SOME OBSERVATIONS ON THE GROWTH OF A STRAIN OF *ESCHERICHIA COLI* IN RAW AND IN PASTEURISED MILK.

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TABLE OF CONTENTS

	Page
Introduction.	1
The organisms of the coli-aerogenes group.	2
Media employed.	3
Methods	4
Tables 1 and 2.	6
Interpretation of tables.	10
Discussion of results.	12
Conclusions.	15
References.	16
Illustrations.	18
Appendix "A"	
Introduction.	20
Media.	20
Methods.	20
Table 3.	23
Interpretation of table.	23
Discussion of results.	24
References.	26
Charts.	
Appendix "B"	
Introduction.	27
Media.	27
Methods.	27
Table 4.	29
Discussion of results.	30
References.	31
Acknowledgments.	32

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INTRODUCTION.

The *Bacterium coli* was first isolated in 1884 by Escherich (5) from the faeces of a cholera patient, subsequent work showing this organism to be a normal inhabitant of the intestinal canal of man and animals (9,11).

The coli-aerogenes group, of which this organism of Escherich is typical, has since occupied a prominent position in the investigations of many bacteriologists, (9,11,12,16) who have demonstrated its widespread distribution. Two very important fields of investigation are those of water supply and milk production, where the coli-aerogenes group has been shown to have outstanding significance (2,7,11). The occurrence of this group in water, milk and milk products is extremely common, and the extent to which these organisms contaminate the water or milk supply has been the subject of much attention, following the realization of their possible origin.

The possible ways of introducing organisms of the coli-aerogenes group into milk are almost innumerable, some workers (2)

concluding that even fresh milk, produced under the best conditions, always contains some organisms of this type, if a sufficiently large amount of milk is examined. On the other hand, the extent of the contamination can be deduced by considering more their absence from specific dilutions, than their presence (13).

The behaviour of these organisms, however, when once introduced, would seem to merit further attention, the prime object of this study being an investigation into the behaviour of a strain of Escherichia coli in raw and pasteurised milk.

THE ORGANISMS OF THE COLI-AEROGENES GROUP.

The Standard Methods of water analysis (17) states - "It is recommended that the coli-aerogenes group be considered as including all gram negative non-spore forming bacilli, which ferment lactose with gas formation, and grow aerobically on standard solid media."

Recognition of the important difference between Bacterium coli and Bacterium aerogenes was made first by Rogers and Clark (12) in 1912. Their classification was based on the measurement of the gas evolved from the growth of the organisms in glucose broth. Bacterium coli gave a constant ratio of one part hydrogen to 1 part carbon dioxide, while Bacterium aerogenes gave a ratio of 1 part hydrogen to 1.4 - 2 parts carbon dioxide. Subsequent work by Levine (10) agreed closely with this classification and emphasised the fact that the Methyl Red positive, Voges Proskauer negative, high gas ratio organism i.e., Bacterium aerogenes, is relatively rare in the faeces

of man and of animals, the supposition being that it is a normal inhabitant of the soil.

Present classification and nomenclature of the main groups, Escherichia and Aerogenes, is given by Bergey (3).

For presumptive, partially confirmed and completed bacteriological tests for the coli-aerogenes group, the Standard Methods of water analysis (17) give detailed instruction both as to the preparation of media and their routine use. Levine (9), covers a wider field, both in regard to modification of existing recipes, and use in routine practice.

MEDIA EMPLOYED

Peptone medium for Methyl Red determination (Standard Methods 17) was used for growing the inoculum of Escherichia coli.

After various experiments, it was decided to use a bile salt agar for obtaining the plate count of Escherichia coli in the milk. As a known culture of Escherichia coli was used to contaminate the milk, it was unnecessary to differentiate between the Escherichia and Aerogenes groups. Prescott and Winslow (11), refer to the advantages of a brom cresol purple agar for the detection of organisms of the coli-aerogenes group, but omit to give the details of preparation.

A modification of the lactose bile salt agar as used by Savage (16) was therefore employed with brom cresol purple as indicator.

Preparation of lactose bile salt agar. (16)

20 grams of dried agar were dissolved by boiling in 1 litre of distilled water, 20 gms. of Bacto peptone and 5 gms. of Bacto

Ox-gall were added with stirring and the solution was then filtered. 10 gms. of lactose and 2 ccs. of a 1.6% alcoholic solution of brom cresol purple were added, the solution was tubed and then autoclaved for 15 minutes at 15 lbs. the total period of subjection to heat being not more than 30 minutes.

METHODS

A 3 year old Jersey cow, 7 month's calved, "Ubyssey Rogue's Betty", from the University of British Columbia dairy herd was selected.

Observing thorough cleanliness and with due precautions, half a pint of fore milk was withdrawn and discarded, the next pint being withdrawn direct into a sterile bottle. This milk was then immediately removed and 100 cc. portions were measured, with a sterile graduate, into four 250 cc. Erlenmeyer flasks.

Flask 1 was then placed in a water bath and heated to 145° F. for 30 minutes with frequent agitation, the temperature being read from a thermometer immersed in a flask containing 100 ccs. of water and placed in the water bath close to the flask of milk. The milk flask was then removed and cooled in a water bath to the required inoculating temperature.

Flask 2 was held as the raw sample at room temperature till the pasteurising of Flask 1 was complete, when it was adjusted to the same inoculating temperature as Flask 1.

Flask 3 was held on ice for 24 hours and then treated the same as Flask 1.

Flask 4 was held on ice for 24 hours and then treated the same as Flask 2 and used for comparative work with Flask 3.

When adjusted to the required temperature each pair of flasks was subjected to equal inoculations, subsequently numerically ascertained by the plate method, of 1 cc. of a specific dilution of a broth culture of Escherichia coli (isolated by Kelly, 8).

In all cases, the absence of other organisms of the coli-aerogenes group from the original raw milk was proved by plating a measured quantity with the bile salt agar.

At definite periods after inoculation, the plate count of the milk in each flask was ascertained by withdrawing 1 cc., diluting in 9 cc. water blanks, and plating the required dilution with the bile salt agar. The plates were incubated at 37° C. and were quite satisfactory for counting purposes when 24 hours old. The colonies were distinctly acid forming as evidenced by the change in colour of the indicator from purple to yellow, their appearance being clearly shown in Figures 1 and 2.

Experimental plates of poor quality milk showed the easily distinguished growth of mould colonies on the media, but all other types of growth, except those of the coli-aerogenes group, were inhibited.

TABLE 1.

THE COMPARATIVE RATE OF GROWTH OF ESCHERICHIA COLI IN PASTEURISED AND RAW MILK HELD AT 37° C.

Expt.	MILK INOCULATED WHEN FRESH				MILK HELD 24 HRS. BEFORE INOCULATION			
	Inoc. per cc. of milk	Growth Hrs.	Plate Count E.Coli per cc. of milk. Past.	Raw	Inoc. per cc. of milk	Growth Hrs.	Plate Count E.Coli per cc. of milk. Past.	Raw
1	63,300	2	620,000	70,000	56,000	3	7,250,000	14,000
		4	3,000,000	1,800		6	102,000,000	7,500
		6	*	10,400		9	300,000,000	55,000
		8	*	114,000		24	600,000,000	400,000,000
		10	*	1,440,000				
		12	*	30,000,000				
		* Uncountable						
2	6,150	2	97,500 Abs. in 1/1000		15,000	2	140,000	2,450
		4	9,900,000 " 1/100*			4	3,240,000	430
		6	37,500,000 " 1/100			6	65,600,000	240
		8	90,000,000 " 1/100			8	270,500,000	65
		10	250,000,000 " in 1/1000			10	345,000,000	Abs. in 1/100
		* One plate (of 2 made) showed 1 colony only.						

TABLE 1.(Con.)

THE COMPARATIVE RATE OF GROWTH OF ESCHERICHIA COLI IN PASTEURISED AND RAW MILK HELD AT 37° C.

Expt.	MILK INOCULATED WHEN FRESH				MILK HELD 24 HRS. BEFORE INOCULATION			
	Inoc. per cc. of milk	Growth Hrs.	Plate Count E.Coli per cc. of milk. Past.	Raw	Inoc. per cc. of milk	Growth Hrs.	Plate Count E.Coli per cc. of milk. Past.	Raw
3	18,900	2	30,500	4050	12,950	2	85,000	3,950
		4	820,000	1470		4	1,665,000	1,200
		6	14,600,000	70		6	24,500,000	185
		8	66,500,000	35		8	203,500,000	60
		10	225,000,000	15		10	405,000,000	25
4	12,700	2	248,000	200	20,400	2	191,000	2,900
		4	10,700,000	10		4	6,060,000	150
		6	65,400,000	15		6	79,400,000	40
		8	306,000,000	30		8	331,000,000	20
		10	730,000,000	35		10	590,000,000	10

TABLE 2. (Cont.)

THE COMPARATIVE RATE OF GROWTH OF ESCHERICHIA COLI IN PASTEURISED AND RAW MILK HELD AT 17° C.

Expt.	MILK INOCULATED WHEN FRESH				MILK HELD 24 HRS. BEFORE INOCULATION			
	Inoc. per cc. of milk	Growth Hrs.	Plate Count E. Coli per cc. of milk.		Inoc. per cc. of milk	Growth Hrs.	Plate Count E. Coli per cc. of milk.	
			Past.	Raw			Past.	Raw
3	18,900	2	21,000	19,000	12,950	2	13,550	12,500
		4	21,000	17,500		4	11,550	15,500
		6	20,500	18,500		6	12,000	14,000
		8	17,000	19,000		8	23,950	10,200
		10	22,500	17,000		10	52,000	9,250
4	12,700	2	16,800	14,400	20,400	2	16,900	21,000
		4	17,900	13,500		4	18,100	18,800
		6	42,000	5,150		6	22,800	19,200
		8	112,000	800		8	38,500	17,100
		10	380,000	1,000		10	112,000	17,500

In all cases no organisms of the coli-aerogenes group were present in 1 cc. of the original raw milk with the exception of Experiment 2, Table 2, where the degree of absence was from 1/10 cc.

INTERPRETATION OF TABLES

If the bacteriological examination of milk for organisms of the coli-aerogenes group is to be of real value, it follows that the behaviour of this type in milk, both raw and pasteurised, merits attention.

By means of controlled experimental inoculation of a specific organism belonging to this group, into raw and pasteurised milk, under laboratory conditions, it is possible to study its behaviour when once introduced. The results obtained, though applicable only to specific cases, would seem to have a suggestive value in the wider sphere of commercial endeavour.

The results in Table 1 may be summarised briefly as follows:-

a) A marked difference is noticeable in the rate of growth of Escherichia coli, when introduced into raw and pasteurised milk held at 37° C.

b) A rapid and continuous growth of this organism in pasteurised milk is seen in every case, whether the milk be fresh or held for 24 hours on ice.

c) A definite reduction in count follows the inoculation of this organism into raw milk in every case, whether the milk be fresh or held for 24 hours on ice. The time taken to reach a minimum count

would seem to vary with the rate of inoculation.

The results in Table 2 may be summarised briefly as follows:-

a) The difference in the rate of growth of Escherichia coli when introduced into raw and pasteurised milk held at 17° C. is not so marked as in Table 1.

b) In the initial stages of growth, the plate counts in both the raw and pasteurised milk agree fairly closely.

c) Later stages of growth show that Escherichia coli increases more rapidly in pasteurised than in raw milk, though the tendency is for the final count to approximate in both cases, as shown in Experiments 1 and 2.

d) The marked reduction in count in the raw milk, apparent in Table 1, is not so evident and does not appear except to a limited extent in Experiment 4, where this reduction coincides with a noticeably rapid increase in the pasteurised milk.

It will be noted that Experiments 3 and 4 in both tables provide a means of gauging the comparative effect of temperature on growth, as the initial inoculation in both pairs of experiments is the same.

DISCUSSION OF RESULTS

Buchanan (4) divides the life cycle of a culture into 7 distinct phases, viz:-

1. Initial stationary phase.
2. Lag phase or positive growth acceleration phase.
3. Logarithmic growth phase.
4. Negative growth acceleration phase.
5. Maximum stationary phase.
6. Accelerated death phase.
7. Logarithmic death phase.

From the purely comparative point of view it is to be seen that these phases for the strain of Escherichia coli used, differ markedly in raw and pasteurised milk.

Alterations of the chemical constituents of various media was found by Salter (15) to have a distinct influence on the various phases of growth of Bacillus communis.

The most important factor controlling the growth of organisms of the coli aerogenes group may be said to be temperature, the practical application being evident in the provision made for thorough cooling in commercial plants. In the estimation of contamination, by means of bacteriological analysis, the difficulty lies in the division of the original contamination from the subsequent increase as influenced by temperature. The investigations of Ayers and Clemmer (2) would seem to indicate that the temperature at which little or no increase in numbers takes place is 10° C. The same workers made a careful study of

the limitations of the colon count as an index of cleanliness in milk production, the conclusions throughout being based on growth in raw milk.

The inhibitive influence which raw milk exerts on germ content has long been noted. Stocking (18) bases this so-called germicidal action more on the inability of certain types of organisms to grow in milk, rather than on their actual suppression.

Comparative work done by Allen (1) clearly indicates the different behaviour of the gas forming Bacillus aerogenes in raw and pasteurised milk. The method used was based on a direct measurement of the gas formed and demonstrated clearly that growth as measured by gas formation was more rapid in the pasteurised milk than in the raw when incubated at temperatures of 30-37° C.

Working along somewhat similar lines but using plate count methods St. John and Pennington (14) also conclude that pasteurisation of milk affects the rate of growth of specific organisms. Bacillus aerogenes was found to increase much more rapidly in pasteurised milk than in raw milk, when incubated at temperatures referred to as "room" and "ice-box". They conclude that the slower rate of increase in raw milk is traceable either to germicidal action or unnatural environment.

Stressing the importance of obtaining absolutely uncontaminated milk from the cow, Evans and Cope (6) clearly demonstrate the bactericidal property of raw milk towards Bacillus coli communis. The milk from different cows was found to vary in this respect, and comparative tests with pasteurised milk showed that the temperature of pasteurisation had a distinct bearing on the subsequent rate of increase

of the organisms in the milk so treated. They found that a decrease of 40% in 4 hours was obtained after inoculation of Bacillus coli communis in raw milk, but in these experiments the importance of the incubating temperature does not seem to be fully appreciated.

These workers all show a reduction in the "colon count" of raw milk or at least a more rapid increase in the growth of coli aerogenes organisms in pasteurised milk. Whatever the activating principle, to which this apparent decrease in raw milk can be attributed, there is no uniform opinion.

Apart from the alterations of the growth phases as shown in the tabulated results, it is seen on examining Table 1, that a definite reduction occurred in the germ content below the number inoculated when the milk was incubated at 37° C. The maximum reduction occurred at a time influenced apparently by the age of the milk and the rate of inoculation, though it is interesting to note that milk kept for 24 hours on ice shows a very marked germicidal action.

On the other hand, as evidenced by the results in Table 2, when the temperature of incubation is lowered to 17° C, this reduction of germ content is not so evident, the tables indicating that the growth phases are altered, but that there is no appreciable reduction in numbers of the strain of Escherichia coli inoculated, except in one case, possibly due to experimental error or faulty incubation.

The indications are that at this temperature, the early stages show little change between the rate of growth of the organisms in raw milk and in milk pasteurised to 145° for 30 minutes, but that later stages show a very much more rapid increase in the latter. At the same

time, however, it is evident that the rate of increase in the raw milk must be maintained steadily as the very late stages show very high and some what similar counts in both milks.

It follows that the arbitrary fixing of a "colon count" standard based on the presence, or even absence, of these organisms in milk is not justified unless a history of the milk is supplied.

CONCLUSIONS

The behaviour of a strain of Escherichia coli in raw milk derived from a specific cow in the University of British Columbia dairy herd and the same milk pasteurised to 145° for 30 minutes is markedly different.

When this organism is introduced into raw milk derived from the above source, and incubated at 37° C. a definite reduction of germ content occurs, followed by an increase. Under the same conditions, in the same milk pasteurised to 145° for 30 minutes, the organism maintains a rapid increase from the start.

When this organism is introduced into raw milk derived from the above source and incubated at 17° C. the reduction in germ content is not apparent or only slightly so. The rate of increase, however, is slower than in the same milk pasteurised to 145° for 30 minutes under the same conditions.

Raw milk has a definite power to inhibit the growth of Escherichia coli between the temperatures of 17° and 37° C. while the pasteurising of milk to 145° F. for 30 minutes destroys all or part of this inhibiting factor.

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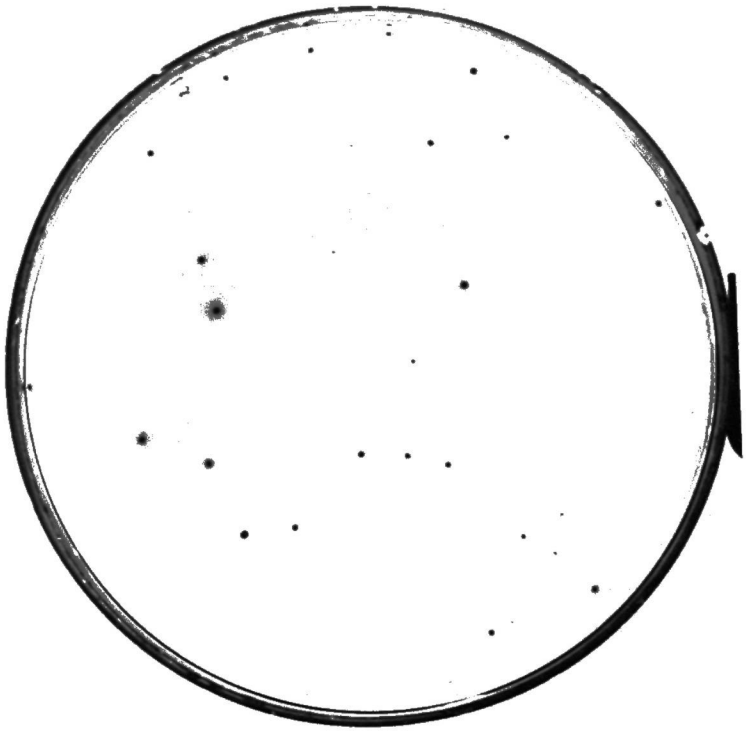
FIGURE 1.

Photographs illustrating the appearance of colonies of Escherichia coli on the media referred to in the text, page 3.

A. 1/1000 dilution. 4 hours after inoculation into raw milk at 17° C.

B. " " 4 " " " " pasteurised milk at 17°C.

Both plates 26 hours old.



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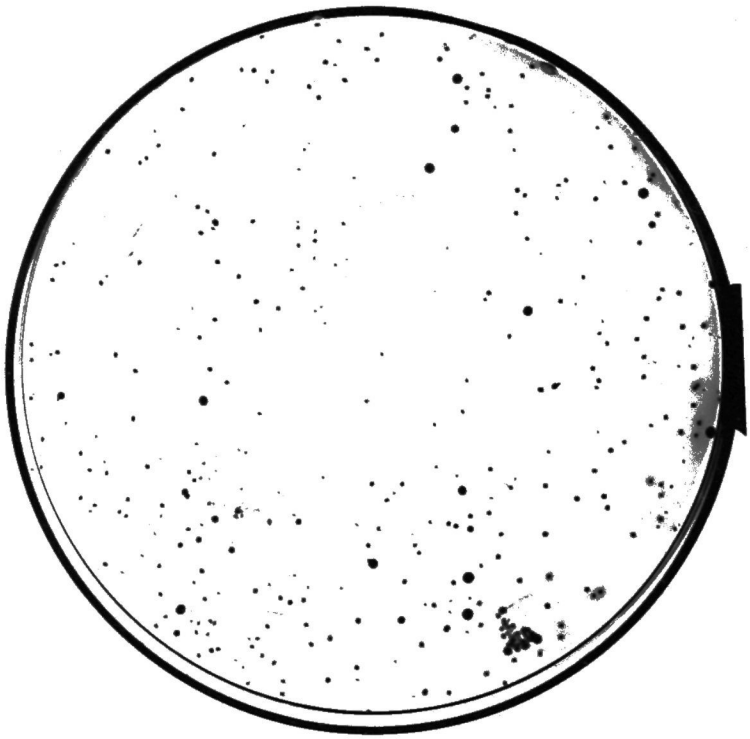

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Figure 2.

FIGURE 2.

Photographs illustrating the appearance of colonies of Escherichia coli on the media referred to in the text, page 3.

C. 1/1000 dilution. 10 hours after inoculation into raw milk at 17° C.

D. " " 10 " " " " Pasteurised milk at 17°C.

Both plates 20 hours old.

APPENDIX "A"

SOME OBSERVATIONS ON THE GROWTH OF A STRAIN OF ESCHERICHIA COLI IN THE MAKING OF CHEDDAR CHEESE.

INTRODUCTION

In view of the fact that organisms of the coli-aerogenes group occur frequently in dairy products other than fluid milk, it was decided to carry the investigation a little further in its application to cheese making, the work being carried out along lines very similar to the previous investigation.

Organisms of this group are often encountered in cheese and have been known and studied for a long time. (20). With modern developments in cheesemaking, however, such as pasteurisation of the milk supply, improvement in starters and the method of their handling, it was thought advisable to investigate briefly the behaviour of the same strain of Escherichia coli, together with other organisms of this group which might be in the raw milk, before, during and subsequent to the manufacture of cheddar cheese.

MEDIA

The same media, as previously described, was employed.

METHODS

One day's bulked milk of the University of British Columbia dairy herd was thoroughly mixed and divided into two equal parts,

one half being heated to 143° F. for 30 minutes in a "Wizard" 50 gallon pasteuriser, the other half remaining raw.

Throughout the experiments every effort was made to obviate contamination. Vats were thoroughly scalded, vat covers used and other factors considered which would tend to ensure a true comparison. The cheese made were of the cheddar type and the process of manufacture was performed as given in the attached charts.

The "colon count" of the raw milk was obtained by plating a specific dilution with the bile salt agar. It was realized that the colonies appearing, though belonging to the coli-aerogenes group, were not all those of Escherichia coli.

In view of the fact that the pasteurising temperature used was well above the thermal death point of the organisms included in the coli-aerogenes group (19) and that the efficiency of the pasteuriser had already been established, the pasteurised milk was considered as being free from organisms of this type.

When the two vats of raw and pasteurised milk had been adjusted to the same temperature, equal quantities of a specific dilution of a broth culture of Escherichia coli (23) were inoculated into each. At the same time definite quantities of starter, composed of equal parts of cultures of "Ericsson"* and"D. 144"* organisms, free from organisms of the coli-aerogenes group were introduced into each vat.

* Stock cultures of lactic acid organisms maintained for use as starters in the Dairy Department, University of British Columbia.

"Colon counts" were made from both vats of:-

(a) The milk just prior to rennetting.

(b) The whey at running.

(c) The cheese 22 hours after putting to press.

The "colon counts" of both milk and whey were carried out as previously described in the first part of this study.

The organisms in the cheese were obtained in suspension dilutions using the method outlined by Kelly (23) these dilutions being plated as in the case of the milk and whey.

TABLE 3.

THE COMPARATIVE RATE OF GROWTH OF ESCHERICHIA COLI IN RAW AND PASTEURISED MILK DURING THE CHEESE MAKING PROCESS.

Rate of Inoc. of		Pasteurised Milk "Colon count"			Raw Milk "Colon count"			
Starter	E. Coli per cc. of milk	Milk when rennet added per cc.	Whey at running per cc.	Cheese 22 hrs. old per gram	Initial "Colon Count" per cc.	Milk when Rennet added per cc.	Whey at running per cc.	Cheese 22 hrs. old per gram
1%	880	1305	1050	500	1200	4000	9000	26,000
2½%	1740	2550	100*	Absent in 1/200	580	2250	500*	3,100

* Lowest dilution made was 1/100 1 colony appearing on each plate from the pasteurised whey and 5 colonies on the raw.

INTERPRETATION OF TABLE 3.

The cheesemaking process depends, among other factors, on the growth of various organisms. It follows that the control, during the process of any strain of organisms detrimental to the cheese, is of paramount importance. The methods of control are essentially dependent on the present state of knowledge concerning the various phases of the growth of such organisms.

The results of the table may be briefly summarised as follows:-

(a) The milk received was contaminated with organisms of the coli-aerogenes group as evidenced by the initial "colon count".

(b) The factor, previously mentioned, in the first part of this study, which inhibits the growth of Escherichia coli in raw milk is not so apparent, as any reduction in germ content in it, occurs also in the cheese made from the pasteurised milk.

(c) The increased amount of starter in the second experiment coincides with a markedly reduced "colon count" throughout the whole process of cheesemaking from both pasteurised and raw milk.

DISCUSSION OF RESULTS

The reaching of definite conclusions based on the somewhat limited scope of the experiments outlined is undesirable, but the results obtained are of interest and suggest various lines of further investigation.

The gas producing properties of organisms of the coli-aerogenes group have long been realized and their exclusion from the milk used in cheesemaking is an essential in the manufacture of good cheese. It follows that their control during the production of the milk and the manufacture of the cheese is of great importance. Work by Hucker (22) points to the fact that the presence of colon and proteus types of organisms in the cheese examined is usually associated with a low grade of cheese.

Once introduced into cheese, through the medium of contaminated milk or faulty manufacturing methods, the growth of organisms of the coli-aerogenes group would seem to be affected by their total number as compared with the other bacterial flora. In addition, the ripening temperature has been shown by Harrison and Connell (20) to have a marked effect on the bacterial flora of cheddar cheese. Examination by Hastings, Evans and Hart (21) of good quality cheddar cheese during

the ripening stages, lead them to conclude that the dominant group present in the early stages is the Bacterium lactis acidi group whose numbers fall off later with an accompanying development of the Bacillus bulgaricus group.

Pasteurisation of milk for cheesemaking on a commercial scale has many advantages according to Stevenson (25) who comments on the superior flavoured product obtained. Murray (24) emphasizes the necessity of minimising post-pasteurisation contamination with gas forming organisms.

The results in both experiments point to the fact that raw milk as used for cheesemaking will be infected with organisms of the coli-aerogenes type. An important controlling factor, apparently, makes its appearance in the amount of starter used. Other factors being as equal as possible, it is seen that the increased amount of starter used from 1% to 2½% coincides with a markedly reduced rate of increase and apparent actual suppression of organisms of the coli-aerogenes type, more especially in the pasteurised milk, where the inoculation of Escherichia coli apparently made little headway, due to the addition of the starter.

As previously stated, definite conclusions based on such shallow investigation cannot be drawn, but efficient control of the organisms of the coli aerogenes type in cheesemaking would seem to include-

(a) Pasteurisation of the milk.

(b) A heavy inoculation of starter, whether or not pasteurisation is practiced.

CHEDDAR CHEESE

RECORD OF PROCESS OF MANUFACTURE

EXPERIMENT

TABLE

	Date Made	Series Number	MILK a.m.	MILK p.m.	MILK Weight lbs.	MILK Fat %	STARTER Acidity %	STARTER Ozs. Per 100 lbs.	STARTER Amount Ozs.	STARTER Time Added	Starter	MILK AT TIME RENNET ADDED Rennet Test secs.	MILK AT TIME RENNET ADDED Acidity %	MILK AT TIME RENNET ADDED Rate of Rennet drams to lbs.	MILK AT TIME RENNET ADDED Amount of Rennet drams	MILK AT TIME RENNET ADDED Time	MILK AT TIME RENNET ADDED Temp. °F.	*CUT TIME	STARTED TO STIR Time	STARTED TO STIR Acidity %	Time Started	Acidity %	CALD Time Finished	CALD Acidity %	CALD Final Temp. °F.	PITCHED Time	PITCHED Acidity %	WHEY DRAWN Hours from Setting	WHEY DRAWN Time	WHEY DRAWN Start Acidity %	WHEY DRAWN Finish Acidity %	CUT IN CUBES AND TURNED Time	CUT IN CUBES AND TURNED Acidity %	TURNED AND PILED Time	TURNED AND PILED Acidity %	TURNED AND PILED Time	TURNED AND PILED Acidity %	TURNED AND PILED Time	TURNED AND PILED Acidity %
Past. 143° F.	Mar. 2nd	67	2	1	190	-	.8	1%	29 ozs.	10.0	Ericcson D 144	-	.2	1-25	7½	10.45	85°	11.25	11.30	.15	11.45	.15	12.25	.155	100°	1.10	.155	3.10	1.55	.16	.185	2.15	.205	2.45	.23	3.30	.28	4.15	.31
Raw	do	68	do	do	190	-	.8	1%	29 ozs.	10.0	do do	-	.21	1-25	7½	10.45	85°	11.25	11.30	.155	11.45	.155	12.25	.16	100°	1.10	.165	3.10	1.55	.165	.18	2.15	.205	2.45	.23	3.30	.29	4.15	.405
Past. 143° F.	Mar.16th	69	16	15	172	-	.76	2½%	4 3/8#	10.20	do do	-	.21	1-25	7	10.55	85°	11.35	11.40	.145	11.55	.145	12.30	.15	102°	1.10	.16	2.45	1.40	.165	.2	2.5	.27	2.30	.34	3.0	.40	3.30	-
Raw	"	70	do	do	172	-	.76	2½%	4 3/8#	10.20	do do	-	.21	1-25	7	10.55	85°	11.35	11.40	.155	11.55	.155	12.30	.155	102°	1.10	.165	2.45	1.40	.17	.205	2.5	.275	2.30	.35	3.0	.40	3.30	-
Average																																							

*Cut Using times allowing 30 minute intervals

Continued on Table

APPENDIX "B".

SOME OBSERVATIONS ON THE GROWTH OF A STRAIN OF ESCHERICHIA COLI IN THE MANUFACTURE OF BUTTER.

INTRODUCTION.

The growth of the same strain of Escherichia coli in the manufacture of butter was briefly investigated, as it was thought possible that such work would prove useful in supplementing that already done on milk and cheese.

The control of the organisms of the coli-aerogenes group in the manufacture of butter centres largely round the pasteurisation of the cream. At the temperature most generally used, (170° F. or higher for 10 minutes or longer) the destruction of organisms of this type may be considered complete. (26). Their presence, however, in butter made from pasteurised cream has been clearly demonstrated (30) and, further, some defects in such butter can frequently be attributed to the action of these organisms.(30). Their presence therefore must be attributed to subsequent recontamination or faulty pasteurisation.

MEDIA

The same media, as previously described, was employed.

METHODS

The mixed morning and evening's milk of the University

of British Columbia dairy herd was separated, the cream obtained being halved. One portion was pasteurised to 170° F. for 10 minutes, by standing the can in a steam heated water bath and stirring the cream constantly. The other half remained raw.

As the temperature used in pasteurising, was well above the thermal death point of the coli-aerogenes organisms, (26) their destruction was considered complete and the "colon count" of the raw cream was determined as previously described.

Throughout the experiments every effort was made to obviate contamination. The churns used (8 gallon Eureka sanitary churns) and butter workers, were thoroughly scalded and other factors considered which would tend to ensure a true comparison throughout.

The two lots of raw and pasteurised cream were cooled in a water bath to the same temperature and inoculated with equal quantities of a specific dilution of a glucose broth culture of the same strain of Escherichia coli (28) as previously employed.

The portions of cream were then held for a definite time at a fixed temperature in running water till ready for churning.

"Colon counts" were then made:-

(a) of the raw and pasteurised cream just prior to churning.

(b) of the buttermilk when removed from churn.

(c) of the finished butter at a definite period after making.

Dilutions of the cream and buttermilk were made in the same way as described in the first part of this study. Dilutions of the butter were obtained by melting at 110° F. diluting in water blanks at the same temperature and proceeding as for the cream and buttermilk.

In all cases a churning temperature of 60° F. was used and two lots of washing water at 56° and 54° F. respectively.

TABLE 4.

THE COMPARATIVE RATE OF GROWTH OF ESCHERICHIA COLI IN RAW AND PASTEURISED CREAM DURING THE BUTTER MAKING PROCESS.

Expt.	Lbs. Cream	Inoc. of E. coli per cc.	Held	Pasteurised "Colon Count" per cc. Cream after inoc. and holding	Pasteurised "Colon Count" per cc. Butter-milk	Pasteurised "Colon Count" per cc. Butter	Raw "Colon Count" per cc. Orig. Count	Raw "Colon Count" per cc. Cream after inoc. and holding	Raw "Colon Count" per cc. Butter-milk	Raw "Colon Count" per cc. Butter
1	9	18,800	2 hrs at 60° F.	15,200	20,900	305 48 hrs	150	15,300	14,500	50 48 hrs.
2	9	18,800	24 hrs at 50° F.	18,500	82,000	Abs.in 1/10 24 hrs	150	16,500	*	Abs.in 1/10 24 hrs
3	13	24,700	24 hrs at 43° F.	16,900	35,500	Abs.in 1/10 96 hrs	40	20,000	42,500	Abs.in 1/10 96 hrs.
4	10	41,700	24 hrs at 43° F.	27,250	54,900	Abs.in 1/10 120 hrs	25	27,250	41,500	Present in 1/10 (1 plate of 2) 120 hrs

* Plates spoilt.

DISCUSSION OF RESULTS

In discussing bacteriological examinations of market butter made from pasteurised cream, the occurrence of organisms of the coli-aerogenes type is of definite significance and is a direct indication of recontamination as evidenced by the work of Sadler and Vollum (30). Realization of this fact is an important aspect of control work. As the temperature of pasteurisation in all the experiments was well above the thermal death point of the organisms investigated, (26) the experimental inoculation is comparable to this recontamination.

The undesirability of having this type of organism present in butter is well recognized and their presence is not indicative of high quality. Examination of the keeping quality of butter conducted by Rosenau, Frost and Bryant (29) points to the fact that Bacillus coli tends to die out on storage, this fact being an important consideration in conducting quantitative and qualitative determinations.

Working along somewhat similar lines, Brown and Peiser (27) found that 30% of the organisms present in cream failed to grow after mechanical agitation in the churn and that washing and salting removed 50% from the unsalted butter.

The reduction in germ content in both the raw and pasteurised cream after the inoculation and holding may be partly accounted for by the wide range of dilutions used, though inhibitive forces may also have been at work.

In all cases, however, the most impressive fact is the tendency of the major portion of the organisms of the coli-aerogenes

group to be removed with the buttermilk. It would seem therefore that the complete removal of the buttermilk and thorough washing of the butter grains is an important step in control work.

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