Reprinted from JOURNAL OF BACTERIOLOGY
Vol. 78, No. 6, pp. 834-838, December, 1959
Printed in U.S.A.

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O. W. KAUFMANN, L. G. HARMON, O. C. PAILTHORP, AND I. J. PFLUG

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O. W. KAUFMANN, L. G. HARMON, O. C. PAILTHORP,[3] AND I. J. PFLUG

Departments of Dairy, Microbiology, and Public Health, and Agricultural Engineering, Michigan State University, East Lansing, Michigan

Received for publication May 28, 1959

In studies on the pasteurization of milk at ultra high temperatures it was observed that the surviving bacteria (less than 100 per ml) exhibited a prolonged lag phase during subsequent incubation of the heat treated milk sample (Kaufmann, 1957). Penfold (1914) concluded that, among other things, the size of the inoculum affects the lag phase of growth as determined by the generation time over the first 2 hr of the growth period. He reported little, if any prolongation of the lag phase when the initial cell level ranged from about 100 to 4000 cells per ml. A lengthening of lag phase was observed, however, when the inoculum was about 2 cells per ml. The prolonged lag phase observed following exposure to ultra high temperatures, therefore, may be attributed in part, to the low level of survivors.

Heat has also been observed by Tobias *et al.* (1955) to affect the rapidity of bacterial recovery; these workers reported slow bacterial development with a heat-resistant *Micrococcus* MS 102 even when the initial level of survivors was greater than 100 per ml. On the basis of this work, the high heat treatment rather than the low survivor level may have induced the prolonged lag. The present study was undertaken to determine the effect of heat on the lag phase observed on subculture of the surviving cells under conditions providing constant initial levels of inocula.

EXPERIMENTAL METHODS

Preparation of test suspensions. Heat-resistant *Micrococcus* strain MS 102, used in this work, was cultured as described by Tobias *et al.* (1955) to assure culture stability with regard to heat resistance. Stability in this respect was particularly desirable to permit the calculation of the initial cell concentration required before heating to obtain the desired cell level after heating. After primary incubation at 32 C for 24 hr and refrigeration at 6 C for 48 hr, a 150-ml prescription bottle containing 30 ml of agar was inoculated and incubated for 24 to 26 hr at 32 C to produce the cells used in the unheated control and heat treated sample. After harvesting the growth in cold sterile water, it was filtered to remove clumps of agar and blended for 30 sec in a Waring Blendor to provide a homogeneous stock suspension containing approximately two billion cells per ml.

This suspension was diluted with sterile milk to obtain 20-ml volumes of test sample having initial cell levels of about 200,000 and 20,000 per ml. Using this procedure, it was possible to secure a cell concentration of about 20,000 and 2000 per ml after heating. Dilutions for the unheated control sample were always designed to provide population levels comparable to the above. Homogenized whole milk, autoclaved at 121 C for 10 min, was used throughout as a diluent since it was desired to conduct the actual heat shock and subsequent recovery of the survivors in a milk medium.

Heat shocking. Duplicate tubes containing 18 ml of sterile milk were immersed in constant temperature water baths held at 87 C and 80 C. When the contents of the tubes reached the desired temperature, 2 ml of inoculum at 43 C were forcibly injected into the sterile milk using a 5-ml hypodermic syringe and an 18-gauge needle. The turbulence created by the force of the injection aided in dispersing the inoculum evenly. Holding time commenced from the instant the inoculum was added; after a 5-sec exposure period in a water bath at 87 C or a 17-sec holding period at 80 C, the test tubes were removed from the bath, promptly plunged into a calcium chloride solution at −23 C as recom-

[1] Published with the approval of the Michigan Agricultural Experiment Station as journal article no. 2430.

[2] Based in part on data from a thesis submitted by O. C. Pailthorp in 1958 in partial fulfillment for the M.S. degree, Michigan State University.

[3] Present address: Foremost Dairies Inc., Los Angeles, California.

mended by Kaufmann *et al.* (1955), and agitated for 15 sec. The holding times are estimated to be accurate to ±1 sec. As a consequence of the cooling which occurred when the inocula were added and in view of the short holding times, the effective exposure temperatures were determined experimentally using a 5-sec interval recording potentiometer. The effective exposure temperatures were calculated to be 82 ± 1 C and 76 ± 1 C; all temperatures hereafter will refer to the effective exposure temperature not to the temperature of the water bath.

Post heat shock recovery. After the heated milks were cooled to room temperature, a 1-ml sample was removed from each tube and the number of viable cells immediately following heat treatment was determined using standard plate counting procedures. All dilutions were plated in duplicate. The remainder of the 20-ml sample in each tube was incubated at 32 C; counts were made periodically, to establish the growth curve of the culture.

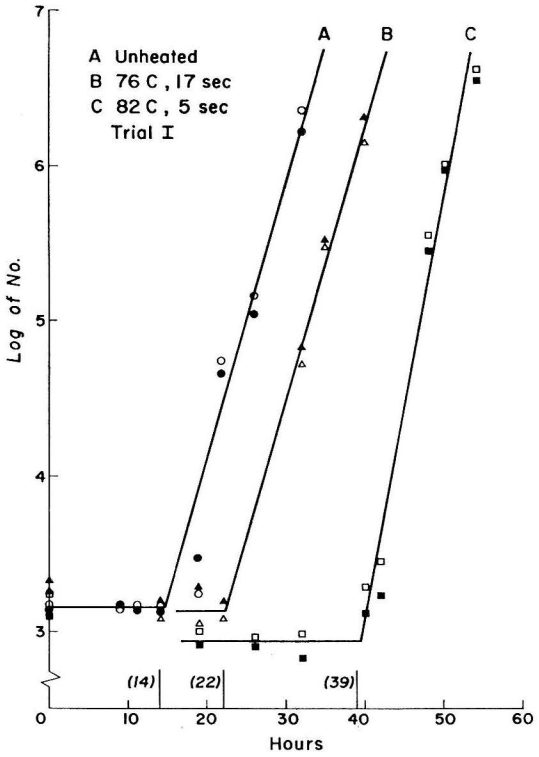

Figure 1. Growth curves of cells surviving 76 C for 17 sec and 82 C for 5 sec showing the effect of heat treatment on the lag phase of development. Initial number of survivors: 2000 per ml.

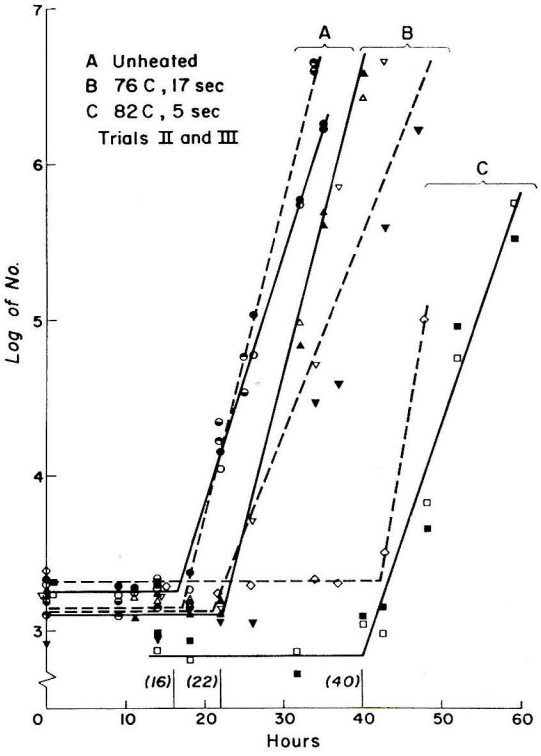

Figure 2. Growth curves of cells surviving 76 C for 17 sec and 82 C for 5 sec showing the effect of heat treatment on the lag phase of development. Initial number of survivors: 2000 per ml.

Unheated control samples having the same initial population level as the heat treated samples were run simultaneously.

An attempt was made to obtain three to four samples for counting while the culture was definitely in the lag phase of the growth cycle and a similar number while the organism was in the exponential portion of the cycle. Using the latter points the best straight line fit for the exponential phase was determined by statistical methods. Plotting the slope of the curve in this manner minimized any bias which may have arisen in visual plottlng. Since the points obtained during the lage phase obviously indicated a straight line in most cases, this portion of the growth curve was fitted by visual methods. The length of the lag period was determined from the intercept obtained by extending the lag and exponential portions of the growth curve. Figures 1 to 4 show the results obtained with an initial population of approximately 2000 and 20,000 cells per ml.

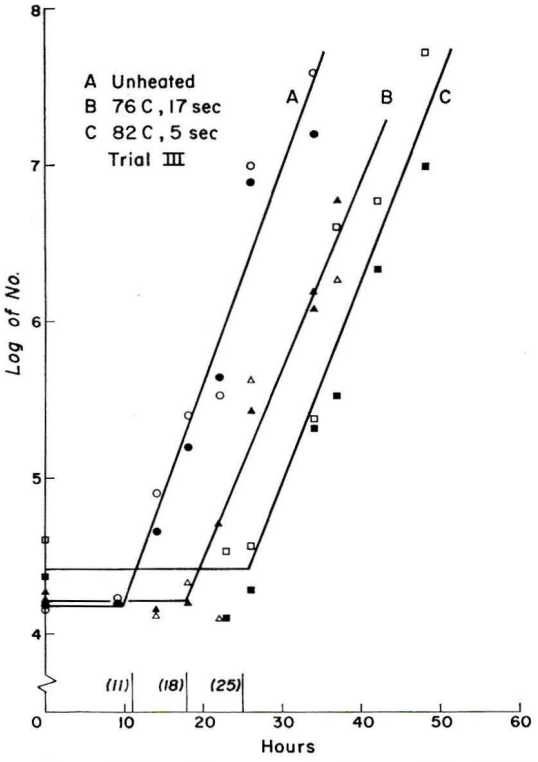

Figure 3. Growth curves of cells surviving 76 C for 17 sec and 82 C for 5 sec showing the effect of heat treatment on the lag phase of development. Initial number of survivors: 20,000 per ml.

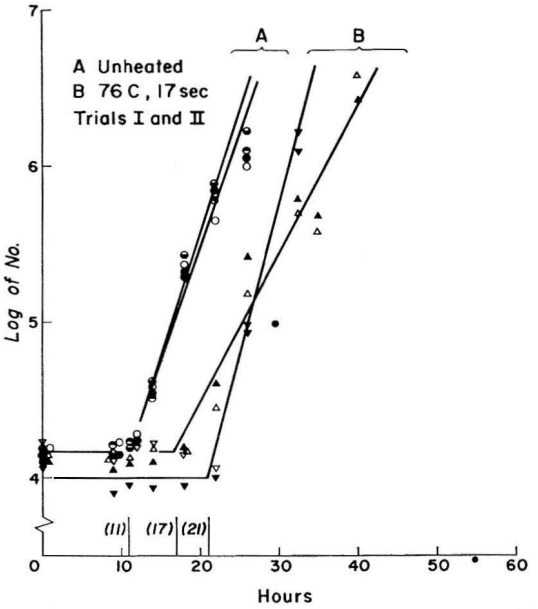

Figure 4. Growth curves of cells surviving 76 C for 17 sec showing the effect of heat treatment on the lag phase of development. Initial number of survivors: 20,000 per ml.

TABLE 1

Plate counts of surviving populations showing the effect of heat on the growth cycle

Trial I

Incubation Time (hr)	Initial Level: 2000/ml			Initial Level: 20,000/ml		
	Unheated control (×1000)	76 C 17 sec (×1000)	82 C 5 sec (×1000)	Unheated control (×1000)	76 C 17 sec (× 1000)	82 C¶ 5 sec (× 1000)
0*	1.4†	2.3	1.3	15.0	12.0	23.0
	1.6	1.8	1.9	13.0	17.0	40.0
9	1.5	—	—	14.0	8.0	—
	1.4	—	—	16.0	13.0	—
11	1.4	—	—	15.0	9.0	—
	1.5	—	—	17.0	15.0	—
14	1.5	1.6	—	42.0	8.6	—
	1.6	1.2	—	33.0	17.0	—
18	3.0	1.8	0.70	270.0	8.7	—
	1.8	1.1	0.94	190.0	14.0	—
22	46.0	1.6	—	760.0	10.0	13.0
	54.0	1.2	—	590.0	12.0	35.0
26	110.0	—	0.63	1700.0	83.0	19.0
	140.0	—	0.82	1300.0	98.0	37.0
32	1700.0	73.0	0.50	—	1300.0	—
	2300.0	52.0	0.92	—	1700.0	—
35	—	340.0	—	—	5200.0	210.0‡
	—	290.0	—	—	6200.0	240.0
40	—	2100.0	1.3	—	—	340.0‡
	—	1400.0	2.1	—	—	4600.0
42	—	—	1.7	—	—	2200.0‡
	—	—	2.6	—	—	6100.0
48	—	—	280.0	—	—	11000.0
	—	—	390.0	—	—	55000.0
50	—	—	860.0	—	—	—
	—	—	1000.0	—	—	—
54	—	—	3500.0	—	—	—
	—	—	4300.0	—	—	—

* Initial count made immediately after heat treatment.

† The paired numbers represent duplicate tests; duplicate plates were also made in each test.

‡ Incubation times here were 34, 37, and 45 hr rather than 35, 40, and 42 hr, respectively.

Typical data from which these curves were obtained are given in table 1.

RESULTS AND DISCUSSION

The growth curves shown in figure 1 represent the bacterial development following incubation of duplicate milk samples which contained initial inocula of about 2000 bacteria per ml. The results obtained on heating at 76 C for 17 sec and 82 C for 5 sec are shown in curves B and C. The unheated control results are represented by curve A. The data presented in figure 1 and summarized in table 2 indicate a lag period of 22 hr after treatment at 76 C and 39 hr after heating at 82 C. This represents an extension of the lag phase by 17 hr under the latter conditions. Trials II and III, shown in figure 2 and summarized in table 2, are presented to substantiate the above. The lag phases ranged from 21 hr after heating at 76 C to 42 hr after heating at 82 C for the prescribed holding periods in trial II and from 23 to 40 hr in trial III. The extension of the lag period was 21 and 17 hr, respectively. The average extension of the lag phase (76 C versus 82 C) for all three trials was 18 hr when the initial inoculum was 2000 per ml. It should also be pointed out that the lag period of the heat treated cells was always longer than that observed with the unheated controls; the average lag extension over the control was 6 hr and 14 hr at 76 ± 1 C and 82 ± 1 C when the initial inoculum was 2000 per ml.

It is apparent that the heat treatment a culture receives influences the rate at which the cells recover during subsequent incubation. The minimum generation times for the control, the 76 C and the 82 C treatments were 37 ± (σ = 16) min, 42 ± (σ = 20) min, and 30 ± (σ = 13) min. In view of the magnitude of the individual variation, it is concluded that the minimum generation time is not affected appreciably by the heat treatment. The difference in the rate of recovery is reflected almost entirely in the lag phase of growth as indicated in figures 1 and 3. The prolonged lag phase, if it is exhibited by all organisms, is of interest as the extension of the lag phase is generally reflected directly in a prolonged shelf life for certain food products.

In the studies carried out with an initial population of approximately 20,000 per ml, a rather marked decrease in the duration of the lag phase was noted when compared to that observed with the lower initial inoculum. At 20,000 cells per ml (figures 2 and 4) the average lag period was 19 hr after treatment at 76 C as compared to 25 hr after exposure at 82 C; this represents an extension of about 6 hr as compared to 18 hr when the inoculum was 2000 per ml.

These data show that under the condition of this experiment, increasing the size of the initial inoculum by 10 times reduced the "lag extension effect" usually observed following heating at 82 ± 1 C for 5 ± 1 sec compared to 76 ± 1 C for 17 sec. This difference might be attributed to the size of the inoculum as described by Penfold (1914) or to some selective process of the different heat treatments which altered the surviving population. If, for example, the inoculum contained cells of variable thermoduric characteristics and the more heat resistant cells, for genetic or chemical reasons, required the longest time to recover, they would also exhibit the longest lag period. Under the conditions of high heat treatment this selective process might be carried out more efficiently and only the more heat resistance cells would be present. If the above is true, the lag extension observed following exposure to the higher temperature could be attributed to strain or variety differences.

The size of the inoculum may or may not exert some influence on the lag phase as has been suggested by Penfold (1914). Based on the results given in table 2 the lag period averaged 16 hr on the control at 2000 per ml as compared to 11 hr at 20,000 per ml. This represents a shortening of the lag phase by only 5 hr when the inoculum is increased 10-fold. After heat treatment at 76 C the lag phases only differed by 3 hr in changing

TABLE 2

Duration of the lag period following various heat treatments at two different population levels

Trial	Duration of Lag Phase in Hr					
	Initial level of survivors:					
	2000/ml			20,000/ml		
	Unheated	76 ± 1 C, 17 sec	82 ± 1 C, 5 ± 1 sec	Unheated	76 ± 1 C, 17 sec	82 ± 1 C, 5 ± 1 sec
I	14	22	39	11	21	—
II	16	21	42	11	17	—
III	17	23	40	11	18	25
Avg...	16	22	40	11	19	25

the inoculum level from 2000 to 20,000 per ml. In view of the inherent experimental error these differences are not considered to represent real differences and one thus may conclude that the lag period is not markedly reduced with unheated or mildly heated cells when the cell level is increased from 2000 to 20,000 per ml.

The data in table 2 indicate that the lag period after heating at 82 C averaged 40 hr when the surviving population was 2000 per ml as compared to 25 hr when the population was 20,000 per ml. This represents a difference of 15 hr. Uncontrollable laboratory accidents complicated trials I and II at 20,000 per ml and 82 C, leaving only a single determination based on duplicate tubes. Unpublished trials at 81 ± 1 C for 5 ± 1 sec carried out during the course of this study, however, indicate a lag period of about 22 hr with an initial population of about 20,000 per ml. This evidence lends support to the single observation of 25 hr noted above. One can conclude, in this instance, that increasing the inoculum 10-fold, reduced the length of the lag phase.

ACKNOWLEDGMENT

The authors wish to thank Dr. W. Baten for his counsel in regard to the statistical phase of this work.

SUMMARY

Heated cells of a heat-resistant *Micrococcus* strain MS 102 exhibit a longer lag phase of development than do unheated cells when the initial cell levels are equal.

At an initial cell population of 2000 per ml, the lag phase of development is increased from 22 to 40 hr when the heating treatment is changed from 76 ± 1 C for 17 sec to 82 ± 1 C for 5 ± 1 sec.

At an initial cell population of 20,000 per ml, the lag period increased only from 19 to 25 hr under the above temperature changes.

REFERENCES

KAUFMANN, O. W. 1957 Ultra high temperature pasteurization. Milk Industry Foundation Conv. Proc., 58–61.

KAUFMANN, O. W., TOBIAS, J., AND WAINESS, H. 1955 A device for collecting and rapidly cooling samples from high-temperature short-time heating units. J. Dairy Sci., **38,** 645–650.

PENFOLD, W. J. 1914 On the nature of bacterial lag. J. Hyg., **14,** 215.

TOBIAS, J., KAUFMANN, O. W., AND TRACY, P. H. 1955 Pasteurization equivalents of high-temperature short-time heating with ice cream mix. J. Dairy Sci., **38,** 959–968.