The Influence of Growth Temperature and Age on the Thermal Resistance of Milk Cultures of *Streptococcus lactis*[1]

T. J. CLAYDON
Iowa State College

IT IS generally considered that *Streptococcus lactis* is readily destroyed by such heat treatments as are employed in the pasteurization of milk. While some acid-producing streptococci survive this treatment, they differ from *S. lactis* in range of growth temperature and certain other characteristics.

The majority of investigations on the thermal resistance of bacteria in milk have been concerned with pathogens, thermophiles and spore formers. While some work has been carried on with types not falling into these classes, only a limited amount of study has been devoted to the conditions that may affect the thermal resistance of *S. lactis*.

Among the factors influencing the thermal resistance of microorganisms are growth temperature and age of the culture. The variations observed in the resistance of organisms, as a result of differences in environment, suggest the possibility of similar effects with *S. lactis*. Whether or not certain conditions of development will enable this organism to withstand pasteurization temperatures is of practical importance. Any consistent difference in its reaction to heat treatments, due to previous environment, would also be significant from the standpoint of classification of the streptococci.

Mattick and Nichols (2) found that, in rail-borne milk, pasteurization efficiencies were greater in warm weather than in cold weather. They attributed this in part to the age of the cells being heated and to the conditions of growth. They suggested that, in warm weather, with rapid growth of some types of organisms, the proportion of young cells would

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be greater than in cold weather when growth would be slow; hence the effect of heat would be greater. Anderson and Meanwell (1) believed that old cells derived from dirty utensils or those grown below their optimum temperature might be largely thermostable and, therefore, survive pasteurization. It was pointed out by Sherman and Hodge (3) that "Slow growth enable an organism to better adapt itself to its environment and hence to exhibit greater viability when exposed to deleterious environmental conditions."

METHODS

Six strains of *S. lactis* were isolated from raw milk and cream delivered to the Dairy Industry Department at Iowa State College. After isolation, cultures of each organism were grown in litmus milk at 37°, 21°, 15° and 10° C.

In studying the influence of the growth temperature and the age of *S. lactis* cultures on the thermal resistance, heat trials were conducted periodically with each of the six organisms which had been carried at 37°, 21°, 15° and 10° C. The trials were commenced after the initial development had begun and continued through the cycle of growth until the numbers of organisms had markedly decreased. With the cultures grown at 37° and 21° C. tests were made daily for about seven days. With the cultures carried at 15° and 10° the intervals between the trials were about two and four days, respectively, since the development of the cultures was slower; the tests continued for about 15 days with cultures grown at 15° C. and for about 30 days with cultures grown at 10° C.

TECHNIC OF HEATING

Thermal resistance trials were conducted at 61.7° C. This temperature was chosen because it is the lowest used in the pasteurization of market milk. Cultures to be heated were diluted 1-1,000 in sterile litmus milk to avoid the effect, on the thermal resistance, of the acid present in the cultures. Throughout the trials it would have been desirable to have equal numbers of organisms in the portions heated to permit more accurate comparisons of heat resistance. However, as the cultures themselves differed in numbers of organisms, it was impossible to obtain equal numbers in the dilutions. Deviations from the 1-1,000 dilution might have resulted in variations in pH values.

The diluted cultures were prepared for heating by placing 2 cc. portions in each of a number of 4 cc. agglutination tubes. These tubes were sealed in a blast lamp and cooled in air. They were then suspended from a metal rod by means of strings and wire hooks so that they hung about 6 inches from the rod. With the exception of the tubes containing cultures grown at 37° C., which were the last to be sealed in comparative tests, all tubes were placed in ice water after sealing to retard bacterial growth.

The length of time between sealing and the beginning of the heating was kept as short as possible and rarely exceeded 25 minutes.

The heating was accomplished by placing the tubes in a galvanized iron tank (20″ x 10″ x 12″) which was filled with water to within 2 inches of the top and heated by two adjustable burners. When the water reached the temperature of 61.7° C., the burners were so adjusted that the variation from this point was not greater than 0.2° C. A thermometer graduated in tenths of a degree Centigrade was suspended in the tank. By the use of a paddle for agitating the water several times a minute, it was possible to keep the temperature of the whole body of water reasonably constant.

In conducting the heat trials the sets of tubes suspended from the metal rods were removed from the ice water and plunged into the bath. The rods rested on the sides of the tank. In the early part of the work, tubes were withdrawn from the bath at intervals of 1, 2, 3, 4, 5, 6, 7, 9 and 11 minutes, respectively; while in later trials the periods of heating were varied as seemed desirable. The periods were timed with a stop watch. One tube of each set was unheated and used as a check. When the tubes were withdrawn from the bath, they were immediately dropped into ice water. After cooling, the cultures which had been previously grown at 37° or 21° C. were again incubated at those temperatures; while the cultures that had been grown at 15° or 10° C. were incubated at 21° or 37° C. in order to speed up development.

OBSERVATIONS

Observations on survival were made frequently for about 9 days with cultures grown at 37° and 21° C. and for about 12 days with cultures grown at 15° and 10° C. Cultures were considered sterile when they failed to change the appearance of litmus milk in the heated agglutination tubes during those periods.

PLATE COUNTS

In most cases plate counts were made on the diluted cultures at the time of heating. Plates were poured immediately after the 1-1,000 dilutions had been prepared. Tomato juice agar of a pH of approximately 7.0 was used throughout. The plates were incubated at room temperature for four days and counted by means of a 6X binocular.

RESULTS

ORGANISM 1

The thermal resistances obtained with organism 1 are graphed in the figure. Probably the most significant feature shown is that the heat resistance of the organism was inversely proportional to the growth temperature. The longest survival time at each growth temperature was 4 minutes at 37° C., 5 minutes at 21° C., 8 minutes at 15° C. and 13 minutes

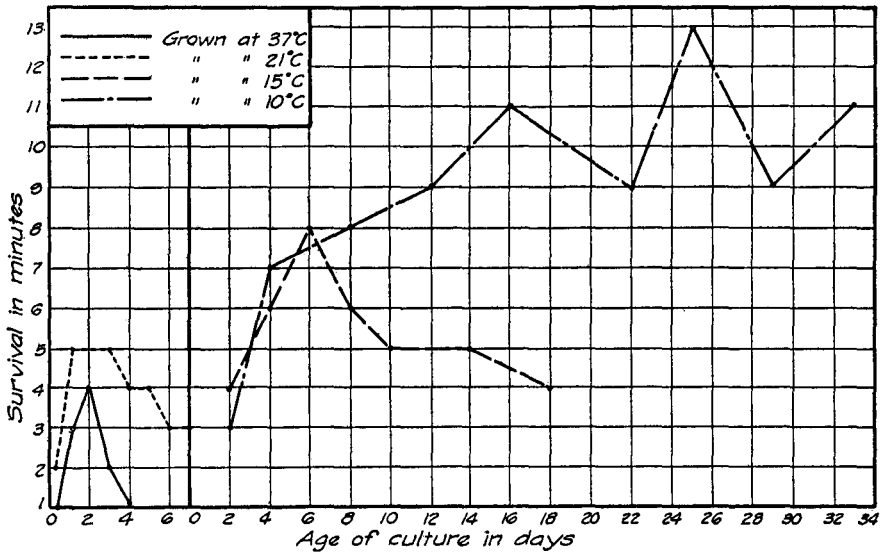

Fig. 1. Survival in minutes of organism No. 1 heated at 61.7° C.

at 10° C. At each temperature the heat resistance of the organism increased with the age of the culture to a maximum and then decreased. With the culture grown at 37° C., the survival time, while only 1 minute at ¼ day of age, reached its maximum of 4 minutes at 2 days and declined again to 1 minute at 4 days. The survival time of the culture grown at 21° C. increased from 2 minutes at ¼ day of age to its maximum of 5 minutes at 1¼ days; it remained at this point until 3 days and then declined to 3 minutes at 6 days. The survival time of the culture grown at 15° C. increased from 4 minutes at 2 days of age to 8 minutes at 6 days and then declined again to 4 minutes at 18 days. With the culture grown at 10° C. the survival time increased rapidly from 3 minutes at 2 days of age to 7 minutes at 4 days and then less rapidly to the maximum of 13 minutes at 25 days. While there was a decline in resistance to 9 minutes at 29 days, there was again an increase to 11 minutes at 33 days. The experiment was not carried on long enough to indicate the rate of decrease in resistance with this growth temperature. In general, the data show that the rate of increase in resistance, the maximum resistance attained and the rate of decrease were associated with the growth temperature of the organism.

Table 1 gives the counts on the diluted cultures at the time of heating and shows that, with the cultures grown at the three highest temperatures, there seemed to be a general relationship between the numbers of organisms and the resistance to heat; when the count was high the resistance was high. However, this did not apply to the culture grown at 10° C.

TABLE 1. *Numbers of bacteria in diluted cultures (1-1,000) at time of heating*

Age of culture in days	Organism No. 1			
	Bacteria per cc. in dilution of cultures grown at			
	37° C.	21° C.	15° C.	10° C.
¼	<1,000	4,000		
1¼	670,000	2,060,000		
2	530,000	2,860,000	1,080,000	<10,000
3	<10,000	1,970,000		
4	<10,000	1,550,000	1,880,000	150,000
5		1,450,000		
6		350,000	1,710,000	
7		32,000		
8			1,410,000	480,000
10			500,000	
12			50,000	620,000
14			35,000	
16				140,000
18			30,000	
22				130,000
25				59,000
29				30,000
33				16,000

At the time of its maximum resistance of 13 minutes the count was the lowest obtained at any time between the fourth and twenty-fifth day. Moreover, this count was much lower than the counts on the other three cultures at their periods of maximum resistance. The table shows that all counts made on the cultures grown at 37° and 10° C. were relatively low, while the counts on the cultures at 21° and 15° C. were relatively high, with those at 21° C. being the higher.

ORGANISM 2

While the heat resistance of this organism was not inversely proportional to the growth temperature, the culture grown at 10° C. was again the most resistant. The longest survival time of the organism at each growth temperature was 9 minutes at 37° C., 7 minutes at 21° C., 7 minutes at 15° C. and 13 minutes at 10° C. As was the case with organism 1, the heat resistance at each growth temperature increased with age to a maximum and then decreased. The same general relationship existed between the number of organisms and the heat resistance as was the case with organism 1.

ORGANISM 3

On the whole, this organism was generally less heat resistant than organisms 1 and 2. With the exception of the culture grown at 21° C., there existed the same relationship between heat resistance and growth

temperature as was the case with organism 1. The longest survival times obtained were: 2 minutes when grown at 37° C., 7 minutes when grown at 21° C., 6 minutes when grown at 15° C. and 9 minutes when grown at 10° C. There was not quite the same relationship between the number of bacteria and the heat resistance with organism 3 as in the case of organisms 1 and 2. With the three highest growth temperatures, the periods of high resistance did not coincide as closely with the high counts, as was the case with the other two organisms. The culture grown at 10° C. attained its maximum resistance at the time of the highest count; in this respect, it again differed from the other two organisms.

ORGANISM 4

This organism did not show the same general relationship between heat resistance and growth temperatures as organisms 1, 2 and 3. The maximum resistances obtained were: 7 minutes when grown at 37° C., 7 minutes when grown at 21° C., 6 minutes when grown at 15° C. and 7 minutes when grown at 10° C. With the cultures grown at 21° and 15° C., the resistances were high when the counts were high. In the case of the culture grown at 37° C. the numbers of bacteria had passed the maximum at the time the greatest resistance was obtained. While the culture grown at 10° C. reached a resistance of 7 minutes at the time the count was high and then became less resistant, it later again survived 7 minutes heating when the count had declined considerably. In this respect it acted similarly to organisms 1, 2 and 3.

ORGANISM 5

As was the case with organism 1, the maximum thermal resistances obtained were inversely proportional to the growth temperatures, being 4 minutes at 37° C., 6 minutes at 21° C., 7 minutes at 15° C. and 9 minutes at 10° C. On the whole, the same relationship between numbers of bacteria and heat resistance existed, as with organism 1. With the cultures grown at the three highest temperatures the resistances were high when the counts were high, but with the culture grown at 10° C. the two highest resistances were obtained near the end of the trials when the counts were very low.

ORGANISM 6

Irregularities in heat resistance were obtained with the cultures grown at 37° and 21° C. and were especially marked with the latter culture. However, the cultures grown at 15° and 10° C. gave results more in accordance with the previous trials, the latter culture finally showing the greatest resistance, as was the case with the other organisms. The highest survival times obtained were 4 minutes when grown at 37° C., 7 minutes when grown at 21° C., 7 minutes when grown at 15° C. and 9 minutes when grown at 10° C. With the exception of the culture grown

at 21° C. there was generally the same relationship between the numbers of bacteria and the heat resistance as with the other organisms. With the culture grown at 21° C., the irregularities in heat resistance did not always correspond to the irregularities in counts. The culture grown at 10° C. again showed the greatest resistance when the numbers of bacteria had greatly decreased.

DISCUSSION

The thermal resistance of *S. lactis* in milk is not high enough to enable the organism to survive pasteurization treatments; and, while such factors as growth temperature and age of the culture modify this resistance to a certain extent, it appears that they do not sufficiently increase the resistance to be of commercial importance.

The relatively high resistance obtained with cultures grown at 10° C. might have been attributable to slower development of the cultures and a correspondingly greater age of the cells. In addition, sufficient acid was not produced in the cultures to effect coagulation; and, while the dilutions of the cultures used in the heat trials were such that the amount of acid present in the heated portions was negligible, there may have been a less detrimental influence prior to heating. The greater resistance secured with cultures grown at 10° C. would seem to confirm the statement of Sherman and Hodge (3) that slowly growing cultures exhibit greater viability under unfavorable conditions than more rapidly growing cultures. It is also in accord with the suggestion of Anderson and Meanwell (1) that organisms, usually destroyed by pasteurization, might be largely thermostable and survive this treatment when grown below their optimum temperature.

SUMMARY AND CONCLUSIONS

1. At 10° C., *S. lactis* cultures grew more slowly than at higher temperatures but attained higher thermal resistances. The highest resistance obtained with an organism grown at 10° C. was 13 minutes.

2. The thermal resistances of *S. lactis* cultures increased with age to a maximum and then decreased. The rates of increase and decrease were influenced by the growth temperature, being slower at lower growth temperatures.

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