STUDIES ON PASTEURIZATION[1]

XII. CAUSE AND SIGNIFICANCE OF PIN-POINT COLONIES FROM PASTEURIZED MILK

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The appearance of so-called pin-point colonies when pasteurized milk is plated has led to much speculation as to what type of organisms they represent. In fact the frequent appearance of the colonies from the pasteurized milk of certain dairies has so disturbed health officials that there has been, in one instance, a disposition to prevent the sale of milk from these dairies. The cause of pin-point colonies is therefore important both to health officials and milk dealers.

PIN-POINT COLONIES

Before taking up the cause of pin-point colonies it must be realized that colonies of almost any organism which forms relatively small colonies may become pin-point in size if there are large numbers of them on a plate. Colonies are called pin points when they are just visible to the naked eye and appear very small under a hand lens. Suppose that a sample of milk is plated using a dilution of 1:100. If the milk contains 15,000 bacteria per cubic centimeter then there will be 150 colonies on the plate and the colonies will appear in their normal size. On the other hand if the milk contains 150,000 bacteria per cubic centimeter then there will be 1500 colonies on the plate and the chances are that a large proportion of them will appear as pin-point colonies. This is due to a restriction in their development by overcrowding.

[1] A list of other papers in this series is given at the end of this paper.

It is a general custom in control work to plate 0.01 cc. of milk when examining pasteurized milk, and under normal conditions this is a satisfactory dilution. If however the count increases, this dilution will not be great enough and through overcrowding of the plates the colonies may become pin-point in size. The relation of this practice of using one dilution of 0.01 cc. to pin-point colonies will be discussed later in this paper.

It is assumed in this discussion that counts are made on standard extract agar with the proper reaction and incubated at 37°C. for forty-eight hours. If the reaction is too high or too low this in itself may cause colonies to become pin-point in size. Incubation temperatures below 37°C. may prevent the appearance of pin-point colonies and slight variations in the composition of the medium may also exert an influence on their appearance.

Yates (1923) has noted the appearance of pin-point colonies in plates from pasteurized milk in Kansas City, Mo., made with standard medium and incubation, and found that the reaction of the medium had some relation to the appearance of such colonies. He also pointed out that another factor came into the local situation, the extensive use of chlorin solution at about the time pin-point colonies were observed. In regard to this factor Yates came to the conclusion that his data pointed strongly to the fact that the presence of pin-point colonies was closely connected with the use of chlorin solutions on the farms and at milk plants. He felt however that his results were not sufficiently comprehensive to warrant definite conclusions.

HISTORY OF TROUBLE WITH PIN-POINT COLONIES AT A MILK PLANT

The trouble from pin-point colonies that was experienced at a milk plant came to our attention in January. At this plant the general method for pasteurizing was as follows: The course of the milk was from storage tanks through a clarifier over a preheater, then through a pasteurizing tank where it was heated to 145°F. and held for thirty minutes. After holding, the pasteurized milk flowed by gravity over a cooler into the bottling machine.

Three tanks of the holder type were used for pasteurizing and were in constant operation from approximately 7:30 a.m. to 3:30 p.m., a period of about seven hours.

It should be kept in mind that as one tank of milk was pasteurized and emptied it was refilled and the process repeated. The ordinary grade of milk was pasteurized until about 2:00 p.m.; then one of the pasteurizing tanks was filled with a *special* grade of milk to be pasteurized. An examination of the raw *special milk* on a certain day in February showed that the average count was 28,000 bacteria per cubic centimeter, while after pasteurization, cooling, and bottling three bottles showed 72,000, 140,000, and 90,000 bacteria per cubic centimeter respectively.

Another series of tests on this special milk was made with the following results:

SAMPLE	BACTERIA PER CUBIC CENTIMETER		
	Raw milk	Pasteurized in laboratory	Pasteurized in plant
1. In weigh can as milk from each shipper was dumped	18,000	2,500	
2. In storage vat	37,000	3,000	
3. In storage vat two hours	65,000	3,600	
4. Through clarifier	72,000	2,500	
5. Over preheater	84,000	4,200	
6. In pasteurizing tank	150,000	130,000	
Out of pasteurizer over cooler			140,000
Bottling machine 1			140,000
Bottling machine 2			180,000
Bottling machine 3			150,000

It will be noted that the samples of raw milk 1 to 5 inclusive showed low counts when pasteurized in sterile bottles in the laboratory, but sample 6 of the special milk when it reached the pasteurizing tank showed a high count which was practically the same after pasteurization in the laboratory. Furthermore the count of the milk, sample 6, pasteurized in the laboratory checked well with that of milk pasteurized in the plant. The colonies on the plates were of the pin-point type.

At this point it is well to mention and to keep in mind that the special milk was run into a pasteurizing tank which had been in use with the ordinary grade of milk for about six hours. The tank was not steamed previous to filling with the special milk.

A few days later, bacterial counts of the ordinary pasteurized milk from the pasteurizing vats were made on standard agar at 37°C., with the following results:

Bacteria per cubic centimeter

Vat 1	2,500	Vat 10	4,700
Vat 2	60,000	Vat 11	100,000
Vat 3	4,400	Vat 12	110,000
Vat 4	11,000	Vat 13	80,000
Vat 5	70,000	Vat 14	240,000
Vat 6	18,000	Vat 15	120,000
Vat 7	15,000	Vat 16	200,000
Vat 8	140,000	Vat 17	180,000
Vat 9	100,000		

The vat numbers refer to the three tanks as they were sampled in series. Therefore vat 1 would be the first tank filled early in the morning, and tank 17 would be one of the three tanks filled later in the day, towards the end of the pasteurizing run. It will be observed that these counts varied, but were generally higher toward the end of the pasteurizing run, when the tanks had been in use for several hours. Pin-point colonies were noted when the counts were high. Under ordinary conditions at this plant a vat run, as shown above, would give an average under 10,000 bacteria per cubic centimeter when determined by standard methods.

FURTHER STUDIES OF THE PLANT INFECTION

Observations made in our laboratories on the development of bacteria at high temperatures led to the belief that the high counts and the pin-point colonies could be attributed to thermophilic bacteria. We are not aware of anything in the literature which shows that thermophilic organisms grow during the pasteurizing process as normally performed. Jensen (1921), however, has advised that skimmed milk be cooled after pasteurization instead of being returned hot to the producer. He found that the length of time in transportation might be several hours and that the skimmed milk during that period might be above 50°C. (122°F.). Jensen found that under such conditions changes were produced in the skimmed milk through the growth of thermophiles. From plates made of pasteurized milk from the plant, a number of bacteria were isolated from pin-point colonies which we found grew readily at 62.8°C. (145°F.).

A special incubator was arranged to operate at 50°C. (122°F.) so that plates could be incubated at that temperature, and composite samples of the special milk were taken at the plant of

TABLE 1

Comparison of bacterial counts made at different temperatures of incubation

SPECIAL-MILK SHIPPERS	BACTERIA PER CUBIC CENTIMETER AT DIFFERENT INCUBATION TEMPERATURES	
	37°C. (98.6°F.)	50°C. (122°F.)
No. 1	76,000	0 in 0.01 cc.
No. 2	6,400	0 in 0.01 cc.
No. 3	230,000	0 in 0.01 cc.
No. 4	423,000	0 in 0.01 cc.
No. 5	78,000	0 in 0.01 cc.
No. 6	463,000	0 in 0.01 cc.
No. 7	6,900	0 in 0.01 cc.
No. 8	5,400	0 in 0.01 cc.
No. 9	8,300	0 in 0.01 cc.
Mixed milk after pasteurization at the plant	202,000	628,000

each shipper of special milk, together with a sample of the same milk after pasteurization. The samples were plated on standard extract agar and on milk-powder agar, and incubated at 37°C. (98.6°F.) for forty-eight hours and at 50°C. (122°F.) for twenty-four hours. Both media support the growth of the thermophilic organism.

Interesting results were obtained as may be seen from table 1. The samples of raw milk, when the plates were incubated at 37°C. (98.6°F.), showed both low and high counts, while those incubated at 50°C. (122°F.) showed no colonies when 0.01 cc.

of milk was plated. After pasteurization at the plant, however, the milk showed a very high count on plates incubated at 50°C. (122°F.). This was proof that thermophiles were present in the special pasteurized milk but not in the raw milk, at least not in 0.01 cc., the dilution used in plating.

It is of further interest to note that plates from milk shippers 4 and 6 showed high counts and pin-point colonies, although it is clearly evident that in this case the pin-point colonies were due to overcrowding and not to thermophiles, since none showed upon incubation at 50°C. (122°F.). This point has been mentioned earlier in this paper.

TABLE 2

Bacterial counts of plant samples on plates incubated at 50°C. (122°F.)

SPECIAL RAW MILK		SAMPLES TAKEN DURING PROCESS	
Shipper number	Bacteria	Source	Bacteria per cubic centimeter
1	0 in 0.01 cc.	Special milk storage vat	0 in 0.01 cc.
2	0 in 0.01 cc.	Special milk through clarifier	0 in 0.01 cc.
3	0 in 0.01 cc.	Special milk over pre-heater	0 in 0.01 cc.
4	0 in 0.01 cc.	Special milk in pasteurizing tank	36,300
5	0 in 0.01 cc.	Special pasteurized milk over cooler	80,000
6	0 in 0.01 cc.	Bottled special pasteurized milk	52,000
7	0 in 0.01 cc.	Ordinary pasteurized milk (1)	7,100
8	0 in 0.01 cc.	Ordinary pasteurized milk (2)	24,700
9	0 in 0.01 cc.	Ordinary pasteurized milk (3)	25,000
10	0 in 0.01 cc.	Ordinary pasteurized milk (4)	37,000

Further data showed clearly that infection of the special milk with heat-resistant types of bacteria took place in the pasteurizing vats, and our results given in table 1 confirmed this view. It was considered desirable, however, to take another series of samples of the special milk during the pasteurizing process, so as to include each step in the process, and to determine the presence of thermophiles by incubating plates at 50°C. (122°F.).

The results in table 2 confirmed our previous experiments and showed that the thermophiles were not present in 0.01 cubic centimeter samples of raw milk from shippers. Neither did they appear in the storage tanks after the milk went through the clarifier and over the preheater. They first appeared when the milk was in the pasteurizing tank and were present to the extent of 36,300 per cubic centimeter. The sample was taken as soon as the tank was filled but before it was held for the thirty-minute holding period at 62.8°C. (145°F.). The special pasteurized milk on the cooler showed a bacterial count of 80,000 at 50°C. (122°F.). This increase was probably due to the growth of the thermophiles. The 50°C. (122°F.) bacteria count of the bottled special pasteurized milk was somewhat lower due probably to the effect of cold on the organisms. This point will be discussed later.

There can be no doubt that the high counts of the special pasteurized milk were due to infection in the pasteurizing tank by thermophilic organisms. As pointed out previously, the special milk went into a tank in which the ordinary grades of milk had been pasteurized. This tank had been in operation for about six hours and was not steamed previous to receiving the special milk.

As shown in table 2, the 50°C. (122°F.) count of the ordinary pasteurized milk ranged from 7100 to 37,000 bacteria per cubic centimeter.

THE ORGANISM CAUSING PIN-POINT COLONIES

Thirty-nine cultures were isolated from 5 samples of pasteurized milk taken at the plant, including the ordinary and the special grades of milk. Of these, 37 cultures were identical, which indicates that at this plant the trouble was due to a single type of organism. Since the same organism has been isolated from pasteurized milk from another dairy, it appears that the organism may be the common cause of so-called pin-point colonies in cases where the trouble is due to a thermophile.

We have termed this thermophilic organism *Lactobacillus thermophilus*, since it apparently has not been described. It has the following characteristics: Morphology, rod, average size 3μ long and 0.5μ in diameter. Cells vary in length from 1.5 to 4.5μ. Stains are irregular, and often show lightly stained bodies in the cells. Spores are not formed. The organism is Gram-positive. It is a facultative anaerobe, but grows best aerobically. Will grow from 30°C. (86°F.) to 65°C. (150°F.).

The optimum temperature for growth lies between 50°C. (122°F.) and 62.8°C. (145°F.). It grows with great rapidity at the pasteurizing temperature.

In litmus milk a slight acidity is developed when the culture is incubated at 50°C. (122°F.) for twenty-four hours. The maximum acidity reached is not over 0.4 per cent calculated as lactic acid. At 37°C. (98.6°F.) several days incubation is required to show the slight acid reaction. Milk is not peptonized and gelatin is not liquified.

The organism when grown at 50°C. (122°F.) for three days in infusion broth with 0.5 per cent of the test substance and with the medium adjusted to pH 7.5 gives the following pH values, which vary slightly with different cultures.

Test substance fermented		*Test substance not fermented*	
Glucose	5.4	Salicin	7.0
Lactose	5.8	Mannitol	7.0
Sucrose	5.4	Raffinose	6.8†
Starch	5.8	Inulin	7.0
Glycerol	6.6*		

* Seems to be slightly fermented.

† May ferment slightly upon longer incubation.

Two other cultures, among the 39 studied, were thermophiles but distinctly different from *Lactobacillus thermophilus*. One was a spore former which was strictly aerobic and fermented only the test substances glucose, sucrose, and glycerol. In milk after forty-eight hours incubation at 50°C. (122°F.) there was no change, but in four days milk was coagulated with an alkaline reaction at the top of the tube. This spore-forming organism does not grow rapidly in milk at the pasteurizing temperature, perhaps because conditions of oxygen tension are not suitable, as this organism is a strict aerobe. It is fortunate that such is the case, for if this thermophile developed as rapidly as *Lactobacillus thermophilus* it would cause a type of plant infection difficult to destroy because of resistant spores.

The remaining thermophile showed no spore formation and gave no evidence of being a factor in causing plant contamination. Both of these cultures were probably stray organisms and of no importance.

TABLE 3

Growth of Lactobacillus thermophilus at different temperatures, in terms of bacteria per cubic centimeter

INCUBATION TEMPERATURE	BACTERIAL CONTENT								
	At start, in cold milk	After fifteen minutes heating to reach incubation temperature	After incubation period						
			One-quarter hour	One-half hour	One hour	Three hours	Four hours	Six hours	Twenty-four hours
71.1°C. (160.0°F.)	650*		0 in 0.1 cc.	0 in 0.1 cc.	0 in 0.1 cc.	0 in 0.1 cc.	0 in 0.1 cc.	0 in 0.1 cc.	
71.1°C. (160.0°F.)	655,000	4,200	0 in 0.1 cc.	0 in 0.1 cc.	0 in 0.1 cc.	0 in 0.1 cc.	0 in 0.1 cc.	0 in 0.1 cc.	
68.3°C. (155.0°F.)	600,000	330,000		2,330	100	0 in 0.1 cc.	0 in 0.1 cc.	0 in 0.1 cc.	
65.6°C. (150.0°F.)	201,000	210,000		76,000	85,000	530,000		7,100,000	
62.8°C. (145.0°F.)	0 in 0.1 cc.†				0 in 0.1 cc.	900	4,000	84,000§	
62.8°C. (145.0°F.)	350				1,700	22,000	86,000		
62.8°C. (145.0°F.)	473,000				2,700,000‡	23,700,000	35,000,000	53,000,000	
62.8°C. (145.0°F.)	620,000	570,000		480,000	860,000	9,300,000		26,800,000	
50.0°C. (122.0°F.)	148,000	174,000		220,000	257,000	6,700,000	16,400,000	23,000,000	
37.0°C. (98.6°F.)	109,000							219,000	9,400,000
30.0°C. (86.0°F.)	125,000							163,000	535,000

* Milk inoculated when at a temperature of 71.1°C. (160°F.).

† Light inoculation. None present in 0.1 cc.

‡ Bacteria count after two hours.

§ After six and one-half hours, count was 471,000.

GROWTH OF LACTOBACILLUS THERMOPHILUS IN MILK AT DIFFERENT TEMPERATURES

Lactobacillus thermophilus is not a strict thermophile, for it will grow slowly at low temperatures. An idea of the growth range of the organism in milk may be obtained from table 3.

A culture of this organism was inoculated into sterile skimmed milk which was then heated to the desired temperature for incubation. Samples were removed at frequent intervals and plates were made which were incubated at 50°C. (122°F.) for twenty-four hours.

As the results show, when the culture was inoculated into milk at 71.1°C. (160°F.) no growth was evident in a six-hour period of incubation. In one experiment a reduction was shown in bacteria in fifteen minutes heating. At 68.3°C. (155°F.) there was a constant reduction in the bacterial count and no evidence of growth. At 65.6°C. (150°F.) there was a reduction during the first one-half hour, then a rapid increase up to six hours, when the experiment ended.

Lactobacillus thermophilus will grow rapidly, therefore, at a temperature as high as 65.6°C. (150°F.). It grows more rapidly, however, at a temperature of 62.8°C. (145°F.), the common pasteurizing temperature, and equally well at 50°C. (122°F.). A number of experiments were made at 62.8°C. (145°F.) with both light and heavy inoculations. It is quite evident that with a light inoculation the count may rise to quite large numbers after six hours of heating at the pasteurizing temperature. Since in milk plants the pasteurizing runs may require a six-hour period, it is probable that some milk remains in the tanks long enough so that a considerable growth of the thermophile may occur. As the milk flows from a pasteurizing tank, therefore, the varying amount of milk left in it, containing variable numbers of thermophiles, will serve as a variable inoculation for the next lot of raw milk going into the pasteurizing tank.

From the table it may also be seen that the thermophile grows slowly at 37°C. (98.6°F.) and more slowly at 30°C. (86°F.). It grows well enough at 37°C. (98.6°F.) to be visible after forty-eight hours incubation on standard extract agar and appears as pin-point colonies only when large numbers are on the plate. With 100 colonies on the plate the colonies are of medium size. Reference is again made to what was said previously about the influence of overcrowding on the size of the colonies.

THE ABSOLUTE THERMAL DEATH POINT OF LACTOBACILLUS THERMOPHILUS

Since the appearance of pin-point colonies in the milk plant under observation was due to a plant infection with a thermophilic organism, it was necessary to know its absolute thermal death-point.

TABLE 4

Thermal death-point of lactobacillus thermophilus

Heated to 71.1°C. (160°F.) in 2½ minutes	Not held	Not killed
Heated to 71.1°C. (160°F.) in 2½ minutes	Held 5 minutes	Not killed
Heated to 71.1°C. (160°F.) in 2½ minutes	Held 15 minutes	Not killed
Heated to 71.1°C. (160°F.) in 2½ minutes	Held 30 minutes	Killed
Heated to 76.7°C. (170°F.) in 2½ minutes	Not held	Not killed
Heated to 82.2°C. (180°F.) in 2½ minutes	Not held	Killed

It has been shown that the organism will grow at 65.6°C. (150°F.) but not at 71.1°C. (160°F.). It is killed by heating at 71.1°C. (160°F.) for thirty minutes, or at 82.2°C. (180°F.) for two and one-half minutes, as shown in table 4.

The most striking feature of these figures, from a scientific standpoint, is the close relation of the maximum growing temperature to the absolute thermal death-point.

From a practical standpoint the fact that the organism is not a spore-former and can be destroyed by heating to 82.2°C. (180°F.) is of great value.

A milk plant can be freed of infection with this thermophile by heating all equipment, with which milk comes in contact, to 82.2°C. (180°F.).

EFFECT OF COLD ON THERMOPHILE

During the studies of the trouble with pin-point colonies and high counts it was observed that while the count after pasteurizing was often high, it was frequently low after storage at refrigerator temperatures for twenty-four hours. The reduction in count was also demonstrated by laboratory experiments, as is shown in table 5. There was a marked decrease in the number when a pure culture of the thermophile was held at 4°C. (39.2°F.) for twenty-four hours.

It may be expected that milk will have a higher thermophile count directly after pasteurizing than it will have at the time of delivery, provided, of course, it has been refrigerated and not immediately delivered.

TABLE 5

Effect of low storage temperatures on Lactobacillus thermophilus

BACTERIA PER CUBIC CENTIMETER			STORAGE TEMPERATURE
Original count after inoculation	After incubation at 62.8°C. (145°F.) for six hours	After storage for twenty hours	
6,400	4,400,000	9,100,000	20°C. (68.0°F.)
8,000	6,500,000	2,800,000	4°C. (39.2°F.)

SOURCE OF LACTOBACILLUS THERMOPHILUS AND PRESENCE IN MILK FROM OTHER PLANTS

Numerous materials from dairy barns have been examined, and while thermophilic organisms have been isolated we have not encountered *Lactobacillus thermophilus*. This organism has however been isolated from raw milk, and 11 cultures so obtained were identical with those isolated from pasteurized milk. It seems probable that the organism may be generally present in raw milk, but in small numbers, which may increase under suitable conditions. The source of the organism is therefore not clear.

This organism has been isolated from pasteurized milk received from two plants, and it seems apparent that it is frequently present in pasteurized milk.

SIGNIFICANCE OF PIN-POINT COLONIES DUE TO THIS THERMOPHILE

Since the organism is present in raw milk in small numbers, and will develop rapidly at the pasteurizing temperature, it is evident that a cumulative infection of tanks and other dairy machinery may take place unless the cleaning and sterilizing process is performed each day in such a manner as to destroy the organism.

If the pasteurizing tanks are properly cleaned and are in perfect condition, without leaks into the insulating walls, and are thoroughly steamed and all parts heated to at least 82.2°C. (180°F.), there should be little trouble from this thermophile. Steaming of the cooler and bottler should of course be included. This sterilizing process must be in daily operation preferably just after the pasteurizing run.

The presence of this thermophile in pasteurized milk appears to indicate improper sterilizing of milk plant equipment. In the milk plant under discussion it seems possible that the process of sterilization was inefficient because of leaks in the pasteurizing tanks. Small leaks were found in the tanks, which probably permitted milk to flow into the insulating walls and reach spaces where it could not be subjected to a temperature sufficiently high to destroy the thermophile. A leaking vat is a factor to be considered in efficient sterilization.

From a sanitary standpoint, except as an indication of improper sterilization of equipment which may result in high counts, the presence of the organism appears to be unimportant. It has, to our knowledge, been consumed for a considerable period of time with no indication of harmful results. *Lactobacillus thermophilus* appears to be a harmless saprophytic nonpathogenic thermophile, which is probably always present in raw and pasteurized milk in variable numbers.

LACTOBACILLUS THERMOPHILUS AND OTHER BACTERIA WHICH MAY GIVE PIN-POINT COLONIES

Pin-point colonies, as has been shown, probably appear as such because of low dilutions and consequent high numbers of colonies on plates. When plating in a routine manner a pasteurized milk running about 10,000 bacteria per cubic centimeter, a 1:100 dilution will give 100 colonies which will appear normal in size. But if the count should suddenly jump to 100,000 then 1000 colonies would appear on the plates and, as we have previously stated, the colonies would be likely to appear as pin-point types. This is just what will happen if the increased counts are due to *Lactobacillus thermophilus*, but it will also happen if the increased count is due to heat-resistant streptococci or other bacteria. Sudden increases in the number of heat-resistant organisms frequently occur in raw milk, and they too may, in routine work at low dilutions, appear as pin-point colonies.

It is of importance to know whether the appearance of pin-point colonies is due to thermophilic or to heat-resistant streptococci or to other heat-resistant organisms which are not thermophilic, because the methods of eradicating the trouble will be different.

If due to a thermophile, the trouble is likely to be in the plant. If due to heat-resistant bacteria, present in large numbers in the raw milk before pasteurization, it is usually necessary to go back to the producing farm to locate the trouble. It can be quite readily determined whether thermophilic or non-thermophilic heat resistant organisms cause the high counts and pin-point colonies. When high counts and pin-point colonies appear, a set of plates should be made and incubated at 50°C. (122°F.) for twenty-four hours. A count in this manner will at once detect thermophiles. Should no colonies or only a few appear at 50°C. (122°F.) it can be assumed that the high counts and pin-point colonies are due to non-thermophilic heat-resistant bacteria. This can be verified by making standard counts at 37°C. (98.6°F.) of the raw milk and of the same milk pasteurized in the laboratory in sterile containers. The presence of a high proportion of a non-thermophilic heat-resistant types will be shown by a low percentage destruction of the bacteria by pasteurization.

Several years ago a sample of milk was sent to these laboratories, which had turned sour while being held for several hours at the pasteurizing temperature. From this milk Dr. Rogers isolated a streptococcus which resembled *Streptococcus lactis*, but which grew rapidly at 62.8°C. (145°F.). It is apparent therefore that other types of thermophiles besides the one described in this paper may cause trouble in milk plants.

SUMMARY

1. Pin-point colonies probably result from overcrowding on plates through the use of dilutions which are too low. The reaction of the medium may also be a contributing factor.

2. The appearance of these colonies from pasteurized milk from one milk plant proved to be due to a thermophilic organism which we have termed *Lactobacillus thermophilus*.

3. A complete description of this organism is given and it is shown that it grows rapidly at the pasteurizing temperature.

4. The thermophile is easily destroyed by heating to 82.2°C. (180°F.).

5. *Lactobacillus thermophilus* appears to be a harmless saprophytic non-pathogenic thermophile probably always present in raw and pasteurized milk, but in variable numbers.

6. Pin-point colonies may also be due to non-thermophilic heat-resistant bacteria. Methods are described for determining whether this type or the thermophile is the cause of pin-point colonies.

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