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Observations on the Counting of Bacteria in Ice Cream by the Plate Method

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SUMMARY

In the plating of ice cream and ice cream mix the use of standard agar plus 1 percent sucrose instead of standard agar was advantageous from two standpoints: (a) the agreement of the counts secured with different dilutions and (b) the ease of counting.

When standard agar was used for plating, occasional samples were encountered with which the different dilutions failed to show a reasonable agreement in count. Serious discrepancies usually involved the 1 to 1,000 and 1 to 10,000 dilutions. Since these discrepancies did not occur when 1 percent sucrose was added to the agar, the dilution of the sucrose present in the ice cream to a very low point may have been a factor in the failure of the dilutions to agree. The addition of 1 percent sucrose to standard agar often resulted in such an increase in the size of the colonies that counting was considerably easier than when standard agar was used. This advantage was evident from the smaller percentage of pin-point colonies encountered with the former medium, but there was also an increase in the size of colonies too large to be classed as pin-points.

In general the addition of sucrose to standard agar had comparatively little effect on the count or the size of the colonies when the ice cream contained only small numbers of bacteria.

Standard agar plus 1 percent sucrose has been adopted at the Iowa Agricultural Experiment Station for the routine plating of ice cream and ice cream mix.

The organisms responsible for the pin-point colonies on plates poured with ice cream were of various types. Streptococci forming acid in milk, some with coagulation and some without when incubated two days at 37° C., were especially prominent although alkali formers and digesters were also encountered in considerable numbers. Many of the cultures secured from pin-point colonies grew better in milk at 45° C. than at lower temperatures. Some of the alkali formers gave large colonies at 21° C. and pin-point colonies at 37° C. The digesters secured from pin-point colonies regularly gave large colonies when the pure cultures were plated.

Observations on the Counting of Bacteria in Ice Cream by the Plate Method

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The development of official or standard methods for various types of laboratory examinations represents a distinct advance from the standpoint of the usefulness of the results obtained. The standard methods for the bacteriological analysis of milk have made it possible to compare, on a satisfactory basis, the results secured in different laboratories and, undoubtedly, have been a factor in extending the use of bacterial counts for the control of milk supplies. The procedure at present required by Standard Methods of Milk Analysis¹ for the macroscopic colony count on milk has been developed over a period of years. It is generally recognized that there are other media and incubation conditions which would give higher counts but none of these is at present standard because of the desire to employ a procedure which is easily carried out and comparatively inexpensive.

The medium required by Standard Methods of Milk Analysis for the macroscopic colony count on milk has been employed with various dairy products. Its use with butter and cheese seems illogical because (a) with butter, organisms which grow poorly on it are often added to the cream and are rather frequently permitted to grow there for the purpose of developing the aroma and flavor that are desired and (b) with cheese, the growth of various types of organisms is necessary for the normal changes in flavor, aroma and texture and, in general, these develop poorly on it. With ice cream the use of the medium seems logical because milk, cream, condensed milk, etc., are the important sources of the bacteria in the finished ice cream and, as with market milk, it is desirable to keep the number contained as low as possible. In a report of the subcommittee on Bacteriological Methods of Examining Ice Cream, American Dairy Science Association,² the wide use of standard agar for the counting of bacteria in ice cream by public health laboratories is pointed out.

¹Standard Methods of Milk Analysis. Fifth edition, Am. Pub. Health Assn. 1929.

²Bacteriological Methods of Examining Ice Cream. Jour. Dy. Sci., 10:460. 1927.

OBSERVATIONS ON THE USE OF STANDARD AGAR³ IN THE COUNTING OF THE BACTERIA IN ICE CREAM

During a period of more than two years bacterial counts have been made at the Iowa Agricultural Experiment Station on a considerable number of samples of ice cream from various dairy plants in the state for the purpose of securing information which would be useful in the formulation of a bacterial standard for Iowa ice cream. Both macroscopic colony and microscopic counts were used originally and, for the former, the medium and incubation conditions which are standard for milk were employed. Plates were frequently encountered which showed many colonies so small they could be counted only with a lens. While this is undesirable from the standpoint of ease of counting, a similar colony development is often encountered on plates poured with milk. Occasionally the colonies developing on the plates poured with ice cream were so tiny they could not be readily recognized even with a lens, and counting was practically impossible.

A more important point, however, was that with certain samples of ice cream the higher dilution plates contained less than the number of colonies which would be expected from the number on the lower dilution plates. For example a set of plates might show rather large numbers of organisms on the 1 to 1,000 dilution plates and many less than one-tenth as many on the 1 to 10,000 dilution plates. When this occurred with a sample for which the numbers of colonies on the plates were such that the higher dilution was counted, the results secured were very evidently too low.

Fay,⁴ in 1926, reported that in making counts on ice cream with standard agar, large numbers of pin-point colonies were noted in low dilutions (1 to 100) while in higher dilutions (1 to 1,000) these were not in proportionate numbers or were absent. On replating the samples on sucrose agar the pin-point colonies were present in all dilutions and in proportionate numbers; essentially the same results were secured by using standard agar and adding sterilized ice cream to the higher dilution plates.

OBJECT OF THE WORK

The object of the work herein reported was to compare the results secured on standard agar and on standard agar plus

³The term standard agar is used to refer to the medium described in Standard Methods of Milk Analysis for the macroscopic colony count.

⁴Fay, A. C., Thermotolerant saccharophilic organisms as a cause of "pin-point" colonies in the bacteriological analysis of ice cream. Jour. Bact., 11:96. 1926.

1 percent sucrose in the plating of ice cream and ice cream mix. The comparison involved (a) the counts secured, (b) the agreement of different dilutions and (c) the percentage of pin-point colonies.

METHODS

The usual procedure in preparing the media employed in a series of comparisons was to make up a quantity of standard agar, divide it into two parts and tube one part without any addition while 1 percent sucrose was added to the other part just before tubing.

The ice cream was plated on a milliliter basis, the dilutions used being poured in duplicate with each medium. The dilutions were prepared using 99 ml. water blanks only and the diluted ice cream or ice cream mix for the two sets of plates came from one set of blanks. The agar was added to the plates as soon as the distribution of the dilution water was completed; in general the order of pouring the media was alternated so as to equalize any influence there may have been from the period of holding in the dilution water. The plates were incubated at 37° C. for approximately 48 hours.

In the counting of the plates an attempt was usually made to secure (a) the total count and (b) the number of pin-point colonies; the pin-point colonies were considered to be those which were so small they could not be definitely recognized as colonies without the use of a lens. A procedure that was commonly followed was to count and check with a wax pencil the colonies which were large enough to be definitely recognized with the unaided eye and then go over the plates with a lens and count the pin-point colonies.

EXPERIMENTAL

Preliminary comparisons of standard agar and standard agar plus 1 percent sucrose were made during 1929 on ice cream samples from a number of dairy plants in the state. The counts were usually somewhat higher when the agar contained the sucrose but the differences were not great. The colonies on the agar containing the sucrose were often larger than on the standard agar and, accordingly, in a number of the comparisons the percentage of pin-points was considerably less when the agar with the sucrose was used; the extreme condition was shown by a comparison in which 95.3 percent of the 170 colonies found on the 1 to 10,000 dilution plates poured with standard agar were pin-points while only 0.5 percent of the 200 colonies found on the 1 to 10,000 dilution plates poured with standard agar plus 1 percent sucrose were pin-points.

The increase in the size of the colonies that resulted from the addition of sucrose to the agar in the preliminary comparisons suggested a more extended trial. The data secured on 84 samples of ice cream from 38 Iowa dairy plants are given in table I; the samples were plated between July 3, 1930, and March 21, 1931, inclusive. Except in six cases, the counts on the standard agar plus sucrose were higher than on the standard agar and in 17 of the 84 comparisons the count on the former medium was more than twice as high as on the latter. Extreme differences in the counts were shown with samples 2, 4, 45, 57, 69, 73 and 74. With some of these there were great discrepancies in the different dilutions poured with standard agar, the 1 to 10,000 dilution plates showing a much smaller number of colonies than would be expected from the number on the 1 to 1,000 dilution plates; in general the number of colonies on the

TABLE I. COMPARISON OF COUNTS OBTAINED ON STANDARD AGAR AND ON STANDARD AGAR PLUS 1% SUCROSE
Ice Cream Samples from 38 Iowa Dairy Plants

Sample no.	Date plated	Bacteria per ml. on		Percent pin-point colonies on	
		Standard agar	Standard agar plus 1% sucrose	Standard agar	Standard agar plus 1% sucrose
1	7- 3-30	2,750,000	4,490,000	80.0	15.0
2	7-19-30	90,000	700,000	22.2	12.8
3	7-24-30	1,120,000	2,040,000	22.3	8.3
4	7-24-30	10,000,000	52,500,000	53.8	11.8
5	7-24-30	2,060,000	2,140,000	17.4	4.4
6	7-24-30	650,000	960,000	32.3	12.4
7	7-24-30	770,000	1,410,000	32.4	10.6
8	7-28-30	66,000	91,000	63.6	18.6
9	7-28-30	36,000	82,000	8.3	12.1
10	7-28-30	44,000	57,000	31.8	19.8
11	7-28-30	17,700	26,000	0.0	0.0
12	7-29-30	147,000	187,000	27.2	5.8
13	7-30-30	925,000	1,615,000	7.5	4.0
14	7-30-30	305,000	445,000	14.7	5.9
15	7-30-30	375,000	425,000	14.6	2.2
16	7-30-30	300,000	440,000	20.0	4.7
17	8- 4-30	370,000	355,000	0.0	0.0
18	8- 7-30	10,000	14,000	45.4	31.0
19	8- 8-30	4,900,000	5,120,000	15.2	0.0
20	8-11-30	1,640,000	1,695,000	0.6	0.9
21	8-12-30	720,000	840,000	20.0	6.0
22	8-13-30	145,000	195,000	28.1	7.6
23	8-13-30	235,000	480,000	4.3	4.1
24	8-15-30	65,000	495,000	16.6	4.0
25	8-16-30	14,000	34,000	42.8	20.5
26	8-16-30	82,000	97,000	14.6	6.2
27	8-23-30	11,600	11,800	8.6	7.6
28	8-23-30	970,000	1,480,000	3.0	8.8
29	9-30-30	1,440,000	2,550,000	16.6	7.0
30	9-30-30	7,800	6,700	2.5	6.0
31	9-30-30	935,000	1,245,000	5.3	0.8
32	10- 2-30	466,000	1,025,000	40.0	14.5
33	10- 3-30	127,000	196,000	36.2	2.0
34	10- 3-30	9,000	9,300	11.1	1.3
35	10- 7-30	70,000	71,000	6.4	8.4
36	10-14-30	113,000	142,000	11.5	1.4
37	10-14-30	191,000	435,000	25.3	0.0
38	10-15-30	108,000	141,000	7.4	0.0
39	10-16-30	226,000	326,000	7.5	2.1
40	10-16-30	257,000	399,000	54.4	7.0
41	10-18-30	68,000	70,000	8.8	1.4

TABLE I. (CONT.) COMPARISON OF COUNTS OBTAINED ON STANDARD AGAR AND ON STANDARD AGAR PLUS 1% SUCROSE

Ice Cream Samples from 38 Iowa Dairy Plants

Sample no.	Date plated	Bacteria per ml. on		Percent pin-point colonies on	
		Standard agar	Standard agar plus 1% sucrose	Standard agar	Standard agar plus 1% sucrose
42	12- 1-30	21,000	56,000	33.4	10.9
43	12- 1-30	382,000	470,000	99.2	0.0
44	12- 3-30	138,000	217,000	88.1	5.5
45	12- 8-30	220,000	1,895,000	42.8	2.6
46	12-10-30	1,730,000	2,750,000	4.6	3.2
47	12-10-30	320,000	405,000	10.9	6.1
48	12-16-30	44,000	60,000	50.0	11.6
49	12-16-30	168,000	231,000	67.2	7.9
50	12-18-30	58,000	60,000	3.0	3.0
51	12-21-30	137,000	316,000	24.5	5.3
52	12-27-30	42,000	46,000	31.2	10.8
53	1-13-31	20,000	44,000	75.0	38.6
54	1-13-31	88,000	128,000	56.5	32.8
55	1-14-31	59,000	62,000	18.6	19.3
56	1-17-31	16,000	15,000	37.5	66.6
57	1-19-31	80,000	2,760,000	100.0	16.6
58	1-19-31	1,560,000	2,480,000	18.0	8.9
59	1-20-31	68,000	88,000	30.8	37.0
60	1-20-31	324,000	350,000	76.5	5.7
61	1-27-31	34,000	35,000	64.7	0.0
62	1-27-31	7,400	10,000	52.7	20.0
63	1-27-31	330,000	390,000	18.1	0.0
64	2- 3-31	61,000	87,000	73.7	1.4
65	2- 3-31	248,000	330,000	71.2	10.0
66	2- 8-31	37,000	24,000	2.1	1.2
67	2- 8-31	*	12,500,000	100.0	12.1
68	2- 8-31	18,300	22,900	6.1	5.4
69	2- 8-31	320,000	2,640,000	86.0	21.2
70	2-17-31	64,000	84,000	2.1	1.2
71	2-21-31	114,000	174,000	1.4	0.5
72	2-21-31	19,000	32,000	0.3	0.3
73	2-21-31	229,000	1,980,000	20.2	12.0
74	2-21-31	470,000	2,675,000	51.2	13.3
75	3- 2-31	4,080,000	4,585,000	0.8	10.2
76	3- 2-31	113,500	129,000	2.0	1.3
77	3- 9-31	111,500	89,500	1.6	1.5
78	3-11-31	620,000	900,000	30.2	1.4
79	3-11-31	9,100	12,000	1.2	1.2
80	3-12-31	132,000	117,000	16.0	14.6
81	3-12-31	7,000	53,000	55.6	2.5
82	3-21-31	34,000	38,000	68.9	30.3
83	3-21-31	36,000	391,000	7.2	7.3
84	3-21-31	160,000	220,000	31.2	18.8

*Only pin-point colonies on the 1 to 100,000 dilution plates and these were so small counting was unsatisfactory.

1 to 1,000 dilution plates appeared to be about the same with the two media.

The colonies on the agar containing sucrose were often distinctly larger than on the agar without sucrose, and this is reflected in the difference in the percentages of the colonies that were pin-points on the two media. The difference in the percentages of pin-points varied a great deal from sample to sample, but whenever it was large enough to be of significance the percentage was smaller when sucrose was present in the agar than when it was not; the extreme condition was shown by

TABLE II. COMPARISON OF COUNTS OBTAINED ON STANDARD AGAR AND ON STANDARD AGAR PLUS 1% SUCROSE

Samples from 3 Iowa Dairy Plants

Run no.	Mix after pasteurization		Mix after homogenization		Mix after holding		Mix in hopper of the freezer		Ice cream	
	Bacteria per ml. on		Bacteria per ml. on		Bacteria per ml. on		Bacteria per ml. on		Bacteria per ml. on	
	Standard agar	Standard agar plus 1% sucrose	Standard agar	Standard agar plus 1% sucrose	Standard agar	Standard agar plus 1% sucrose	Standard agar	Standard agar plus 1% sucrose	Standard agar	Standard agar plus 1% sucrose
1					4,900,000	5,120,000	3,700,000	4,920,000	4,120,000	4,280,000
2	655,000	1,130,000	990,000	1,595,000	1,015,000	1,535,000	1,075,000	3,015,000	775,000	1,410,000
3	1,200,000	2,405,000	6,480,000	5,600,000	5,000,000*	7,000,000*	6,800,000	7,200,000	**	8,680,000
4	66,000	91,500	59,500	75,500	58,500	84,000	58,000	84,000	36,000	82,000
5	588,000	435,000	**	1,150,000	**	805,000	**	975,000	**	765,000
6	345,000	530,000	915,000	1,380,000	425,000	655,000	**	**	915,000	1,805,000
7	3,500	5,000	17,500	25,500	305,000	605,000	305,000	445,000	375,000	425,000

*Estimate.

**Colonies too small to count.

sample 43 with which the percentage of pin-point colonies was 99.2 on the standard agar and 0.0 on the agar plus sucrose.

Table II presents the results secured with standard agar and with standard agar plus 1 percent sucrose on mix taken at various points in the handling and on finished ice cream; the samples came from three Iowa dairy plants that had frequently supplied ice cream with high bacterial counts. In general the counts were somewhat higher in the presence of the sucrose, and the colonies were distinctly larger. In four of the five comparisons in run 5, the colonies on the standard agar were so small it was practically impossible to count them even with a lens, and the same was true with the finished ice cream in run 3. The common effect of homogenizing in increasing the bacterial count, as determined by the plate method, is evident in runs 2, 3, 5, 6 and 7. In run 7 there was apparently an increase in the count during the holding of the mix.

The results presented in table I show a general tendency for the bacterial counts to be influenced less by the addition of sugar when the count on the ice cream was low than when it was high. This relationship is well illustrated by the counts obtained with the two media on 16 samples of ice cream secured from the Iowa State College dairy plant and presented in table III; all but three of the samples were secured between July 23, 1930, and Jan. 17, 1931, inclusive. There were no significant

TABLE III. COMPARISON OF COUNTS OBTAINED ON STANDARD AGAR AND ON STANDARD AGAR PLUS 1% SUCROSE
Ice Cream Samples from Iowa State College Dairy Plant

Sample no.	Date plated	Bacteria per ml. on		Percent pin-point colonies on	
		Standard agar	Standard agar plus 1% sucrose	Standard agar	Standard agar plus 1% sucrose
1	3- 1-28	80,000	71,000	52.5	39.3
2	10- 8-29	28,000	37,000	28.5	24.3
3	10-11-29	50,000	53,000	38.7	39.3
4	7-23-30	20,000	21,000	5.3	3.0
5	7-25-30	35,000	28,000	11.4	14.2
6	7-29-30	17,700	17,800	1.1	1.0
7	8- 1-30	6,200	6,100	3.2	1.6
8	8- 5-30	5,750	5,300	3.5	1.9
9	8- 7-30	7,300	6,400	2.7	3.1
10	8- 9-30	2,700	2,750	5.5	7.2
11	9-18-30	8,650	10,200	5.2	4.4
12	10- 8-30	8,750	9,800	1.0	2.5
13	10-10-30	8,700	8,000	5.8	4.3
14	10-20-30	51,000	38,000	16.6	4.0
15	1-14-31	59,000	62,000	18.6	19.3
16	1-17-31	16,600	15,400	3.1	0.7

differences between the counts on the two media and each medium gave a slightly higher count with eight of the samples. No difference between the two media was noted in the size of the colonies and, in agreement with this, the differ-

ences in the percentages of pin-point colonies were comparatively small. Many of the colonies developing on each medium were large and suggested organisms belonging to the genus *Bacillus*.

Table IV gives the counts secured with standard agar and with standard agar plus 1 percent sucrose on mix taken at various points in the processing and on finished ice cream, all the samples coming from the Iowa State College dairy plant. The counts were regularly low and there was no significant difference between the values secured on the media, although the counts were more frequently higher when the medium contained sugar than when it did not. The size of the colonies was essentially the same on the two media. Homogenization again tended to increase the count as determined by the plate method.

TABLE IV. COMPARISON OF COUNTS OBTAINED ON STANDARD AGAR AND ON STANDARD AGAR PLUS 1% SUCROSE
Samples from Iowa State College Dairy Plant

Run no.	Mix after pasteurization		Mix after homogenization		Mix after holding		Ice cream	
	Bacteria per ml. on		Bacteria per ml. on		Bacteria per ml. on		Bacteria per ml. on	
	Standard agar	Standard agar plus 1% sucrose	Standard agar	Standard agar plus 1% sucrose	Standard agar	Standard agar plus 1% sucrose	Standard agar	Standard agar plus 1% sucrose
1			6,550	7,400	6,250	6,150	6,200	6,100
2	1,000	1,450	6,200	6,900	5,350	6,650	5,750	5,300
3	1,450	1,100	3,300	3,700	3,550	4,000	7,300	6,400
4	2,300	2,800	3,600	3,750	3,150	3,750	4,500	4,600
5	1,900	2,800	3,750	3,400	2,500	3,000	2,200	2,750
6	1,400	6,950	4,250	6,850	6,000	6,650	5,050	4,850

Some outstanding examples of the failure of different dilutions to agree when standard agar was used in the plating of ice cream or ice cream mix are given in table V; the results obtained with the same dilutions when standard agar plus 1 percent sucrose was used are also included. The counts on standard agar were often very much less with the 1 to 10,000 than with the 1 to 1,000 dilution while on standard agar plus 1 percent sucrose the two dilutions showed a reasonable agreement. Sample 1 is a striking illustration of the failure of different dilutions to agree when standard agar was used for the plating; the count according to the 1 to 1,000 dilution was 1,125,000 per ml. (est.) while according to the 1 to 10,000 dilution it was 90,000 per ml. The difference between the counts obtained with two dilutions when standard agar was used varied greatly from one sample to the next. All the results presented in table V deal with 1,000 dilutions and these were

TABLE V. EXAMPLES OF THE FAILURE OF DIFFERENT DILUTIONS TO AGREE WHEN STANDARD AGAR WAS USED IN THE PLATING OF ICE CREAM
Samples from Various Dairy Plants

Sample no.	Bacteria per ml. on standard agar		Bacteria per ml. on standard agar plus 1% sucrose	
	1 to 1,000 dilution	1 to 10,000 dilution	1 to 1,000 dilution	1 to 10,000 dilution
A 1	1,125,000 (est.)	90,000	1,955,000 (est.)	1,430,000
A 2	840,000 (est.)	Very few	Very large numbers	1,500,000
A 3	464,000	90,000	700,000 (est.)	720,000
A 4	720,000 (est.)	50,000	790,000 (est.)	840,000
A 5	146,000	60,000	195,000	180,000
A 6	400,000*	65,000*	450,000†	495,000†
A 7	450,000*	235,000	440,000	480,000
A 8	466,000	240,000	1,025,000 (est.)	920,000
A 9	504,000	Very few	Very tiny colonies	585,000
A10	Very large numbers	50,000	2,760,000 (est.)	2,300,000
A11	1,560,000 (est.)	210,000	2,260,000 (est.)	2,480,000
A12	248,000	76,000	330,000	350,000
A13	330,000	60,000	390,000	390,000
A14	12,000,000 (est.)	None visible	12,500,000 (est.)	12,500,000 (est.)
A15	64,000	10,000	84,000	120,000
A16	320,000	170,000	2,640,000 (est.)	2,400,000
A17	Very large numbers	229,000	1,810,000 (est.)	1,980,000

*Colonies very small.

†Colonies large.

the dilutions generally involved when serious discrepancies were noted. In the results reported by Fay⁵ the discrepancies involved the 1 to 100 and 1 to 1,000 dilutions.

ADDITION OF LACTOSE TO STANDARD AGAR

A number of trials were carried out in which standard agar plus 1 percent lactose was compared with standard agar and with standard agar plus 1 percent sucrose for the plating of ice cream and ice cream mix. In general the addition of lactose eliminated the discrepancies between different dilutions that were noted with certain samples when standard agar was used, and it also increased the size of the colonies. With most samples the agar containing lactose was just as satisfactory as that containing sucrose but with a few samples it gave somewhat smaller colonies. Since sucrose is ordinarily present in ice cream in larger amounts than lactose, it is, perhaps, the more logical sugar to use in a medium intended for the plating of ice cream.

USE OF STANDARD AGAR PLUS 1 PERCENT SUCROSE AT THE IOWA AGRICULTURAL EXPERIMENT STATION

The advantages of standard agar plus 1 percent sucrose for the plating of ice cream and ice cream mix have resulted in the

⁵Fay, A. C., Thermotolerant saccharophilic organisms as a cause of "pin-point" colonies in the bacteriological analysis of ice cream. Jour. Bact., 11:96. 1926.

adoption of this medium at the Iowa Agricultural Experiment Station for the routine plating of these materials. The advantage which was primarily responsible for this adoption is the better agreement of the counts obtained with different dilutions.

GENERAL TYPES OF ORGANISMS RESPONSIBLE FOR PIN-POINT COLONIES ON THE PLATES Poured WITH ICE CREAM

The general types of organisms responsible for the pin-point colonies on the plates poured with ice cream were studied by picking colonies into litmus milk and classifying the cultures thus secured into acid coagulators, acid non-coagulators, alkali formers, digesters and inert after two days incubation at 37° C. A considerable number of the attempts at isolation failed, due undoubtedly to the difficulty of picking the very small colonies even when a lens was used.

A total of 1,016 cultures was secured from 54 samples of ice cream which came from 26 Iowa dairy plants; 445 (43.8 percent) were acid coagulators, 481 (47.3 percent) were acid non-coagulators, 25 (2.5 percent) were alkali formers, 64 (6.3 percent) were digesters and 1 (0.1 percent) was inert. When the acid non-coagulators were transferred to litmus milk and the transfers incubated for two days at 37° C., some of them coagulated the milk and some did not so that the division of the acid formers into coagulators and non-coagulators was not a very definite one with an incubation period of two days at 37° C.

Cultures belonging to each of the general types were studied for the purpose of determining the best growth temperature by making transfers to litmus milk and comparing the rates at which changes were produced at 21°, 37° and 45° C. Of 54 acid coagulators, 52 (96.3 percent) grew best in milk at 45° C. and 2 (3.7 percent) grew best at 37° C.; of 87 acid non-coagulators, 68 (78.2 percent) grew best in milk at 45° C. and 19 (21.8 percent) grew best at 37° C.; of 12 alkali formers, all grew best in milk at 21° C. and of 11 digesters, 6 (54.5 percent) grew best in milk at 45° C. and 5 (45.5 percent) grew best at 37° C.

Cultures belonging to each of the general types were plated on standard agar plus 1 percent sucrose and plates incubated at each of the following temperatures: 21°, 37° and 45° C. Both the acid coagulators and the acid non-coagulators gave very small colonies at all three temperatures, but in general the colonies were slightly larger at 37° C. than at 21° or 45° C. The alkali formers seemed to be of two types; the one type grew well on agar at 21° C. and gave large, transparent, bluish colonies, while at 37° C. it gave typical pin-point colonies, and at 45° C. it failed to develop visible colonies; the other type

gave pin-point colonies at 21° and 37° C. and failed to develop visible colonies at 45° C. The digesters gave colonies which were comparatively large and did not suggest pin-points at all three of the temperatures; the colonies were somewhat larger at both 37° and 45° C. than at 21° C.

The acid coagulators and acid non-coagulators were regularly streptococci. The alkali formers which gave large colonies at 21° C. and pin-point colonies at 37° C. were long, thin rods while those which produced pin-point colonies at 21° and 37° C. were large, thick rods. The digesters were long, thick rods.

DISCUSSION OF RESULTS

The better colony development, both from the standpoint of numbers and size, that often resulted when 1 percent sucrose was added to standard agar used for plating ice cream and ice cream mix suggests the importance of sugar for the development of many of the organisms contained. The variation in the effect of the sucrose with the different samples was presumably due to variations in the flora and in the importance of sucrose to the different types of organisms.

The failure of different dilutions to agree when standard agar was used for the plating of certain samples of ice cream and ice cream mix while a reasonable agreement was secured on standard agar to which 1 percent sucrose had been added, and the value of the latter medium for the plating of ice cream, as shown in the results presented, it is evident that with some samples containing comparatively large numbers of organisms, the counts secured on standard agar were much lower than when 1 to 10,000 dilution was counted than when 1 percent sucrose was added to the agar. This irregularity is corrected by adding 1 percent sucrose to the agar. When less than 1 percent sucrose would have been added to 10,000 dilution plates poured with standard agar, the 1,000 dilution plates contained much less sucrose and gave a reasonably satisfactory count. When 1 percent sucrose is used the small amount of sucrose on the plates from the ice cream does not affect the agar content and, accordingly, the concentration of colonies on the plates is fairly uniform.

The irregularity of the colonies that results from the use of standard agar makes the counting considerably less accurate and of significance especially when large numbers are counted. With the colonies from ice cream, the pin-point colonies becomes

the addition of 1 per-