POPULATION OF CITRATE-FERMENTING BACTERIA IN LACTIC CULTURES

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(Received for publication October 10, 1963)

Summary

The total bacteria and citrate-fermenting bacteria in 72 lactic cultures were enumerated. The numbers of citrate-fermenting bacteria varied from fewer than 10,000 to 2.1 billion per ml. The citrate-fermenting bacteria per ml expressed as per cent of each corresponding total count on Tomato Juice Agar (TJA) had a median of 13%. The mean percentages of citrate-fermenting bacteria were not significantly different among lactic cultures grouped according to their original source, time maintained in the dairy plant, or their particular use.

The general belief seems to be that about 5-10% of the population of lactic cultures as used in the dairy industry are citrate-fermenting bacteria, but the literature contains few data to support this view. Several media for the differentiation of the so-called "aroma" bacteria of lactic cultures have been introduced. In 1933, Benchetrit *(2)* suggested the addition of α bromopropionic acid to tomato juice agar for differentiating the *Leuconostoc* species. Prouty *(7)* suggested the use of brom cresol purple as an indicator for differentiation and Lundstedt *(4)* reported that colonies of these organisms were iridescent on a citrate whey agar. More recently Mayeux *et al. (5)* suggested the use of a medium containing 75 ppm of sodium azide, while McDonough *et al. (6)* added 0.15 μg/ml of tetracycline to tomato juice agar to inhibit the lactic streptococci. However, none of these workers have enumerated the citrate-fermenting bacteria in large numbers of lactic cultures that are in actual use in dairy plants. In this work the medium of Galesloot *et al. (3)* was modified to contain 51g tomato juice agar and 5g calcium lactate per liter and was used with the aroma bacteria indicator of 0.6% calcium citrate sol stabilized with carboxymethyl cellulose *(8)*. Herein is reported the numbers of citrate-fermenting bacteria in lactic cultures in current use.

Procedures

Forty-one dairies submitted lactic cultures for examination in sterile 2-oz. bottles containing approximately 1g of calcium carbonate. In addition, a completed questionnaire with each culture furnished information as to the length of time the culture had been in use in the plant (age), frequency of transfer, carrying medium and its heat treatment, incubation time and temperature, and the products made with the culture.

Each culture was transferred on the day of arrival into Matrix Mother Culture Media[1] and incubated at 21 C for at least 16 hours or until coagulation occurred. The cultures were then cooled and stored at 4 C until the second subculture was made (about 8 to 10 hr). From the second subculture the bacterial populations were determined within 0.5 to 2 hr after the incubation period. Bacterial populations in dilutions of the second subcultures were enumerated by the agar plate method on M-PH medium (MPHM), Tomato Juice Agar (TJA) and TJA containing 0.5% calcium lactate and 0.6% colloidal calcium citrate (TJAC½%). The diluent for the cultures was 0.1% aqueous peptone. Dilutions at 10^{-6} and 10^{-7} were plated on MPHM and TJA. Dilutions at 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} were plated on TJAC½%. Each dilution was plated in triplicate. The plates were incubated at 25 $\pm$ 1 C for 72 $\pm$ 2 hr. Colonies were counted with the aid of a Quebec Colony Counter. When possible, plates were selected for counting and counted according to standard methods *(1)*.

Results and Discussion

Data for the 72 lactic cultures are presented in Table 1. Higher counts were obtained on the TJA than on the MPHM. However, 20 of the 72 cultures had a higher count on the latter. Colony counts per ml on TJA ranged from 3 million to 2 billion with a mean of 540 million. Colony counts on the MPHM ranged from 11 million to 1.4 billion with a mean of 410 million. Analysis by t-test *(9)* indicated the difference between the means of the colony counts on TJA and MPHM to be significant at $P < 0.10$ but not at $P < 0.05$. Colony counts of the citrate-fermenting bacteria on the TJAC½% ranged from fewer than 10,000 to 2.1 billion with a mean of 140 million per ml. Citrate-fermenting bacteria per ml expressed as per cent of each corresponding count on TJA ranged from 1 to 733% with a median of 13%. Culture number 69 (Table 1), used in the plant for 5 yr for making of buttermilk and cottage

[1]Obtained from the Galloway-West Co., Fond du Lac, Wis.

TABLE 1. SOURCE, AGE, USE, TOTAL COUNT AND COUNT OF CITRATE FERMENTERS IN STARTER CULTURES

Culture number	Dairy	Original source[a]	Age - Time maintained in the dairy	Use in dairy[b]	Colony count[c] per ml of culture x 10^{-6}			Colony count on TJAC½% as % of colony count on TJA
					(*MPHM*)	(*TJA*)	(*TJAC½%*)	
2	2	1	5 yr	ChC	220	150	1.3	1
3	2	1	5 yr	ChC	69	68	0.97	1
4	4	1	15 da	BM,CoC	49	340	43	13
7	5	1	10.75 yr	ChC	880	580	37	6
34	19	1	1 yr	ChC	420	650	170	3
46	35	1	1.5 yr	ChC	90	27	42	155
69	41	1	5 yr	BM,CoC	1200	1300	0	0
70	41	1	5 yr	BM,CoC	81	120	1	1
71	41	1	5 yr	BM,CoC	150	160	40	25
72	41	1	3 mo	BM	52	46	22	48
1	1	2	15 da	BM,CoC	420	480	85	18
16	3	2	35 da	BM	560	770	730	95
18	3	2	30 da	BM	580	950	3.6	1
30	20	2	14 da	BM	500	470	19	4
31	20	2	2 da	BM	165	420	24	6
54	28	2	14 da	BM,CoC	110	57	23	40
66	34	2	7 da	BM	380	390	310	79
67	34	2	7 da	CoC	380	350	20	6
15	3	3	20 da	BM	590	450	30	7
17	3	3	20 da	BM	310	430	54	13
19	12	3	14 da	CoC	160	850	5.6	1
21	14	3	10 da	CoC	400	570	59	10
22	14	3	14 da	BM,CoC,SrCm	520	750	74	10
25	15	3	3 da	BM,CoC,SrCm	700	960	440	46
28	21	3	42 da	BM	250	270	740	274
38	18	3	--	StCd	80	610	73	12
52	33	3	7 da	BM,CoC	11	24	36	150
53	33	3	7 da	CoC	13	25	33	132
58	30	3	--		44	66	63	95
59	30	3	1.5 yr	BM,CoC	640	24	42	175
5	6	3a	12 da	ChC	880	980	16	1
10	8	3a	14 da	BM,CoC	740	1000	28	3
13	11	3a	21 da	BM	270	700	54	8
33	19	3a	2 yr	ChC,CmC	640	960	470	49
36	24	3a	21 da	BM,CoC	150	460	13	3
40	26	3a	2 da	BM	520	830	62	7
42	26	3a	40 da	BM	370	860	0.23	1
49	27	3a	2 da	BM,CoC,SrCm	130	17	26	153
68	23	3a	7 da	ChC	1200	1100	73	7
6	6	3b	12 da	ChC	380	390	9	2
12	11	3b	7 da	BM	600	1100	37	3
50	27	3c	2 da	BM,SrCm	22	28	42	150
37	24	3d	21 da	BM,CoC	340	430	27	6
39	23	3d	30 da	ChC	330	600	32	5
61	19	3e	6 mo	ChC	83	1500	87	6
23	14	4	14 da	BM,SrCm	28	42	29	69
43	39	4	15 da	ChC	28	2000	2100	105
48	27	4	2 da	BM,CoC	1000	56	41	73
51	33	4	28 da	BM,CoC	86	11	21	191
8	6	4a	5 da	ChC	1200	1300	96	7
55	28	4a	14 da	BM,CoC	480	74	18	24
57	28	4a	14 da	BM,CoC	74	19	53	28
11	11	4b	7 da	BM	712	860	55	6
41	26	4b	15 da	BM	370	650	31	5
45	38	4c	6 da	BM,SrCm	91	3	22	733
9	8	5	5 da	BM,CoC	25	210	27	13
24	14	5	10 da	BM,CoC,SrCm	50	100	86	86
47	27	5	2 da	BM,SrCm	27	4	24	600
56	28	5	14 da	BM,CoC	53	39	0.76	2
14	13	6	11 mo	ChC,CoC	1300	1600	38	2
26	13	6	7 mo	ChC	610	930	280	30
27	13	6	8 mo	ChC	1400	1600	280	18
60	40	6	7 da	BM	1200	1200	490	41
32	19	6a	3 mo	CmC	320	440	91	21
44	31	6a	28 da	ChC	560	160	340	213
62	19	6a	3 mo	ChC	17	280	110	39
20	12	7	5 mo	CoC	35	320	4.1	1
63	37	8a	2 mo	ChC	1100	1200	710	59
64	37	8b	8 da	ChC	760	820	640	78
65	37	8c	--	ChC	320	520	5	1
29	16	9	4 yr	BM	490	590	120	20
35	17	10	42 da	SwC	340	560	43	8

[a]Number refers to supplier; letter refers to supplier's designation.
[b]ChC = Cheddar cheese, CoC = Cottage cheese, BM = buttermilk, SrCm = Sour cream, StCd = Stirred curd, CmC = Cream cheese, SwC = Swiss cheese.
[c]Counts are rounded to 2 significant figures.

cheese, had less than 10,000 citrate-fermenting bacteria but had one of the higher lactic acid organism counts (1.3 billion). Conversely, the cultures that had the high percentages of citrate-fermenting bacteria had low lactic acid organism counts as indicated by the low counts on the TJA.

The 72 cultures received originated from 9 different sources and the origin of 10 cultures was unknown. Most of the unknown group had been in the dairy for a long period. Culture 7 had been used day after day in the same Cheddar cheese plant for over 10 yrs. Large enough numbers of samples were available from six of the sources for statistical treatment. Analysis of variance by the method of Snedecor (9) indicated no significant differences ($F = 0.855$, $F_{0.05} = 2.41$) among the mean percentages of citrate-fermenting bacteria in cultures from the six sources.

Some interesting comparisons can be made regarding the ages of the cultures. When the three cultures that had no age specified are omitted, it will be noted that of the remaining 69 cultures, 84% had been in the plant for less than 1 yr, 78% for <6 mo, 62% for <30 days, 48% for <15 days and 26% for <8 days. These data imply a high turnover of lactic cultures in the dairy plants participating in this study. Sixty-eight cultures containing citrate-fermenting bacteria were grouped according to the time they had been in the dairy plant. These groups were: less than 8 days, 8-15 days, 16-30 days, 31 days to 6 mo and over 6 mo. An analysis of variance showed no significant differences ($F = 1.69$, $F_{0.05} = 2.51$) among the mean percentages of citrate-fermenting bacteria in the cultures of the five age groups. These data do not support the idea that one of the reasons for changing cultures is that the citrate-fermenting bacteria tend to disappear with repeated transfer in the dairy plant.

Cultures containing citrate-fermenting bacteria were grouped on the basis of their use in the dairy plant. Of these, 59% were used in the making of buttermilk, 36% in Cottage cheese, 30% in Cheddar cheese and 11% in sour cream. Also, two cultures were used in the making of Cream cheese and one in Swiss cheese. An analysis of variance showed no significant differences ($F = 1.26$, $F_{0.05} = 3.11$) among the mean percentages of citrate-fermenting bacteria in the lactic cultures for these three purposes. Presumably some cultures have been classified for buttermilk, cottage cheese, Cheddar cheese, etc. on the basis of the flavor producing organisms but this work shows no significant relationship between the bacterial make-up of the cultures and their use by the dairy plant.

ACKNOWLEDGMENT

We wish to thank Galloway-West Co., Fond du Lac, Wis. for the Matrix Mother Culture Media.

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