J. Milk Food Technol. Vol. 38, No. 2, Pages 87-88 (February, 1975)

A Membrane-Filter Technique to Test for the Significance of Sublethally Injured Bacteria in Retail Pasteurized Milk

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(Received for publication July 5, 1974)

ABSTRACT

A membrane transfer procedure previously described was used to study the possible role of sublethally injured bacteria in the keeping quality of retail pasteurized milk. Trypticase soy broth (TSB) was used as nonselective medium and TSB plus NaCl at pH 6.0 (TSBS 6.0), as the selective medium inhibitory to injured organisms. In pasteurized milk at early stages of storage, colony counts on the latter medium were much lower than on the former. Subsequent transfer of the TSBS 6.0 filters to fresh TSB and further incubation usually increased the counts to about the initial TSB range. Generally the organisms presumed injured and subsequently recovered were the same types as those considered uninjured. They were mainly streptococci and micrococci that produced only slow changes in litmus milk at 5 C, so they are of doubtful significance in the shelf life of retail milk at refrigerator temperatures.

It has been suggested that quality deterioration in certain processed foods could arise from recovery and growth of injured bacteria not initially detected by usual culturing procedures (*4,5*). In pasteurized milk, usually it is considered that poor keeping quality arises from post-pasteurization contamination with psychrotrophic species. It might be questioned if in some instances poor keeping quality is due to the subsequent recovery and growth of heat-injured organisms.

A membrane filter technique was shown to be useful in enumerating and isolating sublethally, heat-injured bacteria (*2*). The original procedure used broth cultures of a mixed flora from milk which were incubated at 32 C. Reported here is a study to test the procedure for investigating heat-injured bacteria in retail pasteurized milk.

METHODS

Sources of samples

Various brands of pasteurized homogenized milk were purchased at local retail stores at different times. "Pull" dates on cartons were used to obtain as fresh samples as available. Samples were taken promptly to the laboratory, held refrigerated, and examined the same day.

Membrane filtering procedure

The procedure used to examine milk samples was the same in priniciple as that previously reported (*2*). The fat content of homogenized whole milk limits the quantity that will pass through bacterial membrane filters (pore size 0.45 μm). Surfactant filtering aids are not satisfactory because they inhibit growth of heat-injured bacteria (*3*). In the method used, 10 ml of warm sterile water were added to the filter suction funnel with filter in place. One milliliter of the appropriate dilution of milk sample (usually 1:10) was added and vacuum applied. After filtering, the lower wall of the funnel was flushed with 10 ml of sterile water drawn through the filter. Occasionally the 1:10 dilutions of milk added to the 10 ml of water in the funnel (approximating 1:100 dilution) were difficult to filter, apparently because membrane porosity varied. Higher decimal dilutions caused no problem. Quadruplicate filters were prepared from each sample.

Trypticase soy broth (TSB) was used as the nonselective medium expected to support growth of uninjured and injured organisms. Prior tests showed that pH 7.5 gave higher counts on filters with pasteurized milk than did pH 7.0. As the selective medium to suppress growth of injured cells in pasteurized milk, TSB plus 2% NaCl at pH 6.0 (TSBS 6.0) was used after testing various medium modifications.

The media were added to absorbent pads in separate 47-mm plastic petri dishes (1.6 ml per dish). After the diluted milk sample was filtered, one pair of the quadruplicate filters was placed in TSB plates and the other pair in TSBS 6.0 plates. Both pairs were incubated at 25 C for 4 days. Plate counts with Standard Methods agar (*1*) also were made with incubation of 25 C for 4 days to provide supporting information on general count level. Although this medium may support growth of both injured and uninjured organisms, it will not differentiate between them. The 25-C incubation temperature was used to permit growth of both psychrotrophs and mesophiles and to avoid longer incubation at lower temperatures.

After colonies on each medium were counted, in some trials, those on TSBS 6.0 were marked by perforating the filters near each one with a sterile needle. The filters then were transferred to fresh TSB plates and incubated four additional days and colonies were counted again.

Types of bacteria

In several trials, after the second incubation, marked and unmarked colonies were picked into litmus milk and incubated at 5 C and room temperature. Cultures were observed for litmus milk reactions and examined for morphology and gram stain.

RESULTS AND DISCUSSION

Fourteen lots of retail pasteurized milk were tested as described, with the results shown in Table 1. From purchase to "pull" date varied from 4 to 10 days. Plate counts at 25 C were generally low, showing no particular relation with time until "pull" date. Most samples had a cooked flavor when tested.

Colony counts on TSB filters tended to be lower than the corresponding plate counts, but agreed reasonably well considering differences in techniques and media. Counts on TSBS 6.0 filters were much lower than on TSB filters. After transfer and further incubation the former counts increased considerably and usually approached the same range as the TSB counts. It should be noted that the TSB and TSBS 6.0 filters are duplicates but are not the same filters. Hence some variation can be expected between initial TSB and transfer counts.

TABLE 1. *Inhibition and recovery of bacteria in retail pasteurized milk by the membrane-filter procedure*

Sample	Days before "pull" date	Plate counts/ml	Membrane filter counts[a] TSB 7.5	TSBS 6.0	Transfer counts
1	8	< 100	8	3	7
2	6	2000	125	85	165
3	5	800	73	17	76
4	4	800	68	36	63
5	10	1900	93	48	—
6	6	500	22	10	34
7	4	1200	68	8	67
8	—	—	86	30	79
9	5	1000	53	24	59
10	—	4300	TNTC	10	75
11	7	1000	50	18	58
12	10	100	15	10	10
13	9	850	56	37	54
14	—	1700	86	30	79

[a]Milk dilution, 10^{-1}. Each value is the average colony count from two filters.

Most colonies on the different filters were small and often difficult to see except with a microscope illuminator. Those on TSBS 6.0 tended to be smaller than those on TSB. These occasionally increased in size after transfer. New colonies after transfer also were usually small and appeared to be the same types as on the TSB and TSBS 6.0 filters. In bacterial populations with thermal resistance at about milk pasteurization temperature some organisms should survive uninjured while others of the same type might be sublethally injured.

It might be questioned if the TSBS 6.0 inhibited uninjured bacteria. As no medium is likely to equally inhibit, at the marginal level, various species in pasteurized milk, TSBS 6.0 may have surpressed some uninjured organisms. However, in three samples subsequently stored for varyious periods and that increased considerably in plate counts, counts on TSB and TSBS 6.0 were about equal (Table 2), which suggests

TABLE 2. *Effect of extended milk storage and bacterial increases on colony counts obtained on two filter media*

Sample	Days after "pull" date	Plate count/ml	Membrane filter counts TSB 7.5	TSBS 6.0
1	6	15,000	146[a]	145[a]
2	3	20,000	150[a]	150[a]
3	0	80,000	105[b]	125[b]

[a]Milk dilution 10^{-2}. Each value is the average from two filters.
[b]Milk dilution 10^{-3}. Each value is the average from two filters.

no inhibition of active cells. Later observations indicated that the increased numbers developed from psychrotrophic contamination during processing and were different types than those detected initially in the low count samples.

Colony types

Most colonies recorded in Table 1 were small and white or faintly yellow, resembling streptococci and micrococci. Morphologically, most were gram-positive cocci with a few gram-positive rods. Occasionally a few larger, colored colonies were present.

Colonies picked into litmus milk caused only slow changes at room temperature. Some were acid-proteolytic. At 5 C little change was evident in two to three weeks and most changes suggested streptococci and micrococci. Some of the species are known to be relatively heat resistant and might be expected to be the types in low-count, pasteurized milk. They also grow slowly at refrigerator temperatures and are rarely associated with defects in retail milk.

In several samples that subsequently developed defects by "pull" date or soon after, the dominant flora differed from types present on initial examination. The organisms were gram-negative rods that caused rapid deterioration in litmus milk at room temperature. They also were heat sensitive, failing to survive laboratory heating at 55 C for 10 min.

Since there are literature reports of psychrotrophic sporeformers in pasteurized milk causing spoilage in some cases, surveillance was maintained for these types. Although some spore types were observed occasionally in this study, they showed little if any increase in numbers during refrigerated storage and did not cause defects at that temperature.

Additional studies with the membrane transfer procedure are in progress to follow changes in bacterial flora and quality during retail milk storage.

CONCLUSIONS

The membrane-filter procedure offers possibilities in detecting sublethally injured bacteria in low-count pasteurized milk. There are problems with milk filtration and with selecting the appropriate medium for marginal inhibition of injured bacteria in a mixed flora.

In early stages of storage the bacterial types considered to be revived, injured organisms generally were the same as those presumed to be uninjured. They were mostly gram-positive streptococci and micrococci. They produced little change in litmus milk at 5 C. From the samples studied, the revival and growth of sublethally injured bacteria seem of doubtful importance in quality changes in refrigerated retail milk.

ACKNOWLEDGMENTS

Contribution No. 899, Department of Dairy and Poultry Sciences, Kansas Agricultural Experiment Station, Manhattan.

REFERENCES

1. American Public Health Ass. 1972. Standard methods for the examination of dairy products (13th ed.). Amer. Public Health Ass., Inc., New York.
2. Goff, J. H., T. J. Claydon, and J. J. Iandolo. 1972. Revival and subsequent isolation of heat-injured bacteria by a membrane filter technique. Appl. Microbiol. 23:857-862.
3. Maxcy, R. B., 1970. Non-lethal injury and limitation of recovery of coliform organisms on selective media. J. Milk Food Technol. 33: 445-448.
4. Speck, M. L., and R. A. Cowman. 1971. Symposium on the restoration of sublethally impaired bacterial cells in foods. II. Injury and recovery of frozen microorganisms. J. Milk Food Technol. 34:548-549.
5. Speck, M. L. 1970. Selective culture of spoilage and indicator organisms. J. Milk Food Technol. 33:163-167.