BACTERIOLOGICAL EVALUATION OF MANUFACTURING-GRADE BULK-TANK MILK

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SUMMARY

Standard, thermoduric, and psychrophilic counts were made on 701 bulk-tank milk samples of manufacturing grade. These samples were obtained from 267 Iowa producers at all seasons of the year. Plates for standard and thermoduric counts were incubated at 32°C. Direct microscopic clump counts were made on 586 samples.

The SPC of 37.7% of the samples exceeded 1,000,000 per milliliter, as compared to 26.5% at this level for the psychrophilic plate count results. Thermoduric counts in excess of 10,000 per milliliter were obtained on 43.6% of the samples.

The direct microscopic clump count was considerably less than the SPC on many samples. Of the 245 samples out of 586 comparisons where the results of these two counts failed to agree, all but 16.3% of the samples had high counts of psychrophilic or thermoduric bacteria. Most psychrophilic bacteria stained readily, but many appeared to grow in clumps. The clumps probably were broken up during preparation of dilutions for plating. Thermoduric organisms apparently did not readily take up the stain, although they were not specifically tested.

Increasing use of farm bulk tanks for handling manufacturing-grade milk means more of this milk is being cooled to 40°F. and below. Much of this milk is picked up every other day, or three times a week. Thus temperature, time of holding, and milk storage equipment are different from those commonly encountered in handling such milk in cans. The microflora which develops in such milk might be expected to be different from that of milk held for shorter periods of time at higher temperatures in cans.

Johnson et al. (6), Atherton (2), and Pearson (9) are among those who have studied the bacteriological aspects of handling Grade A milk in both bulk tanks and cans. They indicated some improvements in bacterial counts for the samples as a whole when a shift was made to bulk tanks, but differences were seldom large. Some producers actually delivered milk with higher counts after converting to bulk tanks.

In the present study, the quality of manufacturing-grade bulk-tank milk was studied where production conditions on the farms, in many cases, did not approximate Grade A requirements. The usefulness of and relationships between

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the direct microscopic clump count, the plate count, and the counts of thermo-
duric and psychrophilic bacteria were studied.

METHODS

The 701 manufacturing-grade bulk-tank samples examined were from 267
producers. These represented deliveries to four Iowa dairies during all seasons
of the year. The samples were collected at the farm by the senior author.
Collection and subsequent laboratory examination were in accordance with
procedures outlined in the tenth edition of Standard Methods for the Examina-
tion of Dairy Products (1). Standard plate count, thermoduric, and psychro-
philic counts were determined. Plates for the standard and thermoduric counts
were incubated at 32°C. Plates for the psychrophilic count were incubated at
5°C. for seven days.

Direct microscopic clump counts were made on 586 of the 701 samples. The
staining technique developed by Levowitz and Weber (7) was used.

Individual colonies from plates for psychrophilic counts were picked onto
plate count agar (PCA) slants, grown at 23°C. for 24 hr., and restreaked for
purification. Cultures to be characterized were picked from a 24-hr. PCA slant
into a carbohydrate-free broth and grown at 23°C. for 20 hr. A broth culture
was used for inoculation purposes. The methods and media used for identifica-
tion were suggested mainly by the Manual of Microbiological Methods (10).

The staining ability of representative psychrophilic cultures was determined
on cultures grown in raw milk. Milk of known low bacterial content was ob-
tained by aseptic means from the Iowa State University dairy farm bulk tank
and immediately taken to the laboratory in a refrigerated container. One drop
of a 20-hr. broth culture was added to 100 ml. of milk in a 6-oz. prescription
bottle and thoroughly mixed. Initial bacterial counts were made on the inocu-
lated milk and an uninoculated control by plating on PCA and incubating the
plates at 23°C. for 72 hr. The inoculated and control bottles were incubated
at 5°C. for 72 hr. The bottles were gently mixed each day. After incubation,
plate counts were made, using incubation at 23°C. for 72 hr. Smears for the
direct microscopic clump counts were prepared as they were for the usual raw
milk samples.

RESULTS

Results of the standard plate counts on the 701 milk samples showed that
37.2% of the total had counts below 200,000 per milliliter, 25.1% fell between
200,000 and 1,000,000 per milliliter, and 37.7% were in excess of 1,000,000 per
milliliter.

Psychrophilic plate count results on the milk samples revealed 59.5% of the
total had counts less than 200,000 per milliliter, 14.0% were between 200,000
and 1,000,000 per milliliter, and 26.5% exceeded 1,000,000 per milliliter.

The 79 cultures of bacteria isolated from the plates for psychrophilic counts
included 51 Pseudomonas, nine Achromobacter, eight Alcaligenes, seven Flavo-
bacterium, three Aerobacter, and one Escherichia, using the criteria of Bergey’s
Manual (3) for differentiation. Fifty-six of the isolates, or 70.9% of the total,
showed evidence of lipase production. These included 42 Pseudomonas, four Achromobacter, eight Alcaligenes, one Flavobacterium, and one Aerobacter. Twenty-eight of the isolates, or 35.5% of the total, failed to grow at 35°C. These included 14 Pseudomonas, six Achromobacter, five Flavobacterium, and three Alcaligenes strains. All others were able to show visible growth on PCA slants within 48 hr.

Thermotolerant counts on the 701 laboratory-pasteurized samples disclosed that 56.4% of the total had counts below 10,000 per milliliter and 29.1% had counts between 10,000 and 100,000 per milliliter, and 14.5% exceeded 100,000 per milliliter.

**TABLE 1**

Comparisons of direct microscopic counts with standard plate counts on bulk-tank milk

<table>
<thead>
<tr>
<th>Total samples</th>
<th>DMC and SPC not in agreement a</th>
<th>PPC high b</th>
<th>TPC high c</th>
<th>PPC and TPC high</th>
<th>Neither PPC nor TPC high</th>
</tr>
</thead>
<tbody>
<tr>
<td>586</td>
<td>(No.) (41.8)</td>
<td>103</td>
<td>69</td>
<td>33</td>
<td>40</td>
</tr>
</tbody>
</table>

a Standard plate count (SPC) at least twice as high as direct microscopic clump count (DMC).

b Psychrophilic plate count 75% or more than the standard plate count (SPC).

c Thermotolerant plate count (TPC) in excess of 30,000/milliliter.

d Per cent of total samples not agreeing.

e Per cent of the 245 samples not agreeing.

Results of the standard plate count and the direct microscopic clump count were compared on 586 milk samples. Table 1 summarizes the data. None of the samples had direct microscopic counts that exceeded the standard plate count to any significant extent. A lack of agreement between the two methods was considered to exist where the standard plate count was at least twice as high as the direct microscopic count. Of the 245 samples (41.8% of the total) showing lack of agreement, 103, or 42.1%, had psychrophilic counts that were 75% or more of the standard plate count. Sixty-nine samples, or 28.1%, had thermotolerant counts that were in excess of 30,000 per milliliter. Counts at this level or above constituted, in this study, one explanation for poor agreement between the standard plate count and direct microscopic count. While the 30,000-per-milliliter level might not be considered adequate to markedly influence the direct microscopic count, it would be assumed that many more thermotolerant bacteria, slightly less heat-tolerant than the survivors of the laboratory-pasteurization heat treatment, were present in the raw sample. It is undoubtedly this large number which contributes to the discrepancy in the standard plate count and direct microscopic count. Thirty-three samples, or 13.5%, had both high thermotolerant and high psychrophilic counts and 40, or 16.3%, had neither a high thermotolerant nor a high psychrophilic count.

Table 2 presents the results of the direct microscopic clump count on 31 psychrophilic cultures grown in low-count raw milk at 5°C. for 72 hr. All
psychrophilic cultures grown in raw milk had higher plate counts than direct microscopic clump counts. Cultures with a wide difference between the results of the two tests tended to grow in clumps, with one exception. The cells of Culture 48 were difficult to see in the stained preparation. All other cultures appeared to stain well enough for relatively easy observation of the cells.

DISCUSSION

The relatively poor bacteriological quality of many manufacturing grade bulk-tank milk samples examined indicates that the bulk tank is far from being

TABLE 2

Direct microscopic counts on several psychrophilic cultures grown in raw milk

<table>
<thead>
<tr>
<th>Culture number and genus</th>
<th>Plate count after 3 days at 5°C</th>
<th>Direct microscopic clump count</th>
<th>Predominant cell distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40T</td>
<td>40T</td>
<td>Clumps</td>
</tr>
<tr>
<td>2 Pseudomonas</td>
<td>40M</td>
<td>16.0M</td>
<td>Clumps</td>
</tr>
<tr>
<td>5 Pseudomonas</td>
<td>21M</td>
<td>5.0M</td>
<td>Clumps</td>
</tr>
<tr>
<td>8 Achromobacter</td>
<td>67M</td>
<td>54M</td>
<td>Pairs</td>
</tr>
<tr>
<td>10 Flavobacterium</td>
<td>1.5M</td>
<td>440T</td>
<td>Small clumps</td>
</tr>
<tr>
<td>12 Achromobacter</td>
<td>320T</td>
<td>60T</td>
<td></td>
</tr>
<tr>
<td>15 Pseudomonas</td>
<td>24M</td>
<td>10M</td>
<td>Clumps</td>
</tr>
<tr>
<td>17 Alcaligenes</td>
<td>250T</td>
<td>220M</td>
<td></td>
</tr>
<tr>
<td>19 Alcaligenes</td>
<td>300T</td>
<td>290M</td>
<td>Pairs</td>
</tr>
<tr>
<td>21 Aerobacter</td>
<td>27M</td>
<td>16.0M</td>
<td>Pairs and singles</td>
</tr>
<tr>
<td>23 Pseudomonas</td>
<td>25M</td>
<td>24.0M</td>
<td>Pairs and singles</td>
</tr>
<tr>
<td>29 Pseudomonas</td>
<td>69M</td>
<td>12.0M</td>
<td>Pairs and clumps</td>
</tr>
<tr>
<td>31 Achromobacter</td>
<td>6.8M</td>
<td>1.6M</td>
<td>Clumps and pairs</td>
</tr>
<tr>
<td>33 Achromobacter</td>
<td>12M</td>
<td>8.9M</td>
<td>Pairs and singles</td>
</tr>
<tr>
<td>36 Pseudomonas</td>
<td>16M</td>
<td>2.6M</td>
<td>Clumps</td>
</tr>
<tr>
<td>38 Flavobacterium</td>
<td>7.8M</td>
<td>4.4M</td>
<td>Small clumps</td>
</tr>
<tr>
<td>40 Pseudomonas</td>
<td>8.3M</td>
<td>780T</td>
<td>Clumps</td>
</tr>
<tr>
<td>42 Achromobacter</td>
<td>560T</td>
<td>260T</td>
<td>Small clumps</td>
</tr>
<tr>
<td>44 Escherichia</td>
<td>700T</td>
<td>420T</td>
<td>Small clumps</td>
</tr>
<tr>
<td>45 Pseudomonas</td>
<td>16M</td>
<td>3.0M</td>
<td>Clumps</td>
</tr>
<tr>
<td>48 Flavobacterium</td>
<td>24M</td>
<td>11.0M</td>
<td></td>
</tr>
<tr>
<td>49 Pseudomonas</td>
<td>65M</td>
<td>16.0M</td>
<td>Clumps</td>
</tr>
<tr>
<td>52 Alcaligenes</td>
<td>14M</td>
<td>240T</td>
<td>Clumps</td>
</tr>
<tr>
<td>54 Pseudomonas</td>
<td>26M</td>
<td>4.4M</td>
<td>Clumps</td>
</tr>
<tr>
<td>56 Pseudomonas</td>
<td>100M</td>
<td>23.0M</td>
<td>Pairs, individuals, clumps</td>
</tr>
<tr>
<td>58 Pseudomonas</td>
<td>18M</td>
<td>4.0M</td>
<td>Clumps</td>
</tr>
<tr>
<td>61 Aerobacter</td>
<td>6.3M</td>
<td>1.6M</td>
<td>Clumps</td>
</tr>
<tr>
<td>62 Achromobacter</td>
<td>8M</td>
<td>1.1M</td>
<td>Clumps</td>
</tr>
<tr>
<td>63 Pseudomonas</td>
<td>42M</td>
<td>25.0M</td>
<td>Pairs and individuals</td>
</tr>
<tr>
<td>67 Pseudomonas</td>
<td>38M</td>
<td>6.7M</td>
<td>Small clumps</td>
</tr>
<tr>
<td>69 Pseudomonas</td>
<td>60M</td>
<td>13.0M</td>
<td>Clumps</td>
</tr>
<tr>
<td>75 Pseudomonas</td>
<td>75M</td>
<td>72.0M</td>
<td>Pairs and individuals</td>
</tr>
</tbody>
</table>

a One drop of a 20-hr. broth culture in 4 oz. of raw milk before incubation. Plates incubated at 23°C for three days.

b Levowitz-Weber staining technique, clumps counted.

c T = Thousand.

d M = Million.
the complete answer to producing and handling quality milk. An inadequate job of cleaning the bulk tank undoubtedly accounts, to a large extent, for many high bacterial counts obtained. The high proportion of psychrophilic bacteria is noteworthy. Because the bulk tank is kept at refrigeration temperature most of the time, the bacteria growing in that environment would be expected to be predominantly psychrophilic. The time between emptying and refilling would not be a long enough interval at a higher temperature that one would expect the character of the microflora to change to any great extent. With the factors of time and warmer atmospheric temperatures, the psychrophilic organisms might increase in numbers very rapidly in the unwashed or poorly washed tank. In spite of the low holding temperatures of the milk subsequently placed in the bulk tank, these residual organisms would slowly increase in numbers and frequently make up a large proportion of the microflora of the milk.

When the producer fails to clean and sanitize his milking equipment which is held at milk-house temperatures between uses, opportunity exists for thermoduric organisms to grow on the equipment, especially during the warmer months. This equipment would become a prime source for contaminating the milk. If a combination of poor care of the bulk tank and of the milking and milk handling equipment prevailed, the microflora of the milk might include large numbers of both psychrophilic and thermoduric bacteria.

The primary factor responsible for the wide discrepancies between plate counts and direct microscopic counts appears to be the tendency for many of the psychrophilic organisms to grow in clumps in raw milk. The necessary shaking of the dilutions during plating procedures may break a bacterial clump into several portions, each of which may produce a colony. The psychrophilic cultures which grew as single cells and pairs had microscopic clump counts comparable to the standard plate count. The comparability of these two tests when the milk contained a large proportion of psychrophilic bacteria would depend to a large degree on whether the majority of the psychrophilic bacteria grew in clumps or as individual cells and/or pairs. The possibility also exists that some of the psychrophilic bacteria stain so poorly they are not counted under routine conditions.

Over 41% of the samples that were not classified properly by the direct microscopic clump count, as judged by the standard plate count, had thermoduric plate counts in excess of 30,000 per milliliter. Although the staining ability of thermoduric isolates was not studied, many thermoduric organisms apparently do not stain readily and, thus, are hard to enumerate. Little (8) found that the microscopic count was not accurate in determining the bacteriological quality of raw milk when the samples contained large numbers of thermoduric bacteria.

The direct microscopic clump count appears to be of limited value for estimating the bacterial population of manufacturing-grade bulk-tank milk where the predominant microflora could be made up of large numbers of psychrophilic or thermoduric organisms. Jezeski et al. (5) and the Committee on Applied Laboratory Methods (4) previously found the direct microscopic count
not as efficient as the standard plate count in picking out unsatisfactory raw milk samples with counts exceeding 200,000 per milliliter.

The plate count with incubation at 32° C., instead of 35° C., appears the procedure of choice for bacteriological classification of bulk-tank raw milk, because the lower temperature permits growth of more psychrophilic bacteria. Modifications of the plate count procedure to make it more applicable to plant routine might prove desirable.

REFERENCES


