be positively identified and were considered atypical strains of closely related species.

NUMBER OF ESCHERICHIA-AEROBACTER ORGANISMS IN DAIRY PRODUCTS

Past studies on the number of Escherichia-Aerobacter organisms in dairy products have been carried out, for the most part, with the object of establishing their sanitary significance. Various factors may affect the number of these organisms. One of the most important of these factors is the temperature at which milk and cream is held. The fact that these organisms grow rapidly at temperatures above 10° C. (50° F.) renders an interpretation of their sanitary significance difficult.

The significance of the presence of these organisms in pasteurized milk and cream and ice cream is still uncertain. A number of workers have found certain strains capable of resisting pasteurization at 62.8° C. (145° F.) for 30 minutes while a number of other investigators have found opposite results. The possibility of contamination following commercial pasteurization as well as faulty pasteurization must also be considered.

The work reported herein has been carried out with the object of determining the number of Escherichia-Aerobacter organisms in raw and pasteurized milk and cream and ice cream with different methods of handling. The survival of these organisms during commercial pasteurization has also been studied.

Review of Literature

In 1918, Ayers and Clemmer (2) made an extensive study of the significance of the colon count in raw milk. They concluded that fresh milk produced under the best conditions always contained some organisms of the colon-aerogenes group but rarely over 2,000 per cc., even when produced under the worst conditions normally encountered. They found that high colon counts could nearly always be attributed to the growth of organisms originally introduced into the milk.

Finkelstein (12) found that where care was used, the number of colon-aerogenes organisms in raw milk averaged less than 100 per cc. and where indifferent methods were used, 588 per cc.

In 1926, the New Hampshire State Board of Health (31) in a study of the raw milk supply of that state found that one-third of all the samples showed a colon value of 0 per 0.01 cc. and sixty per cent of all samples afforded a colon value not exceeding 10 per 0.01 cc. From this they concluded that the colon limit recognized by their department of not exceeding 10 per 0.01 cc. was sufficiently high to be entirely fair to the producer.

Klimmer, Hauft and Borchers (24) in 1929, while investigating 12 market milk samples found that 57 per cent contained more than 10 coli-aerogenes cells per cc., 23.5 per cent being between 10 and 100, ten per cent between

100 and 1,000 and 23.5 per cent over 1,000 cells per cc.

Numerous studies have been made on the survival of Escherichia-Aerobacter organisms during pasteurization. The results reported by the various workers are not in agreement so that the significance of the presence of these organisms in pasteurized dairy products is still uncertain.

In 1915, Ayers and Johnson (3) found that the presence of members of the Escherichia-Aerobacter group in pasteurized milk could not be taken as an indication of unsatisfactory heating. Of 174 cultures studied, 95 (54.5 per cent) survived 60° C. for 30 minutes and 12 (6.89 per cent survived 62.8° C.

The above results were confirmed by Shippen (40) who studied 31 strains of organisms of the Escherichia-Aerobacter group isolated from pasteurized milks of Baltimore. Of the 31 strains, 11 remained viable after 60° C. for 15 minutes; of these one was killed at 68° C. in 15 minutes and at 65° C. for 30 minutes. None of the cultures of A. aerogenes resisted treatment at 60° C. for 15 minutes. Shippen concluded that the presence of E. coli in pasteurized milk was not to be interpreted as an index of improper pasteurization nor of subsequent contamination.

Finkelstein (12) found that the holding method left an average of 42 colon-aerogenes organisms per cc.

of milk. In some cases, no colon-aerogenes organisms survived. The critical temperature for the destruction of these organisms was about 62.8° C.

Tanner and Dubois, in 1925, (43) reported their experimental results indicated that members of the colon-typhoid group in milk, in the numbers in which they occur, were destroyed by pasteurization (30 minutes at 60° C.).

In 1927, Brannon and Prucha (8) found that three colon organisms did not survive pasteurization for 35 minutes at 62.5° C.

Two years later, Tanner and Windsor (44) noted the possibility of resistant strains, or cultures containing some resistant cells, surviving a temperature of 62.8° C. for 30 minutes. They used three methods of treatment; sealed tubes, open flasks and litmus milk tubes and found that they checked closely. Survival was observed for a shorter time in open flasks than in sealed tubes. Only one of 23 cultures of E. coli survived the temperatures of 62.8° C. for 30 minutes in sealed tubes.

In 1930, Beavens (5) found in an examination of 100 samples of pasteurized milk that in 32 per cent of the samples, Escherichia-Aerobacter organisms were able to survive the temperatures used in commercial pasteurization. He concluded from this that the coli test was not a true index to proper pasteurization.

Hammer and Hussong, in 1931, (18) studied three cultures of A. aerogenes, that produced ropiness in milk, for their heat resistance and found it to vary greatly. With one culture, organisms from an old diluted milk or agar slope culture survived 62.8° C. for 10 minutes but those from a young milk culture did not; With another culture organisms from either a young or old diluted milk or agar slope culture failed to survive 61.1° C. for three minutes; with a third culture, organisms from an old diluted milk or agar slope culture regularly resisted 62.8° C. for 10 minutes and in some instances for 20 minutes although organisms from young cultures failed to resist these exposures. Their results indicated that, in some instances, ropiness in pasteurized milk or products made from it is due to contamination following the heating and that this possibility should be considered along with heat resistant causative organisms.

In 1930, Beavens (4) suggested that survival of members of the Escherichia-Aerobacter group in ice cream mixes pasteurized at 62.8° C. (145° F.) for 30 minutes may be caused by the protective action of the high sugar content.

Fabian and Coulter (11) studied in ice cream the thermal death point of 33 cultures of E. coli, 7 cultures of A. aerogenes and 4 cultures of lactose-fermenting organisms isolated from water but not definitely classified.

Four determinations were made. At 62.8° C. for 30 minutes the percentages surviving were 22.7, 6.8, 2.2 and 22.7 respectively for the successive determinations. These investigators found that ice cream had a greater protective action than skim milk, all the cultures being killed in skim milk when held at 62.8° C. for 30 minutes.

Methods Used

Determination of Probable Number of Organisms

The number of Escherichia-Aerobacter organisms was determined by the dilution method using gentian violet lactose peptone bile broth as the enrichment medium. The composition of this medium with a discussion of its advantages and disadvantages has already been given in the section dealing with the identification of cultures.

The usual way of determining the number of organisms per unit volume by the dilution method is to use dilutions in multiples of ten. When a single tube is inoculated with each dilution, the number of organisms present per unit volume may be recorded as the reciprocal of the highest dilution showing growth. If, for example, the highest dilution showing growth is 0.01 cc., the number of organisms may be designated as 100 per cc.

The approximate number of Escherichia-Aerobacter organisms per cc. was determined in this study by

inoculating two tubes with each dilution and recording gas formation after 48 hours incubation at 37.5° C. The probable number of organisms was derived from an adaptation of McCrady's (30) tables given by Buchanan and Fulmer (9) as follows:

Most Probable Number of Organisms With Two Tubes of Each Dilution

Significant Number	Probable Number of Organisms
200	2.5
201	5.0
210	6.0
211	13.0
212	20.0
220	25.0
221	70.0
222	110.0

The significant number may be defined to include the figures representing the highest dilution in which all tubes are positive, and the next two. For example, for a series 1 cc., 0.1 cc., 0.01 cc., 0.001 cc., 0.0001 cc., the results secured might be 22100. The significant number is 210. Examination of the table shows the probable number to be 6. This is the probable number of bacteria in 0.1 cc. of the original sample so that the probable number per cc. is 6 times 10 or 60.

If in the series of dilutions, there is a negative followed by a positive, such as in the series

1 cc.+, 0.1 cc.-, 0.01 cc.+, 0.001 cc.-, the probable number is taken as the reciprocal of the dilution next preceding the last positive result, in this case 10 per cc.

Methods of Studying Heat Resistance

Young and old cultures of the test organisms were prepared as follows: Milk tubes were inoculated from agar slope cultures; after 65 hours incubation at 37.5° C., transfers were made and the newly inoculated cultures incubated for 5 hours at 37.5° C. This resulted in a set of cultures 70 hours old containing old cells and a set of cultures 5 hours old containing young cells.

Heat resistance studies were made on cultures diluted so that approximately the same number of organisms would be present in each case. This was done by adding 1 cc. of the 5 hour culture and 0.1 cc. of the 70 hour culture to respective tubes of milk. The number of test organisms in these dilutions was determined by the standard plate method.

The technic of the open pipette and sealed tube methods used for the study of heat resistance was as follows: Open pipettes were prepared by drawing glass tubing (5 mm.) to a slender tip approximately 30 cm. long, the large ends of which were plugged with cotton and sterilized in pipette cases. The test cultures were then

drawn up into the pipettes for a distance of approximately 5 cm. (one-twentieth to one-fortieth cc. of milk) and the tips sealed. For the sealed tube method, than wall glass test tubes, 7.6 cm. long and of 8 mm. bore were used. These tubes were sealed in a flame after 1 cc. of the test culture was introduced.

The sealed tubes were then fastened to the corresponding pipettes with a rubber band in such a way that the milk levels would be the same. After all were prepared, they were immersed in a water bath preheated to 62° C. (143.6° F.). The variation in the temperature of the water was 0.2° C. Care was taken to have the milk level five or six inches beneath the surface of the water and three or four inches above the bottom of the bath.

Survival of the test organisms was determined at ten, twenty and thirty minute intervals. Individual test samples were prepared for each time interval. As each time interval was completed, the respective tubes and pipettes were withdrawn and immersed in cold water to immediately check the heat effect.

In discharging the heated cultures, the tips of the pipettes were dipped in $HgCl_2$ solution, wiped dry and broken using sterile cotton and the contents blown into sterile milk tubes. In the case of the sealed tubes, the tops were marked with a file, broken and 1.5 cc. of the

heated culture pipetted to sterile milk tubes. Where growth did not appear in the tubes after five days incubation at 37.5° C., it was concluded that the test organisms did not survive the heat treatment.

Results Obtained

Number of Escherichia-Aerobacter Organisms in Dairy Products

Raw Milk

Studies at the College Dairy

Escherichia-Aerobacter and standard plate counts were made on 38 samples of raw milk supplied the College Dairy between January and June, 1930. Nineteen samples of night milk cooled to between 50° F. and 60° F. and nineteen samples of uncooled morning milk were obtained from nine individual patrons. Glass pipettes which would reach to the bottom of a ten gallon milk can were used in securing samples.

The comparative Escherichia-Aerobacter and standard plate counts of the cooled and uncooled milk are reported in table XIV. It is interesting to note that 12 of the 38 samples contained less than one Escherichia-Aerobacter organism per cc. indicating that under careful methods of production, very few of these organisms are present. The data in table XIV have been summarized in tables XV, XVI and XVII.

TABLE XIV

Comparative Escherichia-Aerobacter and Standard Plate Counts of Cooled and Uncooled Raw Milk.

1930 Date	Approx. Air Temp. in Degrees F.	Patron Number	Sample Description: Cooled Night Milk Bacterial Count E-A	Cooled Night Milk Bacterial Count Standard	Uncooled Morning Milk Bacterial Count E-A	Uncooled Morning Milk Bacterial Count Standard
Jan. 17	-10	8	6	25,000	3	9,700
" 26	+20	1	<1*	6,300	<1	7,300
Feb. 3	30	5	<1	1,700	<1	3.100
" 6	35	2	6	2,200,000	250	24,000
" 6	35	4	250	500,000	600	2,400,000
" 8	35	9	25	170,000	60	46,000
" 17	30	5	<1	38,000	<1	17,000
Mar. 15	40	5	<1	15,000	<1	10,000
May 5	70	3	25,000	330,000	2,500	>1,000,000
" 5	70	6	2,500	>5,000,000	2,500	15,000
" 21	70	1	13	36,000	60	190,000
" 21	70	3	250	1,700,000	<1	8,500
" 27	67	4	<1	99,000	3	84,000
" 27	67	7	2,500	250,000	600	170,000
" 29	68	6	3	2,700,000	3	140,000
" 29	68	8	3	22,000	<1	16,000
June 4	66	2	250	1,700,000	<1	35,000
" 4	66	5	6	440,000	250	5,500
" 4	66	9	2,500	>500,000	2,500	71,000

* <1 Escherichia-Aerobacter organisms absent from either one or two 1 cc. quantities of milk.

TABLE XV

Comparative Escherichia-Aerobacter Counts of Cooled and Uncooled Raw Milk.

Sample Source	Escherichia-Aerobacter Counts					
	Less than 10		10 to 100		Over 100	
	Number	Per cent	Number	Per cent	Number	Per cent
Cooled night	10	52.6	2	10.5	7	36.9
Uncooled morning	10	52.6	4	10.5	14	36.9
Total	20	52.6	4	10.5	14	36.9

TABLE XVI

Influence of Season of Year on Escherichia-Aerobacter Counts.

Season of Year	Escherichia-Aerobacter Counts					
	Less than 10		10 to 100		Over 100	
	Number	Per cent	Number	Per cent	Number	Per cent
Winter (Jan. - Mar.)	11	68.8	2	12.5	3	18.7
Summer (May - June)	9	40.9	2	9.1	11	50.0

TABLE XVII

Correlation between Escherichia-Aerobacter and Standard Plate Counts.

E-A Counts	Standard Plate Counts					
	Less than 100,000		100,000 to 1,000,000		Over 1,000,000	
	Number	Per cent	Number	Per cent	Number	Per cent
Less than 10	16	80.0	2	10.0	2	10.0
10 to 100	2	50.0	2	50.0	0	0.0
Over 100	4	28.6	5	35.7	5	35.7

Table XV gives the comparative Escherichia-Aerobacter counts of the cooled and uncooled raw milk. The data show that the number of Escherichia-Aerobacter organisms was less than 10 per cc. in 52.6 per cent of all the samples; between 10 and 100 per cc. in 10.5 per cent; and over 100 per cc. in 36.9 per cent. The Escherichia-Aerobacter counts ranged from less than 1 to 25,000 per cc. The same relative percentages were found for both the night and morning milk indicating that the cooling factor was negligible if it was assumed that all other factors having a bearing on the Escherichia-Aerobacter counts were the same.

The influence of the season of year at which the samples were taken on the Escherichia-Aerobacter counts is shown in table XVI. Sixteen winter samples (January to March) and twenty-two spring and summer samples (May to June) were studied. The data show that 68.8 per cent of the winter samples and 40.9 per cent of the summer samples contained less than 10 Escherichia-Aerobacter organisms per cc.; 12.5 per cent of the winter samples and 9.1 per cent of the summer samples contained between 10 and 100 per cc.; and 18.7 per cent of the winter samples and 50.0 per cent of the summer samples contained over 100 per cc.

The larger number of Escherichia-Aerobacter organisms in the summer samples was undoubtedly due to

the fact that the milk was not as well cooled in the summer, resulting in more favorable conditions for growth.

The correlation between the Escherichia-Aerobacter and standard plate counts is reported in table XVII which shows that 80 per cent of the samples with Escherichia-Aerobacter counts less than 10 per cent cc. had standard plate counts less than 100,000 while 50 per cent with Escherichia-Aerobacter counts between 10 and 100 and only 28.6 per cent with Escherichia-Aerobacter counts over 100 had standard plate counts less than 100,000 per cc. The above results indicate a slight correlation between the Escherichia-Aerobacter and standard plate counts as is to be expected when such counts are due to growth rather than contamination.

Studies from Iowa Pasteurization Plants

Escherichia-Aerobacter and standard plate counts* were made on 20 samples representing the composite raw milk supplies of 11 Iowa pasteurization plants. These samples were secured by workers of the dairy extension department of Iowa State College during April, May and June, 1930. They represented part of a series of samples

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*Standard plate counts determined by Mr. M. Michaelian.

taken before and after pasteurization.

The Escherichia-Aerobacter and standard plate counts are shown in table XVIII. The standard plate counts show that the milk was of poor quality, 70 per cent of the samples having standard plate counts over 500,000 per cc.

The data have been summarized in table XIX to show the correlation between the Escherichia-Aerobacter and standard plate counts. The table shows that 40 per cent of the samples contained less than 100 Escherichia-Aerobacter organisms per cc.

Pasteurized Milk

Studies at the College Dairy

The number of Escherichia-Aerobacter organisms in 53 samples of milk was determined before and after pasteurization and again after bottling. Fifteen pasteurization runs were represented by these samples. The Escherichia-Aerobacter counts of the raw milk samples were not accurate as they were obtained just prior to pasteurization when the milk was at a temperature of 115° F. to 140° F. The pasteurized milk samples were secured from the pasteurizing vat following pasteurization at 142° F. to 144° F. for 30 to 35 minutes. The milk was then cooled to 110° F., pumped over a surface cooler, cooled to approximately 40° F. and bottled. The first

TABLE XVIII

Comparative Escherichia-Aerobacter and Standard Plate Counts of Raw Milk Supplies of Pasteurization Plants.

1930 Date	Plant Source	Bacterial Count	
		E-A	Standard
April 9	A	25,000	5,700,000
" 9	A	250	1,600,000
" 10	B	60	4,000,000
" 10	C	<10	52,000
" 19	D	60	1,600,000
" 24	G	250	320,000
" 26	H	<1	300,000
" 26	I	600	2,100,000
" 26	J	250	1,100,000
" 26	K	6	820,000
May 3	L	250	1,900,000
" 8	B	250,000*	7,000,000
" 22	K	60	360,000
" 22	J	25	1,100,000
" 22	M	60	1,300,000
" 22	H	600	250,000
" 23	G	25,000	870,000
" 30	L	250	500,000
June 6	A	20,000	75,000,000
" 6	A	25,000	15,000,000

* Much higher than other E-A counts and omitted from discussion of results.

TABLE XIX

Correlation between Escherichia-Aerobacter and Standard Plate Counts.

E-A Counts	Standard Plate Counts					
	less than 500,000		500,000 to 5,000,000		over 5,000,000	
	number	per cent	number	per cent	number	per cent
less than 100	3	37.5	5	62.5	0	0.0
100 - 1000	3	42.9	4	57.1	0	0.0
over 1,000	0	0.0	1	20.0	4	80.0

bottle filled and one other were taken as samples. Approximately 800 bottles were filled during a 90 minute period.

The Escherichia-Aerobacter counts before and after pasteurization and again after bottling are reported in table XX. The data show that the number of Escherichia-Aerobacter organisms in the milk before pasteurization was between 100 and 600 in 64.3 per cent of the samples (9 out of 14 samples) with the remaining samples having an Escherichia-Aerobacter count below 100 per cc.

The samples taken from the pasteurizing vat failed to show the presence of Escherichia-Aerobacter organisms in 10 cc. quantities of milk in 93.3 per cent of the cases (14 out of 15 samples). Escherichia-Aerobacter organisms were present in one sample in ten cc. but not in one cc. quantities of milk indicating the survival during pasteurization of a heat resistant strain which was isolated and identified as E. pseudocoloides.

The first milk bottled contained Escherichia-Aerobacter organisms in 81.8 per cent of the samples (9 out of 11) while the milk bottled later contained Escherichia-Aerobacter organisms in only 53.8 per cent of the samples (7 out of 13). The difference in the number of organisms is significant. The first milk bottled contained less than one Escherichia-Aerobacter organism

TABLE XX

Escherichia-Aerobacter Counts Before and After Pasteurization and Again After Bottling.

1930 Date	Before Pasteurization	After Pasteurization			
		Pasteurizing vat	First Bottle	Miscellaneous Bottle	
	E-A Count	E-A Count*	E-A Count	Stage of Bottling	E-A Count
Jan. 12	130	0		last	<1
" 13	25	0		last	<1
" 14	600	0		last	0
" 20	600	0	<1	last	0
" 23	3	0	<1	no sample	
" 29	600	0	3	middle	3
Feb. 4	<1	0	6	last	<1
" 18	250	0	no sample	145th bottle	<1
Mar. 4	250	0	25	last	0
" 11	250	0	6	no sample	
" 17	250	0	0	65th bottle	0
" 18	60	0	<1	60th bottle	<1
April 2	-	0	<1	100th bottle	0
" 6	60	0	0	last	0
" 17	600	<1	3	last	<1

* 0 Escherichia-Aerobacter organisms absent from 10 cc. milk.

<1 Escherichia-Aerobacter organisms present in 10 cc. milk but absent from either one or both 1 cc. quantities.

per cc. in 44.4 per cent of the positive samples (4 out of 9) while the milk bottled later contained less than one Escherichia-Aerobacter organism per cc. in 85.7 per cent of the positive samples (6 out of 7).

The above results indicate that the presence of Escherichia-Aerobacter organisms in the bottled milk was due largely to contamination following pasteurization, undoubtedly from the cooling and bottling equipment, and that the extent of this contamination was gradually reduced by the flow of milk through the equipment. The persistence of slight contamination throughout the entire 90 minute bottling period is significant from the standpoint of coliform standards on bottled pasteurized milk.

Studies from Iowa Pasteurization Plants

Escherichia-Aerobacter and standard plate counts were made on 45 samples of milk from 13 pasteurization plants. These samples were taken before and after pasteurization and again after bottling. They were collected by workers of the dairy extension department of Iowa State College during April, May and June, 1930. The raw milk counts have already been discussed in connection with the number of Escherichia-Aerobacter organisms in raw milk.

The comparative Escherichia-Aerobacter and standard plate counts of samples taken before and after pasteurization and again after bottling are reported in table XXI. The data show that Escherichia-Aerobacter organisms were present in 44.4 per cent of the samples of pasteurized milk (11 out of 25). In the case of samples taken from the pasteurizing vat following pasteurization, 29.4 per cent (5 out of 17 samples) contained Escherichia-Aerobacter organisms while 75 per cent of the samples (6 out of 8) taken from the cooler and bottled milk contained Escherichia-Aerobacter organisms. These results indicate that contamination following pasteurization was largely responsible for the presence of these organisms.

Faulty pasteurization was undoubtedly responsible for the presence of Escherichia-Aerobacter organisms in three of the five positive samples taken from the pasteurizing vat, for the records given in table XXI show that they were pasteurized at 140° F. for intervals ranging from 25 to 50 minutes. One positive sample was pasteurized at 142° F. for 30 minutes while the pasteurization record for the remaining positive sample was not available. These results indicate that the presence of Escherichia-Aerobacter organisms in the pasteurized milk samples was due to faulty pasteurization

TABLE XXI

Comparative Escherichia-Aerobacter and Standard Plate Counts of Samples Taken Before and After Commercial Pasteurization and Again After Bottling.

1930 Date	Plant Source	Raw Milk Bacterial Count		Pasteurized Milk		
		E-A	Standard	Source and Description of Sample	Bacterial Count E-A	Standard
April 9	A	25,000	5,700,000	Vat, 140°F. - 25 min.	250	400,000
"	"			Cooler	60	500,000
"	"			Regular bottle	25	550,000
"	"			In sterile bottle	250	490,000
"	"	250	1,600,000	Vat, 142°F. - 30 min.	0	19,000
"	"			Cooler	0	13,000
" 10	B	60	4,000,000	Vat, 145°F. - 30 min.	0	180,000
"	C	<10	52,000	Vat, 145°F. - 30 min.	0	7,000
" 19	D	60	1,600,000	Vat, 142°F. - 38 min.	0	23,000
"	E			Bottle	0	34,000
"	F			Bottle	25	8,000
" 24	G	250	320,000	Cooler, 143°F. - 30 min.	6	26,000
" 26	H	<1	300,000	Vat, 143°F. - 30 min.	0	13,000
"	I	600	2,100,000	Bottle	250	34,000
"	J	250	1,100,000	Vat, 144°F. - 30 min.	0	84,000

"	"			Cooler	0	13,000
" 10	B	60	4,000,000	Vat, 145°F. - 30 min.	0	180,000
"	C	<10	52,000	Vat, 145°F. - 30 min.	0	7,000
" 19	D	60	1,600,000	Vat, 142°F. - 38 min.	0	23,000
"	E			Bottle	0	34,000
"	F			Bottle	25	8,000
" 24	G	250	320,000	Cooler, 143°F. - 30 min.	6	26,000
" 26	H	<1	300,000	Vat, 143°F. - 30 min.	0	13,000
"	I	600	2,100,000	Bottle	250	34,000
"	J	250	1,100,000	Vat, 144°F. - 30 min.	0	84,000
"	K	6	820,000	Vat, 140°F. - 32 min.	0	4,100
May 3	L	250	1,900,000	Vat, 150°F. - 30 min.	0	56,000
" 8	B	250,000	7,000,000	Vat, 142°F. - 30 min.	6	7,500
" 22	K	60	360,000	Vat	0	16,000
"	J	25	1,100,000	Vat, 144°F. - 30 min.	0	16,000
"	M	60	1,300,000			
"	H	600	250,000	Vat	<10	15,000
" 23	G	25,000	870,000	Vat, 141°F. - 30 min.	0	5,600
" 30	L	250	500,000	Vat, 144°F. - 30 min.	0	90,000
June 6	A	20,000	75,000,000	Vat, 140°F. - 30 min.	<1	45,000
"	A	25,000	15,000,000	Vat, 140°F. - 50 min.	6	120,000

and contamination following pasteurization rather than the survival of heat resistant strains and confirms the findings of the studies at the College Dairy.

Examination of the standard plate counts of the pasteurized samples show that 64 per cent had counts less than 50,000 (16 out of 25 samples); 12 per cent (3 samples) counts between 50,000 and 100,000; and 24 per cent (6 samples) counts over 100,000 per cc. With the exception of the first series of samples from plant A which showed very high Escherichia-Aerobacter and standard plate counts, there was no correlation between the two counts as has also been already reported as true of the samples before pasteurization.

Identity of Escherichia-Aerobacter Cultures

The identification of 21 Escherichia-Aerobacter cultures isolated from pasteurized milk has been already discussed in the section dealing with the identification of cultures. The genus Escherichia comprised 57.2 per cent of all cultures, of which E. pseudocoloides was the predominant species (5 out of 21 cultures) followed by E. coli, E. paragrünthali, E. communior, E. grünthali and E. vesiculiformans. The genus Aerobacter comprised 9.5 per cent of all cultures of which A. cloacae was the only species. The remaining cultures (33.3 per cent) belonged to the genus Citrobacter.

Heat Resistance of Escherichia-Aerobacter Organisms

The ability of two Escherichia-Aerobacter cultures isolated from pasteurized milk to survive pasteurization at 62° C. (143.6° F.) for 30 minutes was studied. The technic used has been described under methods used.

Culture BM73, E. paragrünthali, was isolated from a bottle of milk pasteurized at plant I on April 26, as reported in table XXI. The Escherichia-Aerobacter count was 6 per cc. and the standard plate count 7500 per cc.

The heat resistance of the two organisms at 62° C. (143.6° F.) is reported in table XXII. Young cultures (5 hour) and old cultures (70 hour) were studied at ten, twenty and thirty minute intervals by two different methods; open pipettes and sealed tubes. The data show that the longest survival time was 10 minutes. Old cells of E. paragrünthali survived ten minutes heating by the open pipette and sealed tube methods but not 20 minutes. Old cells of A. cloacae survived ten minutes heating by the sealed tube but not by the open pipette method. Young cells of both E. paragrünthali and A. cloacae did not survive ten minutes heating by either of the two methods.

TABLE XXII

Heat Resistance at 62°C. (143.6°F.) of Two Escherichia-Aerobacter Organisms Isolated from Pasteurized Milk.

Culture	Species	Age of Culture							
		5 Hour Milk Culture				70 Hour Milk Culture			
		Standard Plate Count	Growth After 10 Min.	Growth After 20 Min.	Growth After 30 Min.	Standard Plate Count	Growth After 10 Min.	Growth After 20 Min.	Growth After 30 Min.
		I. Open Pipette Method							
BM73	E. paragrünthali	4,900,000	-	-	-	8,800,000	+	-	-
BM90	A. cloacae	12,000,000	-	-	-	25,000,000	-	-	-
		II. Sealed Tube Method							
BM73	E. paragrünthali	4,900,000	-	-	-	8,800,000	+	-	-
BM90	A. cloacae	12,000,000	-	-	-	25,000,000	+	-	-

The above results indicate that the two organisms studied were unable to survive pasteurization temperature and suggests that their presence in the pasteurized samples was due either to faulty pasteurization or to subsequent contamination. The survival of old cells for longer periods than the young cells confirms the results of Sherman and Stark (39) and Hammer and Hussong (18) who have shown that young cells are more easily killed than are older ones.

Raw Cream

The number of Escherichia-Aerobacter organisms was determined in 24 samples of raw cream supplied the College Dairy for buttermaking during February, March and April, 1930. Thirteen of the samples were sweet cream and eleven sour cream. The cream sampled was approximately two days old, cream deliveries being made three times a week. The majority were composite samples representing cream from a number of patrons.

The Escherichia-Aerobacter counts of the sweet and sour raw cream are given in table XXIII. The data show that 61.5 per cent of the sweet cream samples (8 samples) and 63.6 per cent of the sour cream samples had Escherichia-Aerobacter counts over 10,000. These results indicate that the number of Escherichia-Aerobacter organisms in sweet and sour raw cream is about

TABLE XXIII

Escherichia-Aerobacter Counts of Sweet and Sour Raw Cream.

1930 Date	Grade of Cream	
	Sweet	Sour
	E-A Count	E-A Count
Feb. 17		2,500
" 24	25,000	25
" 24	25	25,000
" 28	25,000	
Mar. 3	60,000	2,500
" 10	25,000	60,000
" 13	250	250,000
" 13	600	
" 13	6,000	
" 14	25,000	250,000
April 4	600	250,000
" 5	60,000	6,000
" 15		60,000
" 21	25,000	250,000
" 25	600,000	

the same. The limited number of samples do not warrant definite conclusions. Five Escherichia-Aerobacter counts were over 100,000 per cc. The counts obtained show that much larger numbers of Escherichia-Aerobacter organisms are present in cream than in milk.

Pasteurized Cream

Four samples of cream taken from the pasteurizing vat following pasteurization at 62.8° C. (145° F.) for 30 minutes failed to show the presence of Escherichia-Aerobacter organisms in two cc. quantities of the pasteurized cream.

Ice Cream

The number of Escherichia-Aerobacter organisms was determined in 20 samples of commercial ice cream from 11 Iowa plants. Samples were taken during January and February, 1931. The Escherichia-Aerobacter counts are given in table XXIV. The data show that 70 per cent of the samples had Escherichia-Aerobacter counts less than 100 per cc. while the range in counts was from 3 to 2500 per cc. The history of the samples was unknown so that it was impossible to interpret the results secured.

Discussion

The range in the number of Escherichia-Aerobacter organisms found in the raw milk (less than 1

TABLE XXIV

Escherichia-Aerobacter Counts of Ice Cream.

Sample Number	E-A Count
197	25
198	25
199	3
200	3
201	25
202	25
203	250
204	25
205	250
206	2,500
207	25
208	25
209	25
210	2,500
211	25
212	250
213	3
214	25
215	25
216	250

per cc. to 25,000 per cc.) together with a lack of correlation between the Eschorichia-Aerobacter and standard plate counts indicates that a correct interpretation as to the sanitary significance of these organisms is difficult. The fact that over 50 per cent of the raw milk samples from the College Dairy contained less than 10 Escherichia-Aerobacter organisms per cc. shows that initial contamination is slight with careful methods of production.

Contamination following pasteurization appears to be largely responsible for the presence of Escherichia-Aerobacter organisms in bottled milk pasteurized at 142° F. to 145° F. for 30 minutes. Inasmuch as the samples studied were obtained from a number of commercial plants using various types of pasteurizing equipment, it is felt that the results obtained were representative of commercial pasteurization.

Summary

1. Of 38 samples of raw milk taken between January and June, from individual patrons of the College Dairy, 56.2 per cent contained less than 10 Escherichia-Aerobacter organisms per cc.; 10.5 per cent contained between 10 and 100 organisms per cc.; and 36.9 per cent contained over 100 organisms per cc. The Escherichia-Aerobacter counts ranged from less than 1 per cc. to 25,000

per cc. There was no difference between the number of Escherichia-Aerobacter organisms in cooled night and uncooled morning milk; winter samples contained smaller numbers than did summer samples; and there was a slight correlation between the Escherichia-Aerobacter and standard plate counts.

2. Of 20 samples taken during April, May and June, from composite raw milk supplies of eleven Iowa pasteurization plants, 15 per cent contained less than 10 Escherichia-Aerobacter organisms per cc; 25 per cent contained between 10 and 100 organisms per cc.; and 60 per cent contained over 100 organisms per cc. The Escherichia-Aerobacter counts ranged from less than 1 per cc. to 25,000 per cc. No correlation was found between the Escherichia-Aerobacter and the standard plate counts except in the case of Escherichia-Aerobacter counts over 1,000 per cc.

3. The number of Escherichia-Aerobacter organisms was determined in 39 samples of pasteurized milk representing 15 pasteurization runs at the College Dairy. All Escherichia-Aerobacter organisms were destroyed in 10 cc. quantities of milk in 93.3 per cent of the samples (14 out of 15) taken from the pasteurizing vat following pasteurization at 142° F. to 144° F. for 30 to 35 minutes. Escherichia-Aerobacter organisms were

present in a larger percentage of samples and in larger numbers in the milk bottled first than in the milk bottled later (81.8 per cent of the samples as compared to 53.8 per cent). These results indicated that the presence of Escherichia-Aerobacter organisms in the bottled milk was due to contamination following pasteurization which was gradually reduced as the milk flowed through the equipment.

4. The number of Escherichia-Aerobacter organisms in 10 cc. quantities of pasteurized milk was determined in 45 samples representing 22 commercial pasteurization runs at 13 Iowa pasteurization plants. While 29.4 per cent of the samples taken direct from the pasteurizing vat showed the presence of Escherichia-Aerobacter organisms, the records showed that 3 of the 5 positive samples were pastourized at 140° F. indicating that their presence was largely due to faulty pasteurization rather than to survival of heat resistant strains. Seventy-five per cent of the samples taken from the cooler and from the bottled milk contained Escherichia-Aerobacter organisms. The results obtained were in agreement with those from the College Dairy.

5. Heat resistance studies of young and old cultures of E. paragrünthali and A. cloacae isolated from pasteurized milk, showed that they were destroyed in

20 minutes at 62° C. (143.6° F.) and indicated that their presence in pasteurized milk was due to faulty pasteurization or subsequent contamination. Old cells were more resistant than young cells.

6. Larger numbers of Escherichia-Aerobacter organisms were present in raw cream than in milk. Over 60 per cent of the 24 cream samples studied had Escherichia-Aerobacter counts exceeding 10,000 per cc. Sweet and sour cream samples showed approximately the same Escherichia-Aerobacter counts. Five cream samples had Escherichia-Aerobacter counts exceeding 100,000 per cc.

7. Escherichia-Aerobacter organisms were absent from four samples of cream pasteurized commercially at 62.8° C. (145° F.) for 30 minutes.

8. The number of Escherichia-Aerobacter organisms in 20 samples of commercial ice cream taken during January and February from eleven plants was less than 100 per cc. in 70 per cent of the cases. The counts ranged from 3 per cc. to 2500 per cc.

RELATION OF NUMBER OF ESCHERICHIA-AEROBACTER ORGANISMS TO THE DEVELOPMENT OF OFF-FLAVORS IN EXPERIMENTAL BUTTER

The isolation of organisms belonging to the Escherichia-Aerobacter group by workers at the Iowa Agricultural Experiment Station from samples of defective butter suggested that these organisms may be responsible for certain defects. The ability of the Aerobacter species to grow better at low temperatures than the Escherichia species foretells that the former are most likely to be found in butter. While the storage temperature at which butter is ordinarily held, -17.8° C. (0° F.), is too low for appreciable bacterial development, butter is often held in retail stores and homes at temperatures of from 7.2° C. (45° F.) to 18.3° C. (65° F.) so that appreciable growth may take place. This is especially true of unsalted butter since salt checks bacterial development to a great extent. With the rapid development of the unsalted butter market within the past few years, the problem of defects caused by bacterial deterioration has become an important one and will undoubtedly be of increasing importance in the future.

Hitherto, little study has been made of the growth or action of the Escherichia-Aerobacter group of organisms in butter. The work herein reported was undertaken to give information on the extent of growth, the

species concerned and the defects caused by this group organisms in butter held at temperatures often found in retail stores and homes.

Methods Used

Sweet cream of good quality was pasteurized in three quart lots at 82.2° C. (180° F.) for 15 minutes, after which it was cooled to 21.1° C. (70° F.). The cooled cream was then equally divided between two sterile glass jars of one gallon capacity. This cream was then held over night at 7.2° C. (45° F.) to solidify the fat globules before churning. Just previous to churning, one jar of the cream was inoculated with three to four cc. of a 24 to 48 hour milk culture of the test organism while the other was left uninoculated as a control. The test organisms represented identified species. In the majority of trials, one species belonging to the genus Escherichia and another belonging to the genus Aerobacter were studied at the same time so that results would be comparable.

The churning process was carried out with a small experimental shaker churn commonly used at the Iowa Agricultural Experiment Station. This churned one jar of cream at a time. Churning usually required from 20 to 25 minutes. After churning, the buttermilk was

poured off, the butter washed twice with sterile distilled water at the same temperature as the buttermilk and the butter removed to a sterile enamel dish in which it was worked with a small butter paddle.

Half of the butter in a churning was salted while the other half was left unsalted. Each lot was divided into two portions and wrapped in sterile parchment paper. One portion was held at 7.2° C. (45° F.) while the other was held at 18.3° C. (65° F.). Holding temperatures varied two or three degrees but not enough to affect results appreciably.

An attempt was made to incorporate two per cent salt in the salted butter but due to leakage and difficulty in working the salt uniformly throughout the butter, the salt content varied considerably as subsequent analyses showed. Salt analyses were carried out (after the butter had been held two to five days) as follows: After removing the surface layer, a ten gram sample was weighed on a small piece of parchment paper. The butter was dissolved by placing paper and butter in 250 cc. of hot water contained in a graduate. After the fat had risen to the surface, 25 cc. of the serum was pipetted off and titrated with $AgNO_3$ using K_2CrO_4 as indicator.

The butter was plated after churning and at two, five and ten day intervals. Agar plates were poured with

eosin methylene blue agar which made it possible to distinguish the Escherichia-Aerobacter group from other groups of organisms while in the case of surface colonies, it was possible to distinguish the Escherichia type from the Aerobacter type. Agar plates were poured in duplicate and incubated for two days at 37.5° C., previous tests having shown that all of the test organisms would grow well at this temperature.

A number of direct microscopic counts were made in conjunction with the plate counts, using the method developed by Hammer and Nelson (19). Briefly, this method was as follows: A representative sample of the butter was melted carefully by heating to 45° C. Ten cc. of the melted butter was centrifuged in a separatory funnel, the serum withdrawn and 0.01 cc. of the mixed serum, measured with a Breed pipette, spread over an area of from one to eight square centimeters and stained as in the microscopic count for milk. An estimation of the number of organisms per cc. of butter was found by determining the number per microscopic field of the serum, then the number per cc. of serum and finally the number per cc. of butter.

Expressed as a formula, the number of individual bacteria per cc. was determined as follows:

$$\frac{\text{average number bacteria per field} \times \text{number of square centimeters examined} \times \text{standardization factor}}{9(\text{ratio of butter to serum})^{*}}$$

After plating, the butter was examined by Dr. B. W. Hammer and the author for the development of off-flavors. Sterile spatulas were used in securing the sample and precautions taken to prevent contamination of the butters.

Results Obtained

In order to gain a definite idea in regard to the growth of the Escherichia-Aerobacter group of bacteria in salted and unsalted butter and their relation to the development of off-flavors, four species belonging to the genus Escherichia and three belonging to the genus Aerobacter were studied. Eight series of experiments were carried out as already outlined, in six of which both Escherichia and Aerobacter species were studied. Table XXV gives the detailed data while tables XXVI to XXIX inclusive, present summaries prepared.

Control butter did not show the presence of colonies on eosin methylene blue agar plates when plated in dilutions 1:10 and 1:100 with the exception of the control in series A which developed a count of 5,200,000 per cc. and a pronounced off-flavor in ten days. These colonies were not typical of the Escherichia-Aerobacter

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*For butter with 15.0 to 15.5 per cent moisture, approximately 1.1 cc. of serum is obtained from 10 cc. of butter giving 9 as the ratio of butter to serum.

TABLE X

Bacterial Counts of Escherichia
Development of Off-Flavors in S
Held at 7.2°C. (45°F.) and 18.3°

Series	Culture	Species	Eosin-methylene-blue Agar Plate Counts per c.c. on Inoculated Cream	Butter Holding Period in Days	Salted E.M.B. Agar Plate Counts	Salted Per Cent Salt
A	C16	A.aerogenes (atypical, indol +)	30,000,000	0 2 5 10		
	Control		<100	0 2 5 10		
B	C23	A.oxytocum	23,000,000	0 2 5 10		
	C8	E.coli	14,000,000	0 2 5 10		
C	C11	A.cloacae	7,600,000	0 2 5 10		
	M2	E.communior	5,300,000	0 2 5 10		

* - = no off-flavor
\+ = slight unclean off-flavor characteristic of Aerobacter
++ = pronounced unclean off-flavor characteristic of Aeroba
+++ = very pronounced unclean off-flavor characteristic of A
n.t. = off-flavors, not characteristic of Aerobacter species

TABLE XXV

of Escherichia - Aerobacter Organisms and
Off-Flavors in Salted and Unsalted Butter
(45°F.) and 18.3°C.(65°F.)

Butter Holding Temperature							
7.2°C. (45°F.)				18.3°C. (65°F.)			
Salted		Unsalted		Salted		Unsalted	
E.M.B. Agar Plate Counts	Per Cent Salt	Flavor*	E.M.B. Agar Plate Counts	Flavor*	E.M.B. Agar Plate Counts	Flavor*	E.M.B. Agar Plate Counts	Fl
			1,100,000	-			1,100,000	-
							56,000,000	-
			71,000,000	-			56,000,000	-
			61,000,000	-			68,000,000	+++
			<100	-			<100	-
							>100,000	-
			12,000	-			11,000,000	-
			<100,000	-			5,200,000	++
			980,000	-			980,000	-
			16,000,000	-			54,000,000	-
			76,000,000	-			>70,000,000	-
			>30,000,000	++			80,000,000	+++
			2,800,000	-			2,800,000	-
			11,000,000	-			17,000,000	-
			20,000,000	-			23,000,000	-
			4,700,000	+ n.t.			33,000,000	++
			420,000	-			420,000	-
			740,000	-			14,000,000	-
			2,600,000	-			290,000,000	-
			4,900,000	+			110,000,000	+++
			300,000	-			300,000	-
			830,000	-			30,000,000	-
			360,000	-			38,000,000	-
			670,000	-			35,000,000	-

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ABLE XXV

ichia - Aerobacter Organisms and
in Salted and Unsalted Butter
18.3°C.(65°F.)

Butter Holding Temperature					
7.2°C. (45°F.)			18.3°C. (65°F.)		
Unsalted			Salted	Unsalted	
Per Cent Salt	Flavor*	E.M.B. Agar Plate Counts	Flavor*	E.M.B. Agar Plate Counts	Flavor*
		1,100,000	-	1,100,000	-
				56,000,000	-
		71,000,000	-	56,000,000	-
		61,000,000	-	68,000,000	+++
		<100	-	<100	-
				>100,000	-
		12,000	-	11,000,000	-
		<100,000	-	5,200,000	++ n.t.
		980,000	-	980,000	-
		16,000,000	-	54,000,000	-
		76,000,000	-	>70,000,000	-
		>30,000,000	++	80,000,000	+++
		2,800,000	-	2,800,000	-
		11,000,000	-	17,000,000	-
		20,000,000	-	23,000,000	-
		4,700,000	+ n.t.	33,000,000	++ n.t.
		420,000	-	420,000	-
		740,000	-	14,000,000	-
		2,600,000	-	290,000,000	-
		4,900,000	+	110,000,000	+++
		300,000	-	300,000	-
		830,000	-	30,000,000	-
		360,000	-	38,000,000	-
		670,000	-	35,000,000	-

bacter species
Aerobacter species
e of Aerobacter species
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TABLE)

Series	Culture	Species	Eosin-methylene-blue Agar Plate Counts per c.c. on Inoculated Cream	Butter Holding Period in Days	Salted E.M.B. Agar Plate Counts	Per Cent Salt
D	C1	E.coli		0	300,000	2.00
				2	130,000	
				5	7,500	
				10	7,900	
	Control		<100	0		
				2		
				5		
				10		
	C16	A.aerogenes (atypical, indol +)		0	88,000	1.53
				2	9,000	
				5	69,000	
				10	>500,000	
E	C8	E.coli	2,100,000	0	110,000	1.69
				2	29,000	
				5	17,000	
				10	6,500	
	Control		<100	0	<100	
				2	<100	
				5	<100	
				10	<100	
	C23	A.oxytocum	2,100,000	0	220,000	1.35
				2	18,000	
				5	5,000	
				10	120,000	
F	M14	E.formica (atypical, indol +)	3,400,000	0	260,000	1.27
				2	21,000	
				5	43,000	
				10	16,000	
	Control		<100	0	<100	
				2	<100	
				5	<100	
				10	<100	

TABLE XXV (continued)

Butter Holding Temperature								
7.2°C. (45°F.)						18.3°C. (65°F.)		
Salted			Unsalted			Salted		Unsalted
E.M.B. Agar Plate Counts	Per Cent Salt	Flavor*	E.M.B. Agar Plate Counts	Flavor*		E.M.B. Agar Plate Counts	Flavor*	E.M.B. Agar Plate Counts
300,000	2.00	-	300,000	-		300,000	-	300,000
130,000		-	530,000	-		1,200,000	-	25,000,000
7,500		-	85,000	-		1,500,000	-	21,000,000
7,900		-	5,130,000	-		1,200,000	-	20,000,000
			<100	-				<100
			<100	+	n.t.			<100
			<100	+	n.t.			<100
			<100	+	n.t.			<100
88,000	1.53	-	88,000	-		88,000	-	88,000
9,000		-	230,000	-		22,000,000	-	100,000,000
69,000		-	75,000	-		23,000,000	-	46,000,000
>500,000		-	>1,000,000	-		6,000,000	++	50,000,000
110,000	1.69	-	110,000	-		110,000	-	110,000
29,000		-	130,000	-		1,200,000	-	18,000,000
17,000		-	97,000	-		2,300,000	-	19,000,000
6,500		-	150,000	-		1,200,000	-	23,000,000
<100		-	<100	-		<100	-	<100
<100		-	<100	-		<100	-	<100
<100		-	<100	-		<100	-	<100
<100		-	<100	-		<100	-	<100
220,000	1.35	-	220,000	-		220,000	-	220,000
18,000		-	190,000	-		2,100,000	+	16,000,000
5,000		-	330,000	-		3,000,000	+	100,000,000
120,000		-	1,000,000	-		11,000,000	+	130,000,000
260,000	1.27	-	260,000	-		260,000	-	260,000
21,000		-	160,000	-		1,200,000	-	16,000,000
43,000		-	99,000	-		1,100,000	-	11,000,000
16,000		-	85,000	+	n.t.	8,300,000	-	22,000,000
<100		-	<100	-		<100	-	<100
<100		-	<100	-		<100	-	<100
<100		-	<100	-		<100	-	<100
<100		-	<100	-		<100	-	<100

XXV (continued)

Butter Holding Temperature						
7.2°C. (45°F.)				18.3°C. (65°F.)		
Unsalted		Salted		Unsalted		
E.M.B. Agar		E.M.B. Agar		E.M.B. Agar		
Flavor*	Plate Counts	Flavor*	Plate Counts	Flavor*	Plate Counts	Flavor*
-	300,000	-	300,000	-	300,000	-
-	530,000	-	1,200,000	-	25,000,000	+ n.t.
-	85,000	-	1,500,000	-	21,000,000	+ n.t.
-	5,130,000	-	1,200,000	-	20,000,000	+ n.t.
	<100	-			<100	-
	<100	+ n.t.			<100	-
	<100	+ n.t.			<100	-
	<100	+ n.t.			<100	++ n.t.
-	88,000	-	88,000	-	88,000	-
-	230,000	-	22,000,000	-	100,000,000	++
-	75,000	-	23,000,000	-	46,000,000	++
-	>1,000,000	-	6,000,000	++	50,000,000	+++
-	110,000	-	110,000	-	110,000	-
-	130,000	-	1,200,000	-	18,000,000	-
-	97,000	-	2,300,000	-	19,000,000	-
-	150,000	-	1,200,000	-	23,000,000	-
-	<100	-	<100	-	<100	-
-	<100	-	<100	-	<100	-
-	<100	-	<100	-	<100	-
-	<100	-	<100	-	<100	++ n.t.
-	220,000	-	220,000	-	220,000	-
-	190,000	-	2,100,000	+	16,000,000	++
-	330,000	-	3,000,000	+	100,000,000	++
-	1,000,000	-	11,000,000	+	130,000,000	+++
-	260,000	-	260,000	-	260,000	-
-	160,000	-	1,200,000	-	16,000,000	-
-	99,000	-	1,100,000	-	11,000,000	-
-	85,000	+ n.t.	8,300,000	-	22,000,000	-
-	<100	-	<100	-	<100	-
-	<100	-	<100	-	<100	-
-	<100	-	<100	-	<100	-
-	<100	-	<100	-	<100	-

TABL

Series	Culture	Species	Eosin-methylene-blue Agar Plate Counts per c.c. on Inoculated Cream	Butter Holding Period in Days	7.2° Salted E.M.B. Agar Plate Counts	Per Cent Salt
G	C11	A.cloacae	9,600,000	0	280,000	1.50
				2	110,000	
				5	330,000	
				10	180,000	
	M2	E.communior	2,900,000	0	490,000	2.00
				2	52,000	
				5	50,000	
				10	60,000	
	Control			0	<10	2.00
				2	<10	
				5	<10	
				10	<10	
H	H1	A.cloacae	14,000,000	0	1,000,000	0.69
				2		
				5	420,000	
				10	800,000	
	H3	A.aerogenes (atypical, indol +)	2,100,000	0	140,000	0.74
				2	20,000	
				5	510,000	
				10	1,800,000	
	BM4	E.paragrinthal	1,800,000	0	160,000	0.79
				2	61,000	
				5	57,000	
				10	40,000	
	Control		<10	0	<10	0.71
				2	<10	
				5	<10	
				10	<10	

TABLE XXV (continued)

Butter Holding Temperature							
7.2°C. (45°F.)					18.3°C. (65°F.)		
Salted			Unsalted		Salted		Unsal
E.M.B. Agar Plate Counts	Per Cent Salt	Flavor*	E.M.B. Agar Plate Counts	Flavor*	E.M.B. Agar Plate Counts	Flavor*	E.M.B. Agar Plate Counts
280,000	1.50	-	280,000	-	280,000	-	280,000
110,000		-	310,000	-	1,900,000	-	32,000,000
330,000		-	3,300,000	-	26,000,000	-	170,000,000
180,000		-	17,000,000	-	18,000,000	+	110,000,000
490,000	2.00	-	490,000	-	490,000	-	490,000
52,000		-	300,000	-	1,900,000	-	30,000,000
50,000		-	830,000	-	17,000,000	-	28,000,000
60,000		-	2,500,000	-	12,000,000	-	29,000,000
<10	2.00	-	<10	-	<10	-	<10
<10		-	<10	-	<10	-	<10
<10		-	<10	-	<10	-	<10
<10		-	<10	-	<10	-	<10
1,000,000	0.69	-	1,000,000	-	1,000,000	-	1,000,000
		-	1,000,000	-	22,000,000	-	70,000,000
420,000		-	6,500,000	-	48,000,000	-	190,000,000
800,000		-	18,000,000	++	28,000,000	+	150,000,000
140,000	0.74	-	140,000	-	140,000	-	140,000
20,000		-	500,000	-	13,000,000	-	70,000,000
510,000		-	4,100,000	-	50,000,000	-	78,000,000
1,800,000		-	9,000,000	+	19,000,000	+	78,000,000
160,000	0.79	-	160,000	-	160,000	-	160,000
61,000		-	210,000	-	37,000,000	-	4,900,000
57,000		-	250,000	-	9,700,000	-	35,000,000
40,000		-	300,000	-	12,000,000	-	33,000,000
<10	0.71	-	<10	-	<10	-	<10
<10		-	<10	-	<10	-	<10
<10		-	<10	-	<10	-	<10
<10		-	<10	-	<10	-	<10

TABLE XXV (continued)

	Butter Holding Temperature						
7.2°C. (45°F.)				18.3°C. (65°F.)			
	Unsalted		Salted		Unsalted		
er : ent: alt:	Flavor*	E.M.B. Agar Plate Counts	Flavor*	E.M.B. Agar Plate Counts	Flavor*	E.M.B. Agar Plate Counts	Flavor*
.50	-	280,000	-	280,000	-	280,000	-
	-	310,000	-	1,900,000	-	32,000,000	+
	-	3,300,000	-	26,000,000	-	170,000,000	++
	-	17,000,000	-	18,000,000	+	110,000,000	+++
.00	-	490,000	-	490,000	-	490,000	-
	-	300,000	-	1,900,000	-	30,000,000	-
	-	830,000	-	17,000,000	-	28,000,000	-
	-	2,500,000	-	12,000,000	-	29,000,000	-
.00	-	<10	-	<10	-	<10	-
	-	<10	-	<10	-	<10	-
	-	<10	-	<10	-	<10	-
	-	<10	-	<10	-	<10	-
.69	-	1,000,000	-	1,000,000	-	1,000,000	-
	-	1,000,000	-	22,000,000	-	70,000,000	+
	-	6,500,000	-	48,000,000	-	190,000,000	++
	-	18,000,000	++	28,000,000	+	150,000,000	+++
.74	-	140,000	-	140,000	-	140,000	-
	-	500,000	-	13,000,000	-	70,000,000	+
	-	4,100,000	-	50,000,000	-	78,000,000	++
	-	9,000,000	+	19,000,000	+	78,000,000	+++
.79	-	160,000	-	160,000	-	160,000	-
	-	210,000	-	37,000,000	-	4,900,000	-
	-	250,000	-	9,700,000	-	35,000,000	-
	-	300,000	-	12,000,000	-	33,000,000	+ n.t.
.71	-	<10	-	<10	-	<10	-
	-	<10	-	<10	-	<10	-
	-	<10	-	<10	-	<10	-
	-	<10	-	<10	-	<10	-

group. It is probable that a number of spore-forming bacteria developed in the butter due to the fact that slight and pronounced off-flavors developed in some of the controls.

Bacterial Counts at 7.2° C. (45° F.)

Bacterial counts of the Escherichia-Aerobacter organisms in salted and unsalted butter held at 7.2° C. (45° F.) are given in table XXVI. The data show that bacterial counts on the fresh Escherichia and Aerobacter butters* were near enough alike to make the studies comparable. Seven Escherichia butters ranged in count from 110,000 to 2,800,000 per cc. with only one butter having a count exceeding 500,000 per cc. Eight Aerobacter butters ranged in count from 88,000 to 1,000,000 per cc. with three butters exceeding 500,000 per cc.

Salted Escherichia butters held for two days gave counts ranging from 21,000 to 130,000 per cc. showing a reduction of over 50 per cent from the number of organisms present in the fresh butters. After holding for ten days, the counts ranged from 6,500 to 60,000 per cc. showing a further decrease in number of organisms.

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* The term Escherichia butters refers to butter (more than one lot) churned from cream inoculated with organisms of species belonging to the genus Escherichia while the term Aerobacter butters refers to butter churned from cream inoculated with organisms of species belonging to the genus Aerobacter.

TABLE

Bacterial Counts o
Organisms in Salte
Held at 7.2° C. (4

A. Species Belong

Series	Culture	Species	Eosin-methy	
			Butter After Churning	2
B	C8	E. coli	2,800,000	
C	M2	E. communior	300,000	
D	C1	E. coli	300,000	130,000
E	C8	E. coli	110,000	29,000
F	M14	E. formica	260,000	21,000
G	M2	E. communior	490,000	52,000
H	BM4	E. paragrünthali	160,000	61,000

B. Species Belon

Series	Culture	Species	Eosin-methy	
			Butter After Churning	2
A	C16	A. aerogenes	1,100,000	
B	C23	A. oxytocum	980,000	
C	C11	A. cloacae	420,000	
E	C16	A. aerogenes	88,000	9,000
F	C23	A. oxytocum	220,000	18,000
G	C11	A. cloacae	280,000	110,000
H	H1	A. cloacae	1,000,000	--
H	H3	A. aerogenes	140,000	20,000

TABLE XXVI

erial Counts of Escherichia-Aerobacter
nisms in Salted and Unsalted Butters.
at 7.2° C. (45°F.)

Species Belonging to Genus Escherichia

Eosin-methylene-blue Agar Plate Counts After Two Days at 37.5°C.					
Salted			Unsalted		
Days Held			Days Held		
2	5	10	2	5	10
			11,000,000	20,000,000	4,700,000
			830,000	360,000	660,000
130,000	7,500	7,900	530,000	85,000	5,200,000
29,000	17,000	6,500	130,000	97,000	150,000
21,000	43,000	16,000	160,000	99,000	85,000
52,000	50,000	60,000	300,000	830,000	2,500,000
61,000	57,000	40,000	210,000	250,000	300,000

Species Belonging to Genus Aerobacter

Eosin-methylene-blue Agar Plate Counts After Two Days at 37.5°C.					
Salted			Unsalted		
Days Held			Days Held		
2	5	10	2	5	10
			--	71,000,000	61,000,000
			16,000,000	76,000,000	>30,000,000
			740,000	2,600,000	4,900,000
9,000	69,000	>500,000	230,000	75,000	>1,000,000
18,000	5,000	120,000	190,000	330,000	>1,000,000
110,000	330,000	180,000	310,000	3,300,000	17,000,000
--	420,000	800,000	1,000,000	6,500,000	18,000,000
20,000	510,000	1,800,000	500,000	4,100,000	9,000,000

Unsalted Escherichia butters held for two days gave counts ranging from 130,000 to 11,000,000 per cc. In the majority of cases, these counts were approximately the same as for the fresh butters showing that little or no growth had taken place. After holding for ten days, the counts ranged from 85,000 to 5,200,000 per cc. Six of the seven counts were appreciably higher than counts for the fresh butters showing that growth of organisms had taken place.

Salted Aerobacter butters held for two days gave counts ranging from 9,000 to 110,000 per cc. and like the salted Escherichia butters, showed a reduction of over 50 per cent from the number of organisms present in the fresh butters. After holding for ten days, the counts ranged from 120,000 to 1,800,000 per cc. showing, unlike the corresponding Escherichia butters, an increase in the bacterial counts.

Unsalted Aerobacter butters held for two days gave counts ranging from 190,000 to 16,000,000 per cc. After holding for ten days, the counts ranged from 1 to 61 millions per cc. with four butters out of eight giving counts exceeding 10 millions per cc. This shows that considerable development of organisms took place in ten days. These counts were several times higher than the corresponding Escherichia butter counts.

From the data obtained, it is evident that (1) Aerobacter species find a temperature of 7.2° C. (45° F.) more favorable for growth in salted and unsalted butters than do Escherichia species; (2) both Escherichia and Aerobacter species first decrease in numbers in salted butters held at 7.2° C. (45° F.) with the Escherichia species continuing to decrease in numbers during a ten day holding period while the Aerobacter species become more salt tolerant and slowly increase in numbers; (3) Escherichia-Aerobacter species develop much more rapidly in unsalted butters than in salted when held at 7.2° C. (45° F.).

Bacterial Counts at 18.3°C(65° F.)

Bacterial counts of Escherichia-Aerobacter species in salted and unsalted butters held at 18.3° C. (65° F.) is reported in table XXVII. The fresh butters were duplicates of those held at 7.2° C. (45° F.) so that counts on the fresh butters are the same as reported in table XXVI.

Salted Escherichia butters held for two days gave counts ranging from 1,200,000 to 1,900,000 per cc. (a count of 37,000,000 is not included as it is not in agreement with the other counts and probably erroneous). After holding for ten days, the counts ranged from

TABLE

Bacterial Counts
Organisms in Salt
Held at 18.3°C. (

A. Species Belon

Series	Culture	Species	Butter After Churning	Eosin-methyle	
				2	D
B	C8	E. coli	2,800,000		
C	M2	E. communior	300,000		
D	C1	E. coli	300,000	1,200,000	1,
E	C8	E. coli	110,000	1,200,000	2,
F	M14	E. formica	260,000	1,200,000	1,
G	M2	E. communior	490,000	1,900,000	17,
H	BM4	E. paragrunthali	160,000	37,000,000	9,

B. Species Belon

A*	C16	A. aerogenes	1,100,000		
B	C23	A. oxytocum	980,000		
C	C11	A. cloacae	420,000		
E	C16	A. aerogenes	88,000	22,000,000	23,
F	C23	A. oxytocum	220,000	2,100,000	3,
G	C11	A. cloacae	280,000	1,900,000	26,
H	H1	A. cloacae	1,000,000	22,000,000	48,
H	H3	A. aerogenes	140,000	13,000,000	50,

* The control in series A developed a count of 5,200,000
of ten days. No explanation for this could be ascertai

TABLE XXVII

erial Counts of Escherichia-Aerobacter
nisms in Salted and Unsalted Butters
l at 18.3°C. (65°F.)

Species Belonging to Genus Escherichia.

Eosin-methylene-blue Agar Plate Counts After Two Days at 37.5°C.					
Salted			Unsalted		
Days Held			Days Held		
2	5	10	2	5	10
			17,000,000	23,000,000	33,000,000
			30,000,000	38,000,000	35,000,000
00,000	1,500,000	1,200,000	25,000,000	21,000,000	20,000,000
00,000	2,300,000	1,200,000	18,000,000	19,000,000	23,000,000
00,000	1,100,000	8,300,000	16,000,000	11,000,000	22,000,000
00,000	17,000,000	12,000,000	30,000,000	28,000,000	29,000,000
00,000	9,700,000	12,000,000	4,900,000	35,000,000	33,000,000

Species Belonging to Genus Aerobacter.

			56,000,000	56,000,000	68,000,000
			54,000,000	70,000,000	80,000,000
			14,000,000	290,000,000	110,000,000
00,000	23,000,000	6,800,000	100,000,000	46,000,000	50,000,000
00,000	3,000,000	11,000,000	16,000,000	100,000,000	130,000,000
00,000	26,000,000	18,000,000	32,000,000	170,000,000	110,000,000
00,000	48,000,000	28,000,000	70,000,000	190,000,000	150,000,000
00,000	50,000,000	19,000,000	70,000,000	78,000,000	78,000,000

: of 5,200,000 per cc. and a pronounced off-flavor at the end
ıld be ascertained.

1,200,000 to 12,000,000 per cc. These counts were several times higher than those of the fresh butters showing that considerable growth of organisms had taken place.

Unsalted Escherichia butters held for two days gave counts ranging from 4,900,000 to 30,000,000 per cc. After holding for ten days, the counts ranged from 20,000,000 to 35,000,000 per cc showing a large increase in the number of organisms, the greater part of which took place during the first two days of holding.

Salted Aerobacter butters held for two days gave counts ranging from 1,900,000 to 22,000,000 per cc. After holding for ten days, the counts ranged from 6,800,000 to 28,000,000 per cc. Three of the five butters gave counts considerably higher than any of the corresponding Escherichia butters showing that conditions were more favorable for the growth of the Aerobacter species than for the Escherichia species.

Unsalted Aerobacter butters held for two days gave counts ranging from 14,000,000 to 100,000,000 per cc. After holding for ten days, the counts ranged from 50,000,000 to 150,000,000 per cc. These bacterial counts were the highest found in the entire study and were several times higher than counts for the corresponding Escherichia butters.

The results secured show that (1) Escherichia-

Aerobacter species increase more rapidly and reach higher numbers in butters held for ten days at 18.3° C. (65° F.) than in butters held at 7.2° C. (45° F.); (2) the Aerobacter species increase more rapidly and reach higher numbers than do the Escherichia species; (3) Escherichia-Aerobacter species increase more rapidly and reach numbers several times as high in unsalted butters as in salted butters.

The development of off-flavors by Escherichia-Aerobacter species in salted and unsalted butters held at 7.2° C. (45° F.) and 18.3° C. (65° F.) is reported in table XXVIII.

Salted control butters did not develop off-flavors either at 7.2° C. (45° F.) or 18.3° C. (65° F.). Unsalted control butters held at 7.2°C. (45° F.) for ten days developed a slight off-flavor in one out of five cases. At 18.3° C. (65° F.) for ten days, two butters developed pronounced off-flavors. The off-flavors produced were not as objectionable as those produced by the Aerobacter species. Due to pasteurization of the cream at a high temperature, 82.2° C. (180° F.) for 15 minutes, a heated flavor was normally present in all of the butter.

Development of Off-Flavors at 7.2° C.(45°F.)

Salted Escherichia butters held at 7.2° C. (45° F.) for ten days did not develop off-flavors.

TABLE

Development of Off-Flavors in

A. Species Belonging

Holding Temperature	Holding Period in Days	Development Name of Species with N				
		E. coli (3)		E. communior (2)		E.
		Salted	Unsalted	Salted	Unsalted	Salt
7.2°C. (45°F.)	2	3-	3-	2-	2-	1-
	5	3-	3-	2-	2-	1-
	10	3-	2- 1+n.t.	2-	2-	1-
18.3°C. (65°F.)	2	3-	2- 1+n.t.	2-	2-	1-
	5	3-	2- 1+n.t.	2-	2-	1-
	10	3-	1- 1+n.t. 1++n.t.	2-	2-	1-

B. Species Belonging

Holding Temperature	Holding Period in Days	Development of Off Name of Species with Number Trial				
		A. aerogenes (3)		A. oxytocum (2)		A.
		Salted	Unsalted	Salted	Unsalted	Salt
7.2°C. (45°F.)	2	3-	3-	2-	2-	2-
	5	3-	3-	2-	2-	2-
	10	3-	2- 1+	2-	1- 1++	2-
18.3°C. (65°F.)	2	3-	1- 1+ 1++	1+	1- 1++	2-
	5	3-	1- 2++	1+	1- 1++	2-
	10	1+ 1++	3+++	1+	2+++	1+

TABLE XXVIII

Off-Flavors in Salted and Unsalted Butters.

cies Belonging to Genus Escherichia

Development of Off-Flavors*						
Species with Number Trials						
(2)	E. formica (1)		E. paragrunthali (1)		Controls (5)	
alted	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted
-	1-	1-	1-	1-	5-	4- 1+n.t.
-	1-	1-	1-	1-	5-	4- 1+n.t.
-	1-	1+n.t.	1-	1-	5-	4- 1+n.t.
-	1-	1-	1-	1-	5-	5-
-	1-	1-	1-	1-	5-	5-
-	1-	1-	1-	1+n.t.	5-	3- 2++n.t.

cies Belonging to Genus Aerobacter

elopment of Off-Flavors*				
th Number Trials				
(2)	A. cloacae (2)		Controls (5)	
alted	Salted	Unsalted	Salted	Unsalted
-	2-	2-	5-	4- 1+n.t.
-	2-	2-	5-	4- 1+n.t.
- ++	2-	1+ 1++	5-	4- 1+n.t.
- ++	2-	1- 1+	5-	5-
- ++ +++	2- 1++	1- 1++ 2+++	5-	5- 3- 2++n.t.

*

- no off-flavor
+ slight unclea flavor charac istic of Aero species.
++ pronounced un off-flavor ch acteristic of bacter specie
+++ very pronounc clean off-fla characteristi Aerobacter sp
n.t. off-flavors n characteristi Aerobacter sp

E XXVIII

n Salted and Unsalted Butters.

g to Genus Escherichia

ent of Off-Flavors*					
Number Trials					
E. formica (1)		E. paragrunthali (1)		Controls (5)	
lted	Unsalted	Salted	Unsalted	Salted	Unsalted
-	1-	1-	1-	5-	4- 1+n.t.
-	1-	1-	1-	5-	4- 1+n.t.
-	1+n.t.	1-	1-	5-	4- 1+n.t.
-	1-	1-	1-	5-	5-
-	1-	1-	1-	5-	5-
-	1-	1-	1+n.t.	5-	3- 2++n.t.

g to Genus Aerobacter

ff-Flavors*			
als			
. cloacae (2)		Controls (5)	
lted	Unsalted	Salted	Unsal ted
-	2-	5-	4- 1+n.t.
-	2-	5-	4- 1+n.t.
-	1+ 1++	5-	4- 1+n.t.
-	1- 1+	5-	5-
-	1- 1++	5-	5-
++	2+++	5-	3- 2++n.t.

*

- \- no off-flavor
- \+ slight unclean off-flavor characteristic of Aerobacter species.
- ++ pronounced unclean off-flavor characteristic of Aerobacter species.
- +++ very pronounced unclean off-flavor characteristic of Aerobacter species.
- n.t. off-flavors not characteristic of Aerobacter species.

Unsalted Aerobacter butters held at 7.2° C. (45° F.) for ten days developed a slight or pronounced unclean off-flavor four times out of seven or in over 50 per cent of the cases. These results are significant and show that it is possible for Aerobacter species to develop objectionable off-flavors in unsalted butters held at 7.2° C. (45° F.) in as short a holding time as ten days.

Development of Off-Flavors at 18.3° C. (65° F.)

Salted Escherichia butters held at 18.3° C. (65° F.) for ten days did not develop off-flavors.

Unsalted Escherichia butters held at 18.3° C. (65° F.) for ten days developed a slight or pronounced off-flavor three times out of seven. As corresponding control butters developed pronounced off-flavors, two times out of five, the foregoing results are not significant. In view of the high bacterial counts at the end of the ten day holding period, between 20,000,000 and 30,000,000 per cc., it was surprising to find that these butters showed so little deterioration as judged by the presence of off-flavors.

Salted Aerobacter butters held at 18.3° C. (65° F.) for two and five days developed a slight off-flavor in one sample out of six. At ten days, all of the butters showed a slight or pronounced off-flavor.

These results are important because they show that organisms belonging to the genus Aerobacter are sufficiently salt tolerant to develop appreciably and cause off-flavors in salted butters held at a temperature 18.3° C. (65° F.) frequently encountered in stores and homes. The ten day counts of the salted Aerobacter butters reported in table XXVII, 6,800,000 to 28,000,000 per cc., accompanied by off-flavor were appreciably lower than ten day counts of unsalted Escherichia butters (with one exception) where the off-flavors produced were not significant. This indicates that the type of organism present is of more importance than mere numbers, from the standpoint of butter deterioration.

Unsalted Aerobacter butters held at 18.3° C. (65° F.) for two days developed a slight or pronounced off-flavor four times out of seven. In ten days, all seven developed a very pronounced unclean off-flavor. These results show a correlation between the number of organisms belonging to the genus Aerobacter and the degree of off-flavor development. From table XXVII, it will be noted that the bacterial counts, 50,000,000 to 150,000,000 per cc., were higher than for any of the other butters while the off-flavors produced were more pronounced.

Per Cent of Organisms of Cream Retained in Butter

The majority of the organisms present in cream are lost in the buttermilk during the churning process. Table XXIX gives the number of organisms retained in the fresh butters after churning cream inoculated with Escherichia-Aerobacter organisms. The data show that from about 3 to about 20 per cent of the bacteria per cc. cream were retained per cc. butter. Eleven of the fourteen butters retained less than 10 per cent of the bacteria per cc. cream. These results agree fairly well with those of Grimes (15) who found that from about 5 to about 30 per cent of the bacteria per cc. cream were retained per cc. butter when the butter was made from sweet cream.

Ratio of the Direct Microscopic to the Eosin Methylene Blue Agar Plate Count of Butter

The development of a direct microscopic technique for estimating the number of bacteria in butter by Hammer and Nelson (19) at the Iowa Agricultural Experiment Station suggested that this method might be of use in determining the dilutions necessary for the agar plate counts. Twelve samples of butter were examined by this method. The ratios of the direct microscopic to the eosin methylene blue agar plate counts are given in table XXX. The data show that the ratios of the direct

TABLE XXIX

Per Cent of Bacteria of Inoculated Cream Retained in Butter.

Series	Culture	Species	Eosin-methylene-blue Agar Plate Counts, 2 Days at 37.5°C.		Per cent of Bacteria Per C.C. Cream Retained Per C.C. Butter
			Inoculated Cream	Butter After Churning	
A	C16	A. aerogenes	30,000,000	1,100,000	3.7
B	C23	A. oxytocum	23,000,000	980,000	4.3
	C8	E. coli	14,000,000	2,800,000	20.7
C	C11	A. cloacae	7,600,000	420,000	5.5
	M2	E. communior	5,300,000	300,000	5.7
D	C1	E. coli	----	300,000	---
E	C16	A. aerogenes	1,300,000	88,000	6.8
	C8	E. coli	2,100,000	110,000	5.2
F	C23	A. oxytocum	2,100,000	220,000	10.5
	M14	E. formica	3,400,000	260,000	7.6
G	C11	A. cloacae	9,600,000	280,000	2.9
	M2	E. communior	2,900,000	490,000	16.9
H	H1	A. cloacae	14,000,000	1,000,000	7.1
	H3	A. aerogenes	2,100,000	140,000	6.7
	BM4	E. paragrunthali	1,800,000	160,000	8.9

TABLE XXX

Ratio of the Direct Microscopic to the Eosin Methylene Blue Agar Plate Count of Butter.

Sample number	Direct Microscopic Count of Individual Bacteria	Eosin-methylene-blue Agar Plate Count, 2 days at 37.5°C.	Ratio of Direct to Plate Count
1	2,700,000	420,000	6.4 : 1
2	590,000	300,000	2.0 : 1
3	5,200,000	740,000	7.0 : 1
4	1,800,000	830,000	2.2 : 1
5	210,000	130,000	1.6 : 1
6	1,500,000	530,000	2.8 : 1
7	2,200,000	360,000	6.2 : 1
8	2,900,000	1,200,000	2.4 : 1
9	34,000,000	25,000,000	1.4 : 1
10	35,000,000	1,900,000	18.4 : 1
11	31,000,000	30,000,000	1.0 : 1
12	31,000,000	1,900,000	16.3 : 1

microscopic to the plate count ranged from 1:1 to 18.4:1.

This wide range in ratios was surprising in view of the fact that the Escherichia-Aerobacter organisms grew well on the plating medium and were present in the microscopic preparations, mostly singly or in pairs, so that the ratios should have been quite narrow. No explanation for this irregularity was found. It was observed, however, in many cases that agar plates prepared from the highest dilutions contained a number of colonies not commensurate with the next lowest dilution, always being too few in number. This indicated considerable error in the plate method and suggested that the wide range in ratios was due to this factor.

Discussion

The data obtained on the growth of organisms of the Escherichia-Aerobacter group in salted and unsalted butter held at 7.2° C. (45° F.) and 18.3° C. (65° F.) show that the Escherichia species find conditions less favorable for growth or survival than do the Aerobacter species. These findings have been confirmed by the fact, already reported, that none of 25 Escherichia-Aerobacter cultures isolated from samples of defective butter by workers at the Iowa Agricultural Experiment Station proved to be

Escherichia species. The temperatures at which butter is held do not favor the Escherichia species as well as the Aerobacter. This becomes evident when it is remembered that the chief habitat of organisms belonging to the genus Escherichia is the intestinal tract of warm blooded animals while the chief habitat of organisms belonging to the genus Aerobacter is soils and grasses.

The growth of both Escherichia and Aerobacter species in salted butters held at 18.3° C. (65° F.) shows that many of the cells are or become salt tolerant. Inasmuch as the salt is dissolved in the moisture present, a butter containing 2 per cent salt and 15.5 per cent moisture has a brine concentration of 12.9 per cent in which the organisms are present.

The peculiar mechanism of the bacterial deterioration of butter is unlike that of other dairy products inasmuch as the bacteria are locked up in the moisture droplets. Rahn and Boysen (34) estimate that there are between 10 and 18 billions of moisture droplets per gram of butter and state that no more than 60 millions of bacteria per gram have been found. Consequently, a large percentage of the droplets must be free from bacteria and remain free, because bacteria can not move from one droplet to another. These workers deduce that in butter with

100,000 bacteria per gram, 88 per cent of the moisture is sterile; with 10,000 bacteria, 99 per cent is sterile; and with 1,000 bacteria, 99.9 per cent is sterile. The last butter cannot be attacked noticeably by bacteria. Consequently, the bacterial content of the fresh butter has an important bearing on the ability with which the butter can be attacked.

Summary

Butter was churned from pasteurized cream inoculated with a pure culture of a species belonging to the Escherichia-Aerobacter group. Eight series of experiments were carried out with four different species belonging to the genus Escherichia and three belonging to the genus Aerobacter. Half of each sample of butter was salted while the other half was left unsalted. The salted and unsalted portions were divided and held at 7.2° C. (45° F.) and 18.3° C. (65° F.) for ten days. Eosin methylene blue agar plate counts and examinations for development of off-flavors were made at two, five and ten day intervals. The results obtained were as follows:

1. Freshly churned Escherichia and Aerobacter butters gave bacterial counts ranging from 88,000 to 2,800,000 per cc.

2. Salted Escherichia and Aerobacter butters held 2 days at 7.2° C. (45° F.) showed a reduction of

over 50 per cent (9,000 to 130,000 per cc.) from the bacterial counts of the butters before holding (88,000 to 2,800,000 per cc.); salted Escherichia butters held 10 days showed a further reduction in count (6,500 to 60,000 per cc.) while corresponding Aerobacter butters showed a slight increase over the two day count (120,000 to 1,800,000 per cc.).

3. Unsalted Escherichia butters held 2 days at 7.2° C. (45° F.) showed no increase in bacterial counts (130,000 to 11,000,000 per cc.) while corresponding Aerobacter butters showed a slight increase (190,000 to 16,000,000 per cc.); unsalted Escherichia butters held 10 days showed an appreciable increase in count over the fresh butters (85,000 to 5,200,000 per cc.) while corresponding Aerobacter butters gave counts several times higher (1,000,000 to 61,000,000 per cc.) than the Escherichia butters.

4. Salted Escherichia butters held 2 days at 18.3° C. (65° F.) gave bacterial counts (1,200,000 to 1,900,000 per cc.) three to five times higher than counts of the fresh butters while corresponding Aerobacter butters gave counts (1,900,000 to 22,000,000 per cc.) seven to twenty times higher than the fresh butters; salted Escherichia butters held 10 days at 18.3° C. (65° F.) gave counts ranging from 1,200,000 to 12,000,000 per cc. while corresponding Aerobacter butters gave counts ranging

from 6,800,000 to 28,000,000 per cc. which were several times higher than for the Escherichia butters.

5. Unsalted Escherichia butters held 2 days at 18.3° C. (65° F.) showed a rapid development of organisms (4,900,000 to 30,000,000 per cc.) while corresponding Aerobacter butters showed a still more rapid development (14,000,000 to 100,000,000 per cc.); unsalted Escherichia butters held 10 days at 18.3° C. (65° F.) gave bacterial counts from 20,000,000 to 35,000,000 per cc. while the corresponding Aerobacter butters gave counts from 50,000,000 to 150,000,000 per cc. which were the highest counts encountered during the entire study.

6. Salted and unsalted Escherichia and salted Aerobacter butters held 10 days at 7.2° C. (45° F.) did not develop off-flavors while unsalted Aerobacter butters developed a slight or pronounced unclean off-flavor (4 times in 7).

7. Salted Escherichia butters held 10 days at 18.3° C. (65° F.) did not develop off-flavors while corresponding Aerobacter butters developed a slight or pronounced unclean off-flavor in all cases.

8. Unsalted Escherichia butters held 2 days at 18.3° C. (65° F.) did not develop off-flavors while corresponding Aerobacter butters developed a slight or pronounced off-flavor four times out of seven; unsalted Escherichia butters held 10 days at 18.3° C. (65° F.) did

not show significant off-flavor development while corresponding Agrobacter butters developed a very pronounced unclean off-flavor in all cases.

9. The per cent of the bacteria per cc. cream retained per cc. butter was from about 3 to about 20 per cent.

10. The ratios of the direct microscopic to the eosin methylene blue agar plate counts ranged from 1:1 to 18.4:1.

ACTION OF ESCHERICHIA-AEROBACTER ORGANISMS ON THE CONSTITUENTS OF MILK

The action of the Escherichia-Aerobacter group of organisms on the fat, casein, lactose and citric acid of milk is of the greatest importance from the standpoint of the changes brought about in dairy products. Unfortunately, milk is a difficult medium with which to work, as samples of milk vary enough at times to cause variations in the actions of bacteria. Besides this, organisms acting in association with other organisms, as they do in milk, do not usually act as they do in pure culture so that care must be taken in the interpretation of results. However, unsatisfactory as milk may be for a culture medium, it is far preferable to using simpler culture media and attempting to apply the results to milk.

In this study, representative Escherichia-Aerobacter species isolated from dairy products were grown in milk for the purpose of determining their action on the different milk constituents.

Methods Used

Determination of Action on Butterfat

The ability of Escherichia-Aerobacter organisms to attack butterfat was determined by streaking broth cultures of the test organisms on nile blue sulphate agar

containing a two per cent emulsion of butterfat. The nile blue sulphate agar was prepared by adding 0.8 cc. of a 0.1 per cent aqueous solution of nile blue sulphate per liter of beef infusion agar.

The principle involved in the use of this medium rests upon the fact that organisms which have the ability to attack butterfat liberate free fatty acids which turn the fat droplets a deep blue color in the presence of nile blue sulphate as an indicator.

Determination of Proteolysis

The proteolytic action of Escherichia-Aerobacter organisms on the proteins of milk was determined as follows: Skim milk in 325 cc. quantities was put into pint milk bottles, the weight of the bottle and the milk recorded and the milk sterilized at 15 pounds pressure for 24 minutes. The milk was then inoculated with a 24 hour milk culture of the test organism and incubated 9 days at 30° C. Distilled water was next added to each bottle until the weight was 1 gram less than the original. In precipitating the curd, 1 cc. of glacial acetic acid was added to each bottle and thoroughly distributed the bottle slowly heated in a water bath to 60° C. and the contents filtered through paper until a clear filtrate was secured.

Amino nitrogen was determined by the Van Slyke method, using 10 cc. of the filtrate. Results are expressed as the number of cc. of nitrogen gas formed over or under that of an uninoculated control treated in the same manner as the inoculated samples.

Soluble nitrogen was determined, using 25 cc. of the filtrate. The filtrate was transferred to a 500 cc. Kjeldahl flask and digested after the addition of 25 cc. of concentrated H_2SO_4, about 5 grams of Na_2SO_4, a small piece of copper wire and about 2 grams of trichloracetic acid. The trichloracetic acid was used to limit foaming. When digestion was complete, 150 cc. of water, a small pinch of paraffin, a small amount of zinc and 80 cc. of caustic NaOH (60 per cent) were added to the Kjeldahl flask and the flask connected to the distillation trap. The distillation was collected in a 500 cc. Erlenmeyer flask containing 40 cc. of distilled water and 12.5 cc. of 0.2006 normal H_2SO_4. Five drops of sodium alizarin sulphonate were added to the flask as an indicator. When distillation was complete, the back titration was made using 0.1001 NaOH. The amount of 0.1 normal H_2SO_4 neutralized by the ammonia distilled over was then determined by difference and the result expressed as the increase per 25 cc. of filtrate over the control.

Duplicate determinations were made on the same filtrate. A negative value represents an instance where there was less amino or soluble nitrogen in the filtrate from milk in which an organism had developed than in the filtrate from the control.

Determination of Volatile Acidity

The amount and nature of the volatile acids produced by Escherichia-Aerobacter organisms in milk were determined as follows: Skim milk in 1200 cc. quantities was sterilized in two liter flasks for 24 minutes at 15 pounds pressure. After inoculation with a 24 hour milk culture of the test organism, the flasks of milk were incubated from 7 to 26 days at room temperature. The large quantity of milk was used so that a sufficient volume of volatile acid would be produced to run a determination of the per cent barium in the barium salt.

At the end of the incubation period, the volatile acids were secured by steam distillation of the fermented milk, after the addition of 40 cc. of normal H_2SO_4 to free any volatile acids that might have been fixed by the milk constituents.

For determinations of the total volatile acidity, the first liter of distillate was collected. A 50 cc.

portion was titrated with 0.1 normal NaOH, using phenolphthalein as an indicator, and the number of cc. required for neutralization multiplied by 20 to give a number representing the total volatile acidity. This number represented the number of cc. of 0.1 normal NaOH required to neutralize the first liter of distillate from the fermented milk.

Where the volatile acidity was sufficiently high (above 50) so that enough volatile acid was present for the preparation of a barium salt, the remaining 950 cc. of the distillate were nearly neutralized with 0.1 normal $Ba(OH)_2$ and the aqueous solution of the barium salt evaporated to dryness on the water bath. This barium salt was then dissolved by adding 50 to 75 cc. of hot water and decolorized by adding a small amount of animal charcoal and filtering while hot. The filtrate was then evaporated and the salt crystallized. Finally, the salt was dried at 100° C. to constant weight.

Per Cent Barium in the Barium Salt

The per cent barium in the barium salt was determined in duplicate as follows: A portion of about 0.3 gram was weighed out, transferred to a 250 cc. beaker, dissolved in 50 cc. of hot water, the water brought to boiling and 3.5 cc. of normal H_2SO_4 added slowly. After digesting over night on the hot plate (under petri dish

lids so that the free volatile acids would not be lost) the $BaSO_4$ was filtered off. Care was taken to transfer all of the precipitate to the filter paper by rinsing the beaker repeatedly with the filtrate and finally with distilled water.

The filter paper containing the $BaSO_4$ was then transferred to porcelain crucibles (whose weight had previously been determined) and ignited to constant weight in a muffle furnace. From the weight of the $BaSO_4$ and the weight of the original salt, the per cent barium in the latter was calculated.

Duclaux Method

For confirmation of the barium values, the Duclaux method was carried out on the filtrate left from the barium determination. The filtrate was made up to a volume of 110 cc. and distilled from a 500 cc. Erlenmeyer flask at the rate of 100 cc. in about 45 minutes. The volume of the solution being distilled was kept constant at 110 cc. After discarding the first 10 cc. of distillate, 10 portions of 10 cc. each were collected and titrated with 0.05 normal NaOH using phenolphthalein as an indicator.

Determination of Volatile Acidity Before and After Addition of Citric Acid

The formation of volatile acidity in milk to which sterile citric acid was added was determined as follows: Skim milk was sterilized in 325 cc. quantities in pint milk bottles. Citric acid at the rate of 0.4 per cent or 0.2 per cent (1.3 grams or 0.65 grams) was sterilized seperately in test tubes containing 5 cc. of water and added to the sterile milk.

The bottles of milk were then inoculated with 48 hour milk cultures of the test organisms and incubated for 9 days at 30° C. Two uninoculated bottles one without the addition of citric acid, were held as controls.

Total acidities were determined , using 20 grams of the fermented milk. Volatile acidities were determined by weighing 250 grams into 3 liter round bottom flasks. Fifteen cc. of normal H_2SO_4 were added to each flask before distillation with steam in order to free any volatile acids that might have been fixed by the milk constituents. The first liter of distillate was titrated using 0.1 normal NaOH and the values expressed as the number of cc. required to neutralize the first liter of distillate.

Results Obtained

Action on Butterfat

Broth cultures of E. coli, E. communior, E. formica, E. paragrünthali, A. cloacae, A. aerogenes and A. oxytocum were streaked on nile blue sulphate agar containing butterfat and incubated for 48 hours at 37.5° C. Examination of the streaks showed that the butterfat was not attacked.

Proteolytic Action

The proteolytic action on the proteins of milk was measured by amino and soluble nitrogen determinations on the filtrate secured after Escherichia-Aerobacter species had been allowed to act upon the milk for nine days at 30° C. The results secured with nine species belonging to the genus Escherichia and three species belonging to the genus Aerobacter are given in table XXXI.

The data show that in the majority of trials, amino and soluble nitrogen values were negative when expressed as the increase over the control. These represent instances in which there was less amino or soluble nitrogen in the filtrate from milk in which organisms had developed than in the filtrate from the controls. In the case of culture C16, A. aerogenes, and H1, A. cloacae, a slight increase in the amino and soluble nitrogen over the control was found. This increase was not large enough to be

TABLE XXXI

Proteolysis By Escherichia-A

I. Species Belonging to the

Culture Number	Species	Amino Nitrogen Per 10 CC. Fil: CC. of N_2 Gas			Increa Over Contro
		Trial 1	Trial 2	Average	
	Control	1.10	1.30	1.20	
C1	E. coli	1.00	1.10	1.05	-.15
M2	E. communior	1.05	1.15	1.10	-.10
BM4	E. paragrünthali	1.15	1.10	1.13	-.12
BM8	E. formica	1.05	1.15	1.10	-.10
BM54	E. pseudocoloides	1.15	1.20	1.18	-.07
M24	E. enterica	1.10	1.30	1.20	0.00
BM5	E. vesiculiformans	1.10	1.15	1.13	-.12
M25	E. grünthali	1.10	1.15	1.13	-.12
BM27	E. anaerogenes	1.10	1.00	1.05	-.15

II. Species Belonging to th

	Control	1.20	1.30	1.25	
RP1	A. aerogenes	1.20	1.15	1.18	-.07
C16	A. aerogenes*	1.25	1.30	1.28	+.08
C23	A. oxytocum	1.10	1.20	1.15	-.05
H1	A. cloacae	1.60	1.50	1.55	+.35
C11	A. cloacae*	1.10	1.25	1.18	-.02

* Atypical

** Distillate collected in 500 cc. Erlenmeyer flasks co
40 cc. of distilled water and 5 drops of sodium aliz

TABLE XXXI

Escherichia-Aerobacter Species.

longing to the Genus Escherichia

Per 10 CC. Filtrate: rage	Increase Over Control	Soluble Nitrogen Per 25 CC. Filtrate: CC. of N/10 NaOH Required ** to Neutralize Distillate: Trial 1	Trial 2	Average	CC. of N/10 H_2SO_4 Neutralized by Distillate	Increase Over Control
.20		14.4	14.0	14.20	10.85	
.05	-.15	15.0	15.1	15.05	10.00	-.85
.10	-.10	14.7	14.8	14.75	10.30	-.55
.13	-.12	14.9	14.6	14.75	10.30	-.55
.10	-.10	15.1	15.1	15.10	9.95	-.90
.18	-.07	14.5	14.5	14.50	10.55	-.30
.20	0.00	14.3	14.6	14.45	10.60	-.25
.13	-.12	14.5	15.2	14.85	10.20	-.65
.13	-.12	15.1	14.5	14.80	10.25	-.60
.05	-.15	14.9	14.7	14.80	10.25	-.60
Belonging to the Genus Aerobacter						
.25		14.4	14.0	14.20	10.85	
.18	-.07	15.0	15.1	15.05	10.00	-.85
.28	+.08	15.8	15.9	15.85	9.20	-1.65
.15	-.05	14.7	14.2	14.45	10.60	-.25
.55	+.35	13.7	13.4	13.55	11.50	+.65
.18	-.02	14.8	15.0	14.90	10.15	-.70

meyer flasks containing 12.5 cc. of 0.2006 normal H_2SO_4
of sodium alizarin-sulphonate as an indicator.

significant. The above results indicate that the Escherichia-Aerobacter group of organisms do not cause appreciable proteolysis in milk.

Volatile Acidity

The volatile acidities produced in milk by six cultures representing three Escherichia species and by three cultures representing two Aerobacter species are given in table XXXII. The values given represent the number of cc. of 0.1 normal NaOH required to neutralize the first liter of distillate from a 1200 gram portion of the fermented milk. The data show that the values for the Escherichia species ranged from 68.0 to 80.5 while the values for the Aerobacter species ranged from 25.9 to 48.0. It is evident from these results that the Escherichia species produced about twice as much volatile acidity as the Aerobacter species.

Hammer and Bailey (17) found that the volatile acidity produced by three cultures of Bacterium aerogenes ranged from 9.7 to 13.2 expressed in cc. of 0.1 normal NaOH required to neutralize the first liter of distillate from a 250 gram portion of the fermented milk.

TABLE XXXII

Volatile Acidities Produced by Escherichia-Aerobacter Species.

Series	Culture Number	Species	Incubation Period at 21°C. in Days	Volatile Acidity**
A	C1*	E. coli	7	68.0
"	C23	A. oxytocum	7	25.9
B	C8*	E. coli	10	68.0
"	M2*	E. communior	10	71.0
"	M14*	E. formica	10	68.0
C	C1	E. coli	26	80.5
"	C8	E. coli	26	76.3
"	C16	A. aerogenes	26	48.0
"	C23	A. aerogenes	26	34.0

* Nature of volatile acids determined by percentages of Ba in barium salts and by Duclaux method. (See tables XXXV, XXXVII, and XXXIX).

** The number given represents the number of cc. of 0.1 normal NaOH required to neutralize the first liter of distillate from a 1200 gram portion of the fermented milk.

Relation of Volatile Acidity to Citric Acid Content of Milk

In order to determine the part played by citric acid in the production of volatile acidity by organisms belonging to the Escherichia-Aerobacter group, cultures of E. coli, A. aerogenes, A. oxytocum and two cultures of A. cloacae were grown in milk with and without the addition of 0.4 per cent sterile citric acid. The total and volatile acidity production after nine days incubation at 30° C. is reported in table XXXIII. E. coli showed an increase in volatile acidity after the addition of citric acid (40.7 to 62.5) while the Aerobacter species showed a decrease in the volatile acidities in each of the four trials; 10.6 to 3.2; 14.3 to 7.6; 24.4 to 19.8; and 11.7 to 10.7. These results suggested that the amount of citric acid added (0.4 per cent) might have inhibited the development of the Aerobacter species so a trial was made using a smaller addition of citric acid (0.2 per cent).

Cultures of E. coli, E. communior, E. paragrünthali, and two cultures of A. aerogenes were grown in milk for nine days at 30° C. with and without the addition of 0.2 per cent sterile citric acid. The total and volatile acidities produced are given in table XXXIV. The data confirm the results reported in table XXXIII where 0.4 per cent citric acid was used. The three

TABLE XXXIII

Volatile Acidities Before and After Addition of 0.4 Per Cent Citric Acid.

Culture Number	Species	Milk Without Addition of Citric Acid		Milk Plus 0.4 Per Cent Citric Acid	
		Total Acidity	Volatile Acidity	Total Acidity	Volatile Acidity
Control		0.11	1.6	0.36	1.5
C1	E. coli	0.35	40.7	0.44	62.5
C11*	A. cloacae	0.43	10.6	0.48	3.2
C16*	A. aerogenes	0.48	14.3	0.51	7.6
C23	A. oxytocum	0.54	24.4	0.47	19.8
H1	A. cloacae	0.43	11.7	0.43	10.7

* Atypical

TABLE XXXIV

Volatile Acidities Before and After Addition of 0.2 Per Cent Citric Acid.

Culture Number	Species	Milk Without Addition of Citric Acid		Milk Plus 0.2 Per Cent Citric Acid	
		Total Acidity	Volatile Acidity	Total Acidity	Volatile Acidity
Control		0.19	2.15	0.40	3.10
C1	E. coli	0.46	46.40	0.51	56.60
M2	E. communior	0.52	57.00	0.55	61.85
BM4	E. paragrünthali	0.52	46.90	0.55	57.75
RP1	A. aerogenes	0.56	6.35	0.60	4.85
C16*	A. aerogenes	0.66	9.50	--	9.65

* C16 - Atypical

Escherichia species gave increased volatile acidities with the addition of 0.2 per cent citric acid; 46.4 to 56.6; 57.00 to 61.85; and 46.90 to 57.75 while Aerobacter species showed a decrease or insignificant increase (6.35 to 4.85 and 9.50 to 9.65).

As culture C1, E. coli, gave an increase in volatile acidity of 21.8 (40.7 to 62.5) after the addition of 0.4 per cent citric acid as compared to an increase of 10.2 (46.4 to 56.6) after the addition of 0.2 per cent citric acid, the increase in volatile acidity was in direct proportion to the amount of citric acid added.

Hastings, Mansfield and Helz (20) found that the citric acid in milk was fermented by E. coli and A. cloacae but was not attacked by A. aerogenes. In this study, the two cultures of A. cloacae studied did not show increased volatile acidities after the addition of citric acid. However, this does not necessarily mean that the citric acid was not attacked as it may have been broken down to non-volatile acids and other additional products.

The above workers point out that organisms which ferment citric acid in milk do not necessarily ferment it in a different organic medium, and that organisms which utilize citric acid in an inorganic medium with no other source of carbon do not necessarily ferment citric acid in milk.

Bosworth and Prucha (7) attribute the destruction of citric acid in souring milk to the action of Bact. lactis aerogenes. However, they overlooked the fact that organisms of the S. citrovorous and S. paracitrovorous types were undoubtedly present which were capable of attacking the citric acid.

Nature of Volatile Acids

The nature of the volatile acids produced by the Escherichia species was ascertained by determining the percentages of barium in the barium salts and by Duclaux values. As the Aerobacter species did not give high volatile acidities (25.9 to 48.0) and the quantity of milk worked with was small (1200 grams) the amount of volatile acid produced was not sufficient for the preparation of barium salts.

The percentages of barium in the barium salts prepared from the volatile acids produced by the four Escherichia cultures listed in table XXXII are given in detail in table XXXV while the percentages of barium in the salts prepared from commercial acetic and propionic acids are given in table XXXVI.

The Duclaux values determined on the free volatile acids remaining after the $BaSO_4$ was filtered off are given in detail in table XXXVII while Duclaux values of commercial acetic and propionic acids are given

TABLE XXXV

Percentages of Barium in Barium Salts Prepared from Volatile Acids Produced by Escherichia Species.

Culture	Species	Det.	Weight				Per Cent Barium in Ba Salt
			Barium Salt	Crucible	Crucible plus $BaSO_4$	$BaSO_4$	
C1	E. coli	A	0.2987	8.6817	8.9434	0.2617	51.57
		B	0.1828	8.6726	8.8324	0.1598	51.46
M14	E. formica	A	0.2749	11.1102	11.3552	0.2450	52.46
		B	0.3502	8.8838	9.1958	0.3120	52.44
M2	E. communior	A	0.3284	9.5008	9.7926	0.2918	52.30
		B	0.2809	8.6476	8.8974	0.2498	52.35
C8	E. coli	A	0.2874	11.1478	11.3992	0.2514	51.44
		B	0.3040	7.5146	7.7801	0.2655	51.41

TABLE XXXVI

Percentages of Barium in Salts Prepared from Commercial Acetic and Propionic Acids.

Salts	Per Cent Barium in Barium Salt Average of Two Determinations*	Theoretical
Ba acetate		
Trial 1	53.56	
2	53.64	
3	53.27	53.78
4	53.32	
5	53.46	
Ba propionate		
Trial 1	47.73	
2	47.65	
3	47.66	48.46
4	48.24	
5	48.17	
Ba salt of propionic and acetic acid		
Trial 1	50.24	
2	51.02	
3	51.00	
Ba butyrate		44.10

* Determinations by Mr. M. Michaelian.

TABLE XX

Duclaux Values of Dis
Acids Produced by Esc

Culture	Species	Det.	10	20	30
C1	E. coli	A	11.96	24.25	36.21
		B	12.44	23.92	35.41
M14	E. formica	A	13.34	25.88	36.08
		B	12.33	24.15	35.96
M2	E. communior	A	12.40	24.80	36.49
		B	11.98	23.65	35.33
C8	E. coli	A	12.54	24.77	36.36
		B	11.82	24.24	36.06

* Percentage of the titration figure for the total 100 c

TABLE XXXVII

Values of Distillates from Volatile
roduced by Escherichia Species.

	Duclaux Valuos* CC. of Distillate							
:	30	40	50	60	70	80	90	100
5	36.21	46.51	57.48	66.78	76.08	84.03	92.36	100.00
2	35.41	46.41	56.46	66.03	75.12	83.73	92.13	100.00
8	36.08	47.06	57.25	66.67	75.69	84.71	92.94	100.00
.5	35.96	46.46	56.17	66.14	75.07	83.73	92.13	100.00
0	36.49	47.44	57.42	67.12	76.28	84.92	92.45	100.00
5	35.33	46.65	56.89	66.76	75.75	84.43	92.21	100.00
7	36.36	46.71	57.05	66.46	75.55	84.01	92.16	100.00
4	36.06	46.36	56.97	66.36	75.46	83.94	92.12	100.00

ıe total 100 cc. of distillate.

in table XXXVIII. The data show that most of the values in table XXXVII are approximately the same or slightly lower than the values obtained for pure acetic acid in table XXXVIII showing that the volatile acids formed were almost entirely acetic.

A summation of the barium and Duclaux values is given in table XXXIX. The data show that in two instances, the barium values were slightly above 51 per cent and in two others slightly above 52 per cent. As these values are much nearer the values obtained for pure acetic acid (53.27 to 53.64, table XXXVI) than for pure propionic acid (47.65 to 48.24, table XXXVI), they indicate that the volatile acids formed were largely acetic with small amounts of propionic. These results are not confirmed by the Duclaux values which indicate that the type of volatile acid formed was entirely acetic.

Summary

Representative Escherichia-Aerobacter species isolated from dairy products were studied in milk with respect to their action on butterfat, action on proteins, the amount of volatile acidity produced with and without the addition of citric acid and the nature of the volatile acids formed. The results obtained were as follows:

TABLE XXXVIII

Duclaux Values of Commercial Acetic and Propionic Acids*.

Acid	No. of runs	Duclaux Values CC. of Distillate									
		10	20	30	40	50	60	70	80	90	100
Propionic acid	12	15.68	29.96	42.70	53.91	64.02	72.97	80.82	88.01	94.42	100
Acetic acid	7	12.82	24.94	36.54	47.37	57.58	66.96	75.81	84.38	92.42	100
Propionic and Acetic mixed	6	13.77	26.62	38.68	49.79	60.01	69.64	78.24	85.27	93.40	100

* Values prepared by Mr. M. Michaelian.

TABLE XXXIX

Summary of Barium and Duclaux Values from Volatile Acids Produced by Escherichia Species.

Culture	Species	Volatile* Acidity	Per Cent Barium in Barium Salt			Results of Duclaux
			Det. A	Det. B	Average	
C1	E. coli	68.0	51.57	51.46	51.52	Acetic
M14	E. formica	68.0	52.46	52.44	52.45	Acetic
M2	E. communior	71.0	52.30	52.35	52.33	Acetic
C8	E. coli	76.3	51.44	51.41	51.43	Acetic

* Number of cc. of 0.1 normal NaOH required to neutralize the first liter of distillate from a 1200 gram portion of the fermented milk.

1. Escherichia and Aerobacter species did not attack butterfat.

2. Escherichia and Aerobacter species failed to show appreciable proteolysis in milk.

3. Escherichia species produced volatile acidities ranging from 68.0 to 80.5 (number of cc. of 0.1 normal NaOH required to neutralize the first liter of distillate from a 1200 gram portion of fermented milk) while Aerobacter species produced volatile acidities ranging from 25.9 to 48.0.

4. With the addition of 0.2 and 0.4 per cent citric acid, Escherichia species produced increased volatile acidities while Aerobacter species showed a slight decrease in most cases.

5. The volatile acids produced by Escherichia species were largely acetic with small amounts of propionic as determined by the percentages of barium in the barium salts while they were entirely acetic as determined by Duclaux values.

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