BACTERIA THAT SURVIVE AND GROW DURING THE PASTEURIZATION OF MILK AND THEIR RELATION TO BACTERIAL COUNTS

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BACTERIA THAT SURVIVE AND GROW DURING THE PASTEURIZATION OF MILK AND THEIR RELATION TO BACTERIAL COUNTS

PAUL S. PRICKETT1 AND ROBERT S. BREED

ABSTRACT

The serious problem produced by the irregular development of "pin-point" colonies on agar plates made for the purpose of controlling the sanitary quality of pasteurized milk is complicated by the variety of the bacteria that survive or actually grow during the pasteurization of milk. The problem is further complicated in that certain variations in laboratory technic, most important of which are variations in the composition of the nutrient agar and in the temperature of incubation, play an important part in causing the appearance of these pin-point colonies.

In the present study, it has been found that the direct microscopic examination of pasteurized milk is of great value as a means of detecting the presence of heat-resistant and heat-loving bacteria. When microscopic examination shows large numbers of streptococci or of rod-shaped bacteria, then the routine methods of making agar plate counts are quite likely to fail to reveal all of the living bacteria present. Under these conditions special media and temperatures of incubation adapted to special types of bacteria must be used in order to secure the growth of these bacteria. When grown under proper conditions, the bacteria that appear in pin-point colonies on routine plates usually grow colonies of normal size.

A study of the distribution of the various types of heat-resistant and heat-loving bacteria supports the idea that the original source of these bacteria is in the dust and dirt that may get into the milk on the farm. The original contamination is small in amount but the numbers are increased rapidly where ever

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temperatures warm enough for growth are found. These usually appear during the pasteurization process.

It was found that the best method by which a farmer could reduce the number of these troublesome bacteria to a minimum was by reducing contamination from feed, hay, stable dirt, etc., to a minimum. Under some conditions milk cans and possibly other utensils may be a source of trouble.

The following precautions have been found valuable by milk plants in preventing the excessive growth of heat-loving bacteria in the plant: Return of clean, sterile, dry cans to producers; no repasteurization of drainings or drippings from pasteurizing or bottling equipment; no repasteurization of returns from milk routes; keeping the length of the pasteurizing run with any given equipment within the limit that permits excessive numbers of thermophilic bacteria to develop; not allowing milk to be held longer than 30 minutes at warm temperatures (above 100°F); flushing and scalding the walls of pasteurizing vats between batches where batch type pasteurizers are used; the scrupulous cleaning and steaming of equipment with complete removal of any milk cooked on to the equipment; and flushing of equipment with hot water just before use. A reduction in the number of heat-resistant and heat-loving bacteria was found where bottled pasteurized milk was held over night in cold temperatures (below 45°F).

INTRODUCTION

One of the most popular and prosperous agricultural activities of the farmer of New York State is the production of milk. When it is remembered that about 75 per cent of the milk produced in the State is consumed as fluid milk or cream, the larger part of which is pasteurized, it will be realized that an investigation of the bacteria surviving pasteurization and of the methods of bacteriological control of pasteurized milk is of importance to the dairy industry of the State. These same questions are also of importance to the various public and private milk control laboratories charged with maintaining milk quality. Those industries manufacturing dairy products, in which the milk is subjected to heat-treatments, are also affected by the activities of the bacteria that resist high temperatures.
Doubtless investigators have noticed small, grayish-white, dense (punctiform) colonies appearing on agar plates (Fig. 1) prepared from heated milk almost from the very beginning of the use of the plate technic in examining substances for their bacterial content. Certainly some of the more common streptococci found in milk would have given such colonies. However, it was not until shortly after the close of the World War that serious attention was paid to the appearance of such colonies on agar plates poured from pasteurized milk. This was because of the suddenness with which they appeared in large numbers and their equally sudden disappearance. Due to their size.
shape, and appearance these colonies were soon given the descriptive name of "pin-point" colonies, or shortened merely to "pin-points."

The so-called pin-point colonies have caused much discussion, part of which has been due to a varied use of the term by different workers. It is used here to denote those very small, punctiform, grayish-white, surface and sub-surface colonies often encountered on plates poured from milk, especially pasteurized milk, the causal organisms of which are heat-resistant enough so that at least 90 per cent of them survive or even grow on being heated 30 minutes at 145°F (62.8°C). In general, this is what most workers have in mind when they refer to pin-point colonies. However, there are those who also apply the term to the small colonies found on plates poured from raw milk, and occasionally pasteurized milk, which are produced by certain of the more or less common streptococci, the majority of which are not heat-resistant. While it is recognized that the term pin-point colonies is not a desirable term, is inaccurate, and is capable of more than one interpretation, still it is felt that its use is so widespread and that it so aptly describes the condition, that it is not likely to be entirely discarded. Consequently, it is suggested that to save confusion certain definite limits, as suggested, be kept in mind when using the term. Fay (1926, 1927)\(^2\) also stresses this point.

To avoid confusion, definitions for certain other terms used in this report are given here. *Obligate thermophiles* (heat-loving bacteria) are those bacteria which will develop at 133°F (56°C), but not at 98.6°F (37°C). *Facultative thermophiles* will grow at both 56° and 37°C. Heat-resistant bacteria are those which will endure high temperatures, such as are reached in pasteurizing, but they do not grow at these temperatures.

Among the first studies and reports of the sporadic appearances of the pin-point colonies, and the consequent abnormally high counts in the pasteurized milk affected, were those made by Dotterrer (1923) and Yates (1923). Two explanations soon were offered for the appearance of pin-point colonies. One was that it was due to a change in the ingredients used in the preparation of the standard nutrient agar medium used for plating, plus a changed method for adjusting the reaction of the standard medium. The other was that it was due to the use of certain chlorine compounds in the disinfection of dairies and milk plants. Another point of view held by many public health officials was

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\(^2\)Refers to Literature Cited, page 24.
that the appearance of pin-point colonies on plates poured from pasteurized milk indicated unsanitary practices in the milk plant.

Dotterrer pointed out that high counts after pasteurization often indicated the presence of an unusually large percentage of heat-resistant organisms in the raw milk supply, rather than the presence of unsanitary practices in milk plants. Ayers and Johnson (1924), who, in their study of *Lactobacillus thermophilus* were probably the first workers in this country to isolate and name an organism responsible for producing pin-point colonies, confirmed the claims made by Dotterrer.

Soon the complaint concerning pin-point colonies was countrywide, and the committee on Standard Methods of Milk Analysis of the American Public Health Association requested its referee to stimulate research on the problem. Study of the relationship between pin-point colonies and heat-resistant organisms was undertaken at this Station. The results of an investigation of the non-spore-forming organisms that play a part have been published by Robertson in Technical Bulletins Nos. 130 and 131 of this Station and in Bulletins Nos. 274 and 275 of the Vermont Station. A study of certain of the thermophilic spore-formers involved has been published by Prickett in Technical Bulletin No. 147 of this Station. A summary of these and related bulletins has been prepared by Breed as Bulletin No. 559 of this Station. A description of the results of work done to control an outbreak of thermophilic bacteria in a pasteurizing plant is given by Yale in Technical Bulletin No. 156.

**EXPERIMENTAL IMPORTANCE OF MICROSCOPIC EXAMINATION**

An important fact demonstrated in this study is that plate counts obtained by incubation at 37°C (98.6°F) gave little idea as to the real bacterial content of the milk if thermophilic, and to a less extent, if heat-resistant organisms are present. In one sample of milk examined, the 37°C plate count was 3,000,000 per cc, the 56°C (133°F) plate count was 40,000,000 per cc, and the direct microscopic count showed 58,000,000 per cc. These typical figures, taken from Table 1, demonstrate that the microscope is of value in detecting thermophilic and heat-resistant organisms when these are present in pasteurized milk. It not only allows the analyst to determine quickly the number of organisms present, but of equal if not greater importance, it also allows him to determine simultaneously, the types of organisms present. This information is often of great importance to the public health official.
<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Acidity of milk as percentage of lactic acid</th>
<th>Direct microscopic examination</th>
<th>Number of colonies per cc on agar plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.13</td>
<td>Rods (plump, medium length, clumped)</td>
<td>Dehydrated Bacto-nutrient agar</td>
</tr>
<tr>
<td>2</td>
<td>0.13</td>
<td>Spores?</td>
<td>48 hours at 37°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 hours at 56°C</td>
</tr>
<tr>
<td>3</td>
<td>0.16</td>
<td>Rods (more single and paired than in No. 1)</td>
<td>Dehydrated Bacto-nutrient agar</td>
</tr>
<tr>
<td>4</td>
<td>0.18</td>
<td>More spores in No. 4</td>
<td>48 hours at 37°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 hours at 56°C</td>
</tr>
<tr>
<td>1H</td>
<td>0.28</td>
<td>Rods (large and small types, big clumps); spores; streptococci</td>
<td>Dehydrated Bacto-nutrient agar</td>
</tr>
<tr>
<td>2H</td>
<td>0.28</td>
<td>Very few large rods or spores; streptococci</td>
<td>48 hours at 37°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 hours at 56°C</td>
</tr>
<tr>
<td>3H</td>
<td>0.41</td>
<td>Rods (small, few clumps)</td>
<td>Dehydrated Bacto-nutrient agar</td>
</tr>
<tr>
<td>4H</td>
<td>0.38</td>
<td>Few streptococci</td>
<td>48 hours at 37°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48 hours at 56°C</td>
</tr>
</tbody>
</table>

*1 = Milk from No. 1 pasteurizer at beginning of run, examined as received.
2 = Milk from No. 2 pasteurizer at beginning of run, examined as received.
3 = Milk from No. 1 pasteurizer at end of run, examined as received.
4 = Milk from No. 2 pasteurizer at end of run, examined as received.
H indicates that the milk was heated for 12 hours at 63°C before plating.
FACTORS INFLUENCING PRODUCTION OF PIN-POINT COLONIES

It was found in studying this problem that, in general, two factors largely influence the production of pin-point colonies. These factors which may operate independently or together, are laboratory technic and the type of organisms present in the milk.

Laboratory technic affecting the production of pin-point colonies.—Certain variations in the laboratory technic may produce pin-point colonies on the plates. The two most important are the composition of the media and the temperature of the incubator.

It has only recently been generally realized that thermophilic bacteria in milk, especially the spore-bearing rods, may be very particular in their nutritive requirements. Hussong and Hammer (1928) have described a thermophilic spore-former that grows very well on glucose nutrient agar but which refuses to grow on nutrient or whey agar. Robertson, in Technical Bulletin No. 130, reports a thermophilic spore-former that required raw, unsterilized milk for good growth. In the present investigation, organisms have been isolated that grew very poorly or not at all on ordinary nutrient agar but would grow luxuriantly in peptonized milk media, while others grew best on yeast-extract agar. Certain organisms have also been isolated that grew very poorly on ordinary nutrient agar, but grew abundantly in a synthetic medium containing glucose. The heat-resistant streptococci grew very poorly on nutrient agar.

The importance of slight variations in the reaction of the media as affecting the appearance of certain types of pin-point colonies was pointed out by Cooledge (1924). Knudsen and Sørensen (1929) have recently shown that buffering media with phosphates and citrates enable some of the lactic streptococci to develop visible colonies on certain media.

From the work of others and that of this laboratory, it seems that no one medium is suitable for the detection of all thermophilic and heat-resistant organisms. The most suitable medium depends on the flora of the milk under examination. If the medium on which the milk is plated is not favorable either in reaction or nutrient materials, such organisms either will not grow or will form pin-point colonies due to restricted growth.

The incubator temperature is also important. Improper filling of an incubator with petri plates has been shown to influence the count, and the differences in temperatures which usually exist in different
parts of incubator also play their part in causing otherwise invisible colonies to appear as pin-points. If the temperature remains constant at 37°C (98.6°F), only the facultative thermophiles will develop on the plates; but if the temperature raises 2°C, or even 5°C as may happen, then the lower limit of growth for obligate thermophiles is reached and they start development. If the temperature drops again before full development is reached, these colonies must appear as pin-points. Even facultative thermophiles may find 37°C so cold that they will not grow normal size colonies.

An incubator so regulated that its temperature is 37°C during the day normally has a higher temperature during the night when the door remains closed at all times. This situation is even more likely to occur over a week-end. Use of maximum-minimum or recording thermometers in incubators reveals many unsuspected variations in temperature of these types.

Sediment in the medium used may also cause a report of the presence of pin-points. This frequently happens where dehydrated media are used without taking proper precautions for bringing this material into solution. Difficulties were experienced in this work from this cause until the trick of bringing this material into complete solution was learned. This is done by adding a relatively small amount of water to the powder in a large flask which is then shaken until the powder is thoroly wet. Then the remainder of the water is added and the material dissolved in a steam chest rather than the autoclave. A short period of contact between the medium and the water with frequent shaking before it is placed in the steam chest seemed to aid in bringing the material into complete solution. The length of time required for the medium to go into solution in the steam chest seemed to depend also upon the size of the flask and the amount of medium it contained. Usually 20 minutes was sufficient. If the flask was removed and shaken during this period the process was hastened. After this treatment it seldom was found necessary to filter the medium and no precipitate appeared on sterilization in the autoclave. The results secured by this method of preparation were much more satisfactory than those secured where the medium was dissolved in the autoclave.

It is especially difficult to distinguish between sediment from dehydrated agar and pin-point colonies even under a microscope. It frequently happens in such agar that objects which may be regarded as colonies cannot be made to produce growth on transfer even when special
methods adapted to growing the types of bacteria producing pin-point colonies are used.

Crowding often produces pin-point colonies, and this condition may arise regularly in the routine laboratory examination of pasteurized milk as routine samples frequently are not plated in dilutions higher than 1:10,000. If thermophilic and heat-resistant organisms are present in their usual numbers in these routine samples, a plate poured from a 1:10,000 dilution would be crowded and the colonies probably would be of the pin-point variety, altho it is quite possible that in higher dilution and with less crowding the colonies would be considerably larger.

ORGANISMS CAUSING PIN-POINT COLONIES

Sources of materials studied.—To study the flora of milk which would cause the production of pin-points, samples were obtained of raw milk from various sources; of pasteurized milk in the various stages of pasteurization; of foam on milk in pasteurizers; of scrapings of "milk-stone" from the walls of pasteurizing equipment; of rinsings of supposedly sterile cans at the pasteurizing plants; and of dairy utensils on dairy farms.

A survey of the raw milk supply of certain pasteurizing plants in Buffalo showed that certain of their producers were delivering raw milk which contained heat-resistant and thermophilic organisms. Visits were made to representative farms supplying such milk, and samples were collected of materials which were suspected of contributing their quota of thermophilic organisms to the raw milk produced on that farm. A few samples were obtained from other sources. Some cultures were also obtained from other laboratories, both as subcultures and from plates containing pin-point colonies.

Methods of isolating the cultures.—It was found that the methods of isolation employed markedly affected the type of organisms isolated, consequently, the methods were varied to secure a variety of cultures. An understanding of this fact is thought to be of special importance in comprehending the pin-point problem and in interpreting the results obtained. 3

Types of organisms isolated.—From all of the samples examined, a total of 480 cultures were isolated. This number was made up of seven different groups of organisms which are listed in the order of the

3Details of the methods employed throughout this study are given in Technical Bulletin No. 147 of this Station.
frequency of their isolation in this study as follows: Spore-forming rods; streptococci; non-spore-forming rods; micrococci; Actinomyces; sarcinae; and yeast. Of these organisms, only the spore-forming rods, the Actinomyces, and a few of the non-spore-forming rods were found to be truly thermophilic. The remainder were merely heat-resistant.

The thermophilic and especially heat-resistant spore-forming bacteria isolated in this investigation were studied in detail. Ten types of species were recognized as follows: Bacillus michaelisii Pickett (probable synonym B. thermophilus aquatilis liquefaciens Michaelis), B. calidus Blau, B. thermoalimentophilus Weinziril, B. aerothermophilus Weinziril, B. nondiastaticus Bergey, B. thermoliquefaciens Bergey, B. calidolactis Hussong and Hammer, B. kaustophilus Pickett, B. terminalis Migula var. thermophilus Pickett, and B. subtilis (Ehrenberg) Cohn, as described by Ford.

All of the seven different main groups of bacteria were isolated at one time or another from pin-point colonies, altho they did not all continue to give this type of colony when cultivated on various media. Other workers have described a variety of heat-resistant and thermophilic organisms isolated from pin-point colonies. Ayers and Johnson (1924) described a Lactobacillus, while Robertson, in Technical Bulletin No. 131, describes the same or a closely related bacterium. He also found a heat-resistant streptococcus and a heat-resistant sarcina as organisms which commonly form pin-point colonies.

Clark and Dougherty (1926) report the isolation of thermophilic spore-forming rods and non-spore-forming rods from pin-point colonies. Work on non-spore-forming organisms that form pin-point colonies has been reported by Seibel (1927) and by Hucker in Technical Bulletin No. 134 of this Station. The latter discusses the effect of cooling milk before pasteurization on the relative numbers of the heat-resistant streptococci. It has been found that the treatment the milk receives previous to plating and the methods and plating media used exert a marked influence on the types of cultures obtained. It is felt that an appreciation of this is necessary to understand and interpret the problems that arise when pin-point colonies are encountered on plates poured in the sanitary control of milk.

In some cases at least the heat-resistant organisms are more frequently responsible for the appearance of pin-point colonies on milk control plates than truly thermophilic organisms. This conclusion, reached from the results of this study, is borne out by the experience of the routine milk control work on the milk supply of the City of
Geneva carried out in this laboratory. *Streptococcus thermophilus* Orla-Jensen (1919) has been isolated from the majority of the pin-point colonies appearing on the control plates poured from pasteurized milk. However, it is realized that facultative thermophiles are also capable of causing pin-point colonies under the conditions attending the preparation and incubation of plates for sanitary milk control, even when such conditions are strictly standardized. In this connection, it is interesting to note the types of organisms obtained from the Board of Health Laboratory of Buffalo which were considered typical pin-point colony producers. Out of nine cultures, four were thermophilic spore-formers and incidentally all four were found to be of the same species, *B. thermoalimentophilus* Weinzirli. Of the remainder, all of which were heat-resistant rather than thermophilic, one was a streptococcus, one a micrococcus, and three non-spore-forming rods.

It is thought that one reason why more heat-resistant streptococci were not included in the series of 480 cultures isolated is because the most common species in pasteurized milk, *Streptococcus thermophilus* Orla-Jensen, is a very difficult organism to carry in the laboratory. It is a delicate and sensitive organism when cultivated in artificial culture media.

As pointed out by Breed in Bulletin No. 559 this organism answers the description of the species that some investigators have found difficulty in isolating. It does not grow on ordinary standard media unless significant amounts of milk are added to the media as e. g., thru the dilution water; it is very sensitive to cold so that it dies out readily if held at temperatures below 68°F (20°C); and it is not easy to secure viable transplants from milk plates. In this study it was successfully picked from the plates by excising whole a small block of agar containing the colony, transferring the block to a tube of semi-solid, pptonized-milk-yeast-extract medium, crushing the block against the inner wall of the tube, mixing it with medium, and incubating at 98.6°F (37°C).

The obligate thermophilic organisms will play no part in forming pin-point colonies unless the incubation temperatures are warmer than 37°C, and if this occurs, the methods used are no longer standard.

**DISTRIBUTION OF TYPES OF THERMOPHILIC AND HEAT-RESISTANT ORGANISMS ISOLATED IN THIS STUDY**

In Table 2, the distribution of the organisms isolated from milk, milk plants, and dairies has been tabulated. The fact that Plant BD
Table 2.—Distribution of the Types of Thermophilic and Heat-resistant Organisms Isolated in This Study.

<table>
<thead>
<tr>
<th>Source of culture</th>
<th>B. calves</th>
<th>B. thermophilus</th>
<th>B. thermophilus menthophilus</th>
<th>B. pseudepolioides</th>
<th>B. thermolactis</th>
<th>B. michaelis</th>
<th>B. terminalis</th>
<th>B. kaustophilus</th>
<th>B. subtilis</th>
<th>Streptococci</th>
<th>Micrococci</th>
<th>Non-spore-forming rods</th>
<th>Actinomyces</th>
<th>Sarcinae</th>
<th>Yeasts</th>
<th>Not identified (probably spore-forming rods)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm BA</td>
<td>13</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>12</td>
<td>1</td>
<td>49</td>
<td>19</td>
<td>40</td>
<td>5</td>
<td>29</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>2</td>
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<tr>
<td>Farm BM</td>
<td>14</td>
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<td>9</td>
<td>2</td>
<td>3</td>
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<td>1</td>
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<td>2</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>16</td>
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<td>19</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pasteurizing plant BD</td>
<td>4</td>
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<td>3</td>
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</tbody>
</table>

*Number of cultures isolated from source indicated.
received the most intensive study accounts for the largest number of cultures having been obtained from it. It can be seen at a glance that *B. calidus* was the type most frequently isolated from the materials collected on farms BA and BM, and that *B. nondiastaticus* was the dominant type found on farm BS. It would seem from the information presented in Table 2 that *B. calidus*, *B. acrothermophilus*, and *B. nondiastaticus* were the dominant types of thermophilic spore-formers in the materials examined from these farms. Further investigation might or might not confirm this. Additional study of farms is necessary before generalizations can be drawn. Another interesting fact is that spore-forming rods and *Actinomyces* were the only thermophilic organisms isolated from these materials. The information gained from this phase of the study sheds no light on the source of the heat-resistant organisms isolated from milk.

It is interesting to note in Tables 2 and 3 the number of cultures of *B. subtilis* that were isolated from pasteurized milk. The samples secured from city I (Ithaca) yielded a majority of *B. subtilis* cultures. It seems significant that a large number of the cultures isolated from the Ithaca milk and from the State Fair samples were *B. subtilis*, when it is learned that the former milk was refrigerated 24 hours and the latter for at least five days before it was examined. Furthermore, these samples represent widely diverse sources, suggesting *B. subtilis* to be one of the most widely distributed of the organisms that are of importance in this connection.

In Table 3 is found a comparison between the number of cultures of each group and species of organisms isolated from raw and pasteurized milk. It is suggested that little importance be attached to the results reported for plant BG, since only a few samples were obtained at that plant. When the figures for the other three plants are examined, it will be noted that in many of the cases the same organisms were isolated from both the raw and the pasteurized milk. There are two notable exceptions in the figures for plant BD. These will be noted in the columns headed "*B. kaustophilus*" and "Non-spore-forming rods." This is explained by the fact that, due to the conditions under which the majority of samples were obtained, no samples of the raw milk were available for comparison with these samples of pasteurized milk. In nearly all other cases comparable samples of raw and pasteurized milk were obtained. The figures given in the column "Streptococci" are interesting. It is thought that one reason why more cultures of these
<table>
<thead>
<tr>
<th>PLANT</th>
<th>MILK</th>
<th>B. caldus</th>
<th>B. thermacidophilus</th>
<th>B. aerothermophilus</th>
<th>B. nonadactyloides</th>
<th>B. thermodilobiicola</th>
<th>B. michaelisii</th>
<th>B. terminalis</th>
<th>B. kurstaki</th>
<th>E. subtilis</th>
<th>Streptococc</th>
<th>Micrococc</th>
<th>Non-sporforming rods</th>
<th>Actinomyces</th>
<th>Sarcinae</th>
<th>Yeasts</th>
<th>Not identified (probably spore forming rods)</th>
</tr>
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<tr>
<td>BD</td>
<td>Raw Pasteurized</td>
<td>4*</td>
<td>1</td>
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<td></td>
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<tr>
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<td>Raw Pasteurized</td>
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<td>G</td>
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<td>2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>11</td>
</tr>
</tbody>
</table>

*Number of cultures isolated from the source indicated.
organisms were isolated from pasteurized milk than from raw milk is because in the raw milk many other types were present and that the heat-resistant streptococci did not constitute so large a part of the flora as they did after pasteurization had killed the less heat-resistant types. This explanation seems more logical when it is known that the number of colonies picked from plates was governed by the number of types of colonies that developed, and their relative numbers.

**SUGGESTIONS ON CONTROLLING THERMOPHILES**

Practically all who have studied the pin-point problem agree that the organisms responsible originate outside of the milk plant, probably on the farm; that these organisms are present in the raw milk, usually in relatively low numbers; and that these organisms, if present in the raw milk, undergo extraordinarily rapid multiplication in the milk during the pasteurizing process unless certain precautions are observed. Thus, any suggestions for controlling these organisms would come under one of two heads, viz., control measures on the farm and control measures in the milk plant.

**SUGGESTED CONTROL MEASURES FOR FARMS**

The control of heat-resistant organisms in market milk is a difficult problem at best because of the nature of the organisms themselves and because of the diversity of types. Usually, the pasteurizing process destroys over 95 per cent of the ordinary bacteria found in raw milk. However, raw milk has been found by Dotterrer (1923), Mudge,4 and others the flora of which was made up so largely of heat-resistant organisms that only a comparatively small per cent of the organisms in the raw milk were killed. If such raw milk constitutes a comparatively large portion of the milk pasteurized in a plant, satisfactory reduction of the total number of bacteria present in the raw milk can not be achieved even by the most careful control of the pasteurizing process.

The following figures obtained in this laboratory are interesting. A sample of raw milk showed 640,000 colonies per cc when the plate was incubated at 37°C (98.6°F), and 360,000 colonies per cc when duplicate plates were incubated at 55°C (131.0°F). Another sample showed 8,000 at 37°C and 42,000 at 55°C. It was found during this study that usually organisms which will develop on plates incubated at 55°C will also withstand pasteurization. Added to this is the

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probability that an appreciable percentage of the organisms that develop at 55°C will also develop at least as pin-point colonies in 48 hours' incubation at 37°C.

Mudge reports a case where an extraordinarily thorough cleanup of the farms of the milk producers of a certain plant solved the pin-point colony problem of that plant. In the course of his study, a certain plant found to be troubled with thermophiles conducted a cleanup campaign with their producers after which it was impossible to demonstrate the presence of thermophilic organisms in samples of raw milk collected from each producer's milk. A noticeable portion, however, of the colonies developing on plates poured from the pasteurized milk after the cleanup were heat-resistant in type.

Since thermophilic organisms grow little, if at all, at low temperatures, the farmer can prevent their multiplication in the milk by keeping it cool. Hucker, in Technical Bulletin No. 134, reported that he found larger numbers of heat-resistant streptococci in uncooled raw milk than he did in cooled raw milk as delivered at pasteurizing plants.

Several farms around Buffalo that were considered to be producing milk with an abnormally high thermophilic count were visited to observe their practices and to study the source of the organisms. In several instances strains of the same spore-bearing organism were isolated from pasteurized milk of a pasteurizing plant and from materials from a farm which was delivering its raw milk to that plant.

Eckford (1927) also has reported finding a thermophilic spore-former from materials from farms that correspond to a type she isolated from pasteurized milk. Thermophilic or heat-resistant non-spore-forming organisms were not isolated from farm sources in this study. The source of most of these types has yet to be determined.

All of the farms visited had certain things in common. Three of them were only a few miles apart and delivered milk to the same plant, and it is possible that these were receiving unsterile milk cans. However, conditions were found on the farms which might have contaminated the milk with thermophiles. At all the farms visited where samples were collected (Table 4) the feeding practices were such that the air contained feed and hay dust at the time of milking. The bedding was dirty and well mixed with manure in all cases. All of the stables showed unusual amounts of dust, cobwebs, etc., on window sills, ledges, and such places. Only one of the stables (farm BA) inspected could be classed as fairly clean and this applied chiefly to the floor. At two of the farms, BM and BJ, milk had been shown to be heavily con-
taminated and the conditions of the cows was very bad. The latter showed no evidence of having been brushed. Their hides were filthy, also heavily coated with dandruff-like material, and the hair of some of them would come away in great bunches at the slightest pull. The sanitary condition of one stable inspected (farm BM) was particularly bad and the milk produced in this stable was shown to be consistently heavily contaminated with thermophilic organisms. All of the farms used single service cotton strainer discs, but two of the farms used strainer cloths in addition.

Table 4.—Distribution of the Thermophiles Isolated from the Materials Collected on the Farm.

<table>
<thead>
<tr>
<th>Cultures isolated from</th>
<th>B. caldus</th>
<th>B. aerothermo-philus</th>
<th>B. nondia statistens</th>
<th>B. thermoliquefaciens</th>
<th>B. michaelisitii</th>
<th>B. terminalis var. thermophilus</th>
<th>Actinomyces</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Dry, ground feed (bran, etc.)</td>
<td>A-M*</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Ensilage</td>
<td>A-M</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
<td></td>
<td>M</td>
</tr>
<tr>
<td>Hay and hay dust</td>
<td>M-S</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Barn and equipment:</td>
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<td></td>
<td></td>
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<td></td>
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<tr>
<td>Barn floor scrapings</td>
<td>A-M-S</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Dust from ledges</td>
<td>A-M-S</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Milking-machine air hose</td>
<td>M-M</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
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<td></td>
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<tr>
<td>Milking-machine brine solution</td>
<td>M-M</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rinsings from milk cooler</td>
<td>A-M</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Wash rag for pails</td>
<td>A-M</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cow:</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Bedding for cow</td>
<td>A-M</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cow's trough water</td>
<td>A-M</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Scrapings from cow's bodies</td>
<td>A-M</td>
<td>M</td>
<td>S</td>
<td>A</td>
<td></td>
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<tr>
<td>Raw milk on farm</td>
<td>A-M</td>
<td>M</td>
<td>S</td>
<td>A</td>
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*Letters refer to the farms on which the materials were collected from which the cultures were isolated.

From a closer inspection of Table 4, it is seen that with the exception of two species, *B. thermoliquefaciens* and *B. terminalis* var. *thermo-philus*, every species isolated was obtained from the feed as well as from other materials. It is of interest to note in passing that the samples of feed were obtained from sacks, bins, etc., before it was brought into the stables. An important relationship disclosed by Table 4 is that the same species found in the dry, ground feed and the hay and hay-dust, both of which produce a dust that settles over all objects in the barn, were found in other materials collected from the
barns. The only indication offered that one of the species isolated might not have come from the feed is in the case of *B. terminalis* var. *thermophilus* which was isolated from several producers' raw milk when a survey was made of the raw milk delivered in Geneva for its thermophilic bacterial content, as is seen in Table 2. However, the source of these organisms was not found at Geneva as no visits were made to the farms.

Some effort has been made to control thermophiles by chemical sterilization. In fact the use of chlorine compounds in dairies and in milk plants was widely blamed at one time for the appearance of pin-point colonies. Yates (1923) and Clarke and Dougherty (1926) in discussing this question agree that the use of such disinfectants causes the producer and dealer to use less heat and hot water in cleaning their equipment, and they believe that to be one of the causes of pin-point colonies.

Clarke and Dougherty (1926) investigated the amount of chlorine necessary to sterilize dairy equipment infected with thermophilic organisms. They consider that 28 parts of available chlorine per million parts of water, when applied at a temperature of 82.2°C (180°F), provides a safe margin for ordinary work when non-spore-forming bacteria are to be dealt with. For the spore-forming thermophiles, they conclude that the "critical" strength of chlorine sufficient for sterilizing at room temperature lies somewhere between 25 and 35 parts of chlorine per million parts of water.

They state "... that while these tests conducted under laboratory conditions have allowed for unusual concentrations of bacteria, various conditions may arise in plant operation that would require a still heavier concentration of chlorine to be effective. Excess of organic matter, such as might be found in carelessly cleaned piping, would absorb considerable quantities of chlorine and prolonged exposure to metallic surfaces, particularly copper, would also result in a lessening of the effective strength of the chlorine solution used."

The authors of this bulletin feel that in regard to the use of chemical disinfectants on dairy equipment infected with thermophiles the same objections obtain and the same precautions must be observed as with any other types of bacteria found in milk. Dairy control officials object to the use of chemical sterilizers on the ground that the user of them is liable to slight the cleaning operations in the belief that the disinfectant makes thorough cleaning unnecessary. This fallacy is especially pernicious where thermophilic and heat-resistant organisms are concerned,
since they are so much more resistant to heat than other types, and consequently are not killed by pasteurizing. From the foregoing discussion it would seem that cleanliness plays a very important, if not the greater, part in controlling the number of thermophilic organisms in milk as produced on the farm.

It is suggested that feeding practices planned to keep dust from the stable, particularly at milking time, are helpful. Immediate cooling of the milk and maintenance of it at a cold temperature is important. It is also suggested that the milk utensils should be scalded or steamed and dried quickly and thoroly. The combination of moisture and warmth are favorable for the development of bacteria, and if moisture is present and the temperature is comparatively high (35°C to 45°C —95.0°F to 113°F), there is danger of the development of thermophilic and heat-resistant organisms.

SUGGESTED CONTROL MEASURES FOR PASTEURIZING PLANTS

There are certain precautions possible at the pasteurizing plant that will greatly aid in the control of pin-point organisms. Important among these is the degree of sterility of the cans returned to the producers. By their very nature the heat-resistant bacteria are the last ones killed when a can is washed and steamed. If some organisms of this type are left in a can that is returned to a producer, they will inoculate the milk put in that can and it is quite likely that they will multiply in the milk. Temperatures and other conditions favorable for the development of thermophiles are found where milk cans are left in the sun with covers on. Thermophilic and heat-resistant organisms have been isolated in this work from supposedly sterile cans ready to be returned to the producers.

Another practice occasionally carried out at some plants is the repasteurization of milk. The milk thus treated may be returns from milk routes or drainings from the pasteurizing equipment. The latter ordinarily contains the largest number of heat-resistant and thermophilic organisms. This milk is usually repasteurized at the beginning of the next day’s run, in which case the entire pasteurizing equipment is almost certain to be plentifully seeded with heat-resistant and thermophilic organisms, no matter how carefully it was cleaned and steamed before use. Certain plants known to follow this practice were found to

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5The authors are under obligation to N. E. Lazarus of Buffalo and to M. W. Yale (see Technical Bulletin No. 156) for suggestions in regard to this portion of this bulletin.
be troubled with thermophiles. In some cases when the practice was discontinued the thermophilic problem solved itself.

From numerous tests made both in this study and in others, it has been found that the longer the pasteurizing run, the more likely it is that thermophiles will develop. It seems that these organisms are ordinarily present in the raw milk in relatively small numbers but develop very rapidly under the peculiarly favorable conditions supplied by the pasteurizing process. The film of milk left on the walls of the vat is seeded with the thermophiles and this inoculates the incoming milk (Fig. 2). Plants using the batch type pasteurizers have the advan-

![Microscopic Appearance of Milk Stone Scrapped from Pasteurizing Vats](image)

**Fig. 2.—Microscopic Appearance of Milk Stone Scrapped from Pasteurizing Vats.** Numerous bacilli and spores are evident.

...tage that they can easily rinse out and scald a vat between pasteurizing runs, thus ridding the vat of material that would inoculate the incoming milk. This method has been used commercially and found satisfactory.

Several factors have to be considered in connection with the continuous flow type pasteurizer. First, the run should be less than the length of time found by observation to be long enough to develop thermophiles in a given milk supply. As a corollary to this, after pasteurization has once started there should be no delays of any sort in
which the milk is held in the pasteurizing train at a high temperature. It is also dangerous from the standpoint of thermophilic troubles to allow the milk to remain for any considerable length of time at 45° to 55°C (113° to 141°F) in pre-warmers or dead-ends. If a continuous flow type pasteurizer is in use for a long pasteurizing run with the usually attendant thermophilic flora, it may be necessary in order to control the thermophiles, to stop pasteurizing in the middle of the run, tear down the pasteurizing train, wash and scald it, then reassemble and complete pasteurizing. However, if a plant is doing enough business to justify the installation of two continuous flow units, it is unnecessary to stop the flow of pasteurized milk. One unit can be started before the other, and then after it has been in use as long as possible, it can be rinsed and scalded while the other unit is still in operation; then it can be put back in operation before it is time to rinse and scald the second unit.

![Microscopic Appearance of Foam from Pasteurizing Vat, Showing Thermophilic Bacilli](image)

Foam is an ideal breeding place for heat-resistant and thermophilic bacteria. Whitaker, et al., (1927) have shown that in pasteurization foam is usually several degrees below the temperature of the milk and contains a large number of bacteria. That part of the equipment in
which foam forms usually is filled with and emptied of milk several times during a single pasteurizing run, yet the foam remains in the equipment, and it is from this source that subsequent batches of milk are inoculated with heat-resistant and thermophilic organisms. Fig. 3 demonstrates the microscopic appearance of foam containing organisms that produce pin-point colonies.

It has been pointed out previously that many thermophiles are sensitive to cold, and that the count of milk containing them is often lowered by holding the milk in the refrigerator over night before delivering. This phenomenon was observed in this study.

CONCLUSIONS

1. The pin-point problem is complex due to the variety of heat-resistant and thermophilic organisms which may cause such colonies as well as the influence upon them of certain elements in the laboratory technic.

2. This complex problem can be simplified, the types as well as the numbers of bacteria present can be determined, and more intelligent control measures can be adopted by officials thru the use of the direct microscopic count in examining milk. The number of colonies appearing on plates incubated at 56°C (132.8°F) contributes valuable additional information.

3. Heat-resistant and thermophilic organisms may be controlled partially at least by farmers and milk plant operators who carefully observe the rules of cleanliness and certain corrective measures.

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