TEMPERATURE AND LAMBDA PHAGE REPRODUCTION

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ABSTRACT

GROMAN, NEAL B. (University of Washington, Seattle) AND GRACE SUZUKI. Temperature and lambda phage reproduction. J. Bacteriol. **84**:431-437. 1962. — The effect of temperature on lambda phage production in *Escherichia coli* K-12 was studied, and a temperature-phage yield relationship established. Phage yield declines slowly below optimal temperature and rapidly above the optimum. An extensive comparison of phage reproduction at 37 and 44 C was made when it was observed that at 44 C phage yield was reduced to 2 to 5% that at 37 C in the absence of any significant effect on bacterial growth. The reduced yield is manifest rather uniformly in every infected cell, and the reduction is due primarily to a speed-up of the lytic process. Temperature shift-up and shift-down experiments established that the temperature-affected step or process is initiated about 25 min after adsorption. This is close to the time the first intracellular phage appears. The data indicate that heat inactivation, lysogenization or major blocks in adsorption, penetration, replication, and maturation make minor contributions, if any, to the depression of the yield. Phenotypic adaptation of phage and host to the production of a greater yield at 44 C was not observed. There was no correlation observed between the enhanced thermoresistance of free λ*vir*, tr_1 phage and ability to reproduce at 44 C.

Lwoff's (1959) work on temperature and animal-virus production provided the stimulus for the present study with bacteriophage. The influence of temperature on phage reproduction has been studied by a number of workers. D'Herelle (1921) observed that lysis by phage was delayed if the incubation temperature was below 37 C. Doerr and Grüninger (1922) reported that a coliphage failed to reproduce at 43 C, although the uninfected bacterial strain grew well at this temperature; there were other reports (Krueger and Pucheu, 1941; Luria, 1943; Linz, 1948) that staphylophage and coliphage production was inhibited in the range of 42 to 45 C. Krueger and Fong (1949) and Buzzell, Trkula, and Lauffer (1954) observed that phage-bacterium complexes were inactivated at approximately 47 C, and in both cases the complex proved to be more sensitive to heat than the uninfected host or the free phage. The most detailed analysis of the effect of temperature on the kinetics of phage production was made by Maaløe (1950) and Weiss-Bentzon, Maaløe, and Rasch (1952), who studied changes in the latent period and in the rate of T4 phage production when infected cells were shifted from 36 to 19 C at various times. Discussion of their findings will be deferred until later. In the present work, the effect of temperature on the production of temperate, coliphage lambda has been studied, with emphasis on temperatures above 37 C. An effort has been made to relate the observed inhibition of phage production to the effect of temperature on known steps in the phage cycle.

MATERIALS AND METHODS

Bacteria and phages. Escherichia coli K-12 and K-12 (λ) were obtained from Allan Campbell, and a streptomycin-resistant mutant of K-12 was obtained from Francois Jacob. The latter strain was used as indicator for plaque counts. When required, streptomycin was incorporated into the overlay medium at 200 μg/ml of overlay.

Temperate phage λ was obtained from Allan Campbell. It has a half-life of approximately 45 min at 44 C when suspended in broth. A virulent thermoresistant mutant, λ*vir*,tr_1, was isolated by selection at 55 to 60 C. It reproduces on K-12 (λ) and shows no decline in titer after 2 hr at 50 C.

Media and methods. All experiments were carried out in Trypticase Soy Medium (BBL) supplemented with 2.5 g/liter of dipotassium phosphate and glucose and 10 g/liter of yeast extract (TSY). This rich medium was employed

to minimize the effects of any nutritional imbalances which might occur at elevated temperatures. Dilutions were also made in this medium.

The standard methods and media employed by Kaiser (1955) for phage assays were used. In one-step growth-cycle experiments, adsorption was carried out at 37 C for 10 min in 0.01 M $MgSO_4$, after which samples were diluted into growth medium held at the appropriate temperature. The initial phage-bacterium ratio was 1:20. The per cent adsorption was determined by assaying in the presence and absence of streptomycin. During the reproductive cycle, phage assays were also made, after lysis by the chloroform technique of Sechaud and Kellenberger (1956). Samples were pipetted into 5 ml of medium containing three or four drops of chloroform, were shaken vigorously, and allowed to stand for 20 min at room temperature prior to assay.

Ultraviolet-light induction of lysogenic cultures was carried out as follows. Cells were grown in TSY medium to a concentration of 4-5 $\times$ 10^8/ml; then they were centrifuged and resuspended in 0.01 M $MgSO_4$ to a concentration of 2 $\times$ 10^8/ml. This cell suspension (5 ml) was pipetted into a flat-bottomed petri dish (9-cm diam) and irradiated for 40 sec at a distance of 57 cm with a Hanovia 83A1, 25-w lamp. The dishes were agitated on a rotary shaker throughout the irradiation period.

Bacterial growth studies and phage one-step growth cycles were carried out in water baths controlled to $\pm$0.1 C. All cultures were aerated on a reciprocal shaker at 100 strokes per min (1½-in. strokes). Bacterial growth was measured by changes in optical density. A Klett-Summerson colorimeter with a no. 54 filter was employed.

RESULTS

Growth of K-12 at various temperatures. The growth rate of K-12 in TSY medium was determined at 37, 44, 46, and 48 C. A typical set of data is given in Fig. 1. Growth at 44 C appeared to be slightly more rapid than at 37 C. The break in the curve at 46 C was consistently seen, as was the slow increase in optical density at 48 C. Bacterial counts showed that, after an early lag, the rate of cell division at 44 C was slightly higher than at 37 C, at 46 C the rate was materially reduced, and at 48 C cell counts remained almost unchanged. Cells grown for three to four

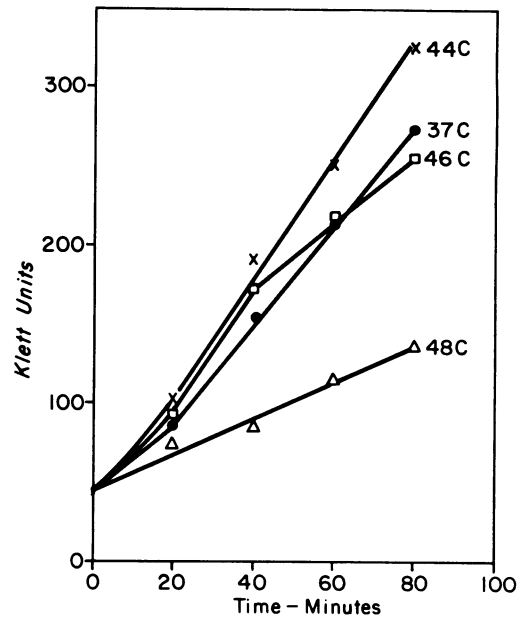

FIG. 1. *Growth of Escherichia coli* K-12 *at various temperatures.*

generations at 44 C were significantly smaller than those grown at 37 C, whereas at 48 C cells were longer and some "snake" forms appeared. From these limited observations it is apparent that 44 C is the maximal temperature at which growth is similar to that at 37 C.

Phage yield at various temperatures. Phage production at various temperatures was determined for both λ and λ*vir*,tr_1. Log-phase cells were infected as described in Materials and Methods, and samples were distributed to media at temperatures ranging from 25 to 46 C. Assay times for phage were varied for each temperature according to experience gained in preliminary experiments, and were arranged to minimize inactivation at elevated temperatures. Assays were carried out until maximal yields were obtained. Completion of lysis was verified by chloroform treatment of terminal samples. Infection of K-12 rather than induction of K-12 (λ) was utilized to determine λ production, since an accurate comparison of infective centers was difficult with induced cells incubated at different temperatures. Phage λ*vir*,tr_1 acted as a partial control for the possible effect of lysogenization and heat inactivation on the yield of λ phage.

The temperature profile for λ is given in Fig.

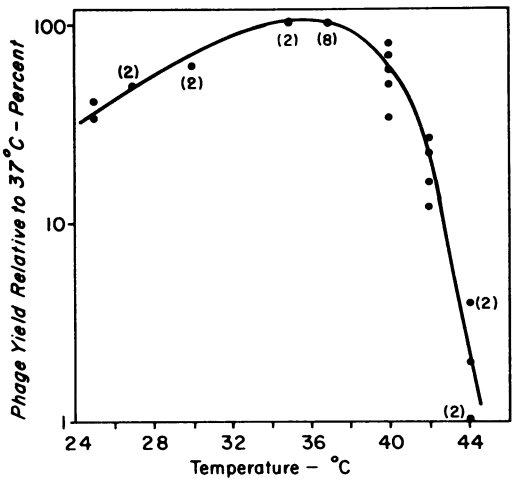

FIG. 2. *Effect of temperature on λ phage production. Each point represents data from a single experiment, unless the number in parentheses indicates differently.*

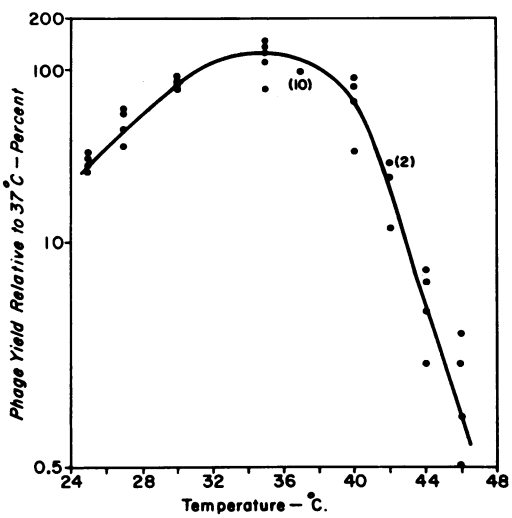

FIG. 3. *Effect of temperature on λvir,tr₁ production. Each point represents data from a single experiment, unless the number in parentheses indicates differently.*

2 and that for λ*vir*,*tr*₁ in Fig. 3. It is evident in both cases that a slow decline in phage yield occurred at temperatures below optimum, and a rapid decline occurred at temperatures above the optimum. Stable final yields were reached more rapidly at higher than at lower temperatures. In contrast to a maximal completion time of 60 to 70 min at temperatures above 37 C, sampling continued up to 130 min at 30 C and for 240 min at 25 and 27 C. Aside from a slight shift in temperature optimum, there was very little difference between virulent or temperate phage over this range of temperatures. This finding indicates that neither heat inactivation nor lysogenization, under conditions of single infection, are significant factors in λ-phage productivity.

As a result of the data obtained on bacterial growth and phage yield at various temperatures, a comparative study of phage reproduction was carried out at 37 and 44 C. At these temperatures, bacterial growth rates were similar, but at 44 C phage yield was only 2 to 5% that at 37 C. The general question to be answered was: what was the basis for the drop in phage production at 44 C?

Kinetic studies. The kinetics of phage λ reproduction at 37 and 44 C was described previously (Groman and Suzuki, 1961), so that only a resumé of the significant findings will be represented. From one-step growth curves, it was observed that the number of infective centers present at 44 C during the latent period was only slightly lower than the number at 37 C, a difference, if any, that cannot account for more than a minor fraction of the loss in phage yield. It was also noted that the first intracellular phage appears at the same time at both 37 and 44 C, indicating that the higher temperature did not exert its effect on processes related to phage synthesis, insofar as the appearance of intracellular phage reflects these processes. Finally, it was observed that lysis began 5 to 6 min earlier at 44 C and terminated in about one-half the time it took at 37 C. This suggests that it was the lytic process that was most affected by the higher temperature.

Single-burst analyses. Additional information on the temperature inhibition of phage production was obtained from single-burst analyses of cells incubated at 37 and 44 C. The results of three experiments are given in Table 1. The fact that the numbers of bursts at 37 and 44 C were nearly equal supports the contention that the depression of yield at 44 C was not due to loss of infective centers, nor was it due to an unexpected differential rate of lysogenization by λ at the two temperatures. The observation that all bursts at 44 C were in a low range is also of interest. This shows that the temperature effect operated rather

uniformly on all infected cells. The alternative hypothesis, of a few cells producing a normal yield and most cells no phage at all, is thus excluded. Finally, the relative yields at 37 and 44 C were similar to those obtained from the one-step growth-cycle data, indicating that the conditions in both types of experiments were similar.

Effect of a shift-up in temperature (37 to 44 C) on phage reproduction. Further evidence concerning the effect of temperature on phage yield was sought in temperature shift-up and shift-down experiments. The results of two shift-up experiments are given in Fig. 4. Transfer from 37 to 44 C as late as 25 to 30 min after infection still led to maximal inhibition of phage yield, indicating that the temperature-affected step(s) occurred after this interval. Shift-up at subsequent times resulted in a partial but decreasing inhibition of yield. Some phage maturation occurred at 44 C, even when transfer from 37 C occurred as late as 50 min; nevertheless, when the various yields were compared to the final yield at 37 C, the deficit resulting from even a short period at 44 C was evident. Beyond 50 min, transfer to 44 C was followed by an immediate cessation of phage production, as if lysis occurred immediately. Transition to the temperature-affected step occurs at about the same time the first mature phage appears in infected cells (Groman and Suzuki, 1961).

TABLE 1. *Single burst analysis of lambda phage**

Expt no.	Temp	Plates with bursts/total no. plates	Range of plaques per plate	Total phage yield	Average burst size	Phage yield†
	C					%
1	37	7/25	154–235	1,286	157	2.5
	44	10/25	2–12	47	4	
2	37	11/40	223–932	5,270	400	3.8
	44	10/40	3–34	172	15	
3	37	14/31	21–1053	5,918	320	2.8
	44	11/31	3–36	124	9	

* Cells were singly infected during a 10-min adsorption period and diluted into TSY medium at 37 and 44 C. During the latent period and after further dilution, a series of 1-ml samples was prepared at each temperature. Each sample contained an average of less than one infected cell per tube. The 44-C samples were assayed for total phage at 65 min, and those at 37 C were assayed at 80 min. To calculate the average burst size, the average number of bursts per plate (x) was determined from $P = e^{-x}$, where P is the proportion of plates without bursts.

† Yield at 44 C as a percentage of the yield at 37 C.

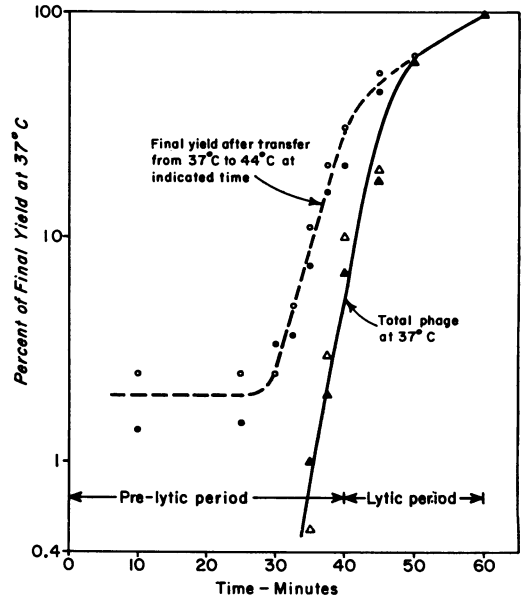

FIG. 4. *Effect of a shift-up in temperature (37 C to 44 C) on production of lambda phage. Cells were infected at 37 C, diluted into TSY medium at the same temperature, and assayed for total and free phage. At selected times, samples were removed and assayed for phage by the chloroform lysis technique, and a second sample was diluted into medium at 44 C and assayed for final phage yield 70 min after the initiation of the experiment.*

Effect of a shift-down in temperature (44 to 37 C) on phage production. The results of two shift-down experiments are given in Fig. 5. When infected cells were incubated at 44 C for as long as 25 min prior to transfer to 37 C, there was no appreciable effect on the final phage yields, and the average burst size was similar to that of cells infected and maintained at 37 C. Transfer at subsequent intervals led to a progressive decrease in final yield. Beyond 40 min, there was no increase in yield as a result of incubation at 37 C. Since, at 44 C, lysis began at 40 min, this sharp cut-off in productivity was not surprising.

Although phage yields were not depressed by a period of 20 to 25 min at 44 C prior to shift-down

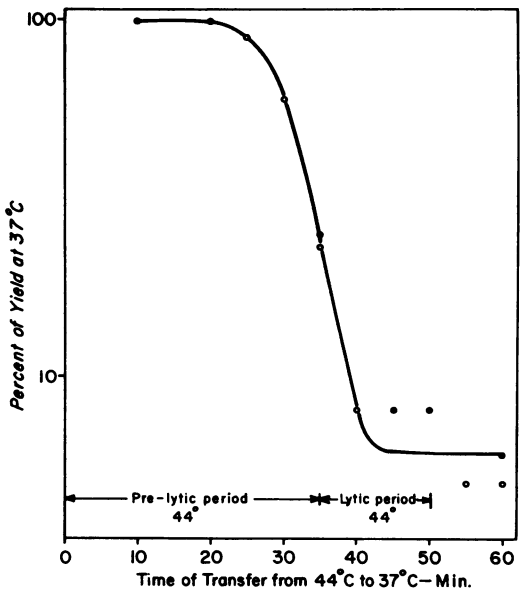

FIG. 5. *Effect of a shift-down in temperature (44 C to 37 C) on production of lambda phage. Cells were grown at 37 C but infection and dilution were carried out in TSY broth at 44 C. At selected times, samples were removed and diluted into medium at 37 C. Assays for final phage yield were made 80 min after the start of the experiment. The base line value (100% value) is the yield obtained from the sample transferred to 37 C at 10 min.*

to 37 C, these data gave no information about the effect of this early period on the timing of the various events in the one-step growth cycle. To examine this, the one-step growth cycle of cells transferred from 44 to 37 C at 30 min was determined. As seen in Fig. 6, intracellular phage appeared between 30 and 35 min, and the rise period was about 35 min long. These values are similar to those of an infection carried out entirely at 37 C. As expected, there was a slight shortening of the latent period and some depression of burst size, but these data show that a sojourn at 44 C during a major part of the cycle did not seriously alter the timing of phage development.

Kinetics of ultraviolet inactivation of phage produced at 37 and 44 C. Is the depressed yield at 44 C due to clumping of viable phage particles at the higher temperature, rather than to a true decrease in output? If this were true, one would expect phage from a 44-C lysate to exhibit multiple-hit inactivation kinetics when exposed to ultraviolet light. Both 37-C and 44-C lysates from a single experiment in which the expected ratios of phage were obtained were diluted into 0.01 M $MgSO_4$ and irradiated. Phage survival was determined at various times, and a semilogarithmic plot of survival vs. dosage made. Both lysates gave identical survival rates, and in each case the straight line intersected the origin at 100% survival. Single-hit kinetics prevailed, and therefore the hypothesis that clumping might occur at 44 C was discarded.

Is phage phenotypically altered with regard to temperature effects after a single passage at 44 C? To test this possibility, a lysate produced at 44 C was used as the phage inoculum for a second cycle at 37 and 44 C. In two such experiments, the 44 C/37 C yield was 3 and 4%, respectively, and the burst size at 37 C was normal. There was no evidence that phage was phenotypically altered during reproduction at 44 C.

Does growth of the host cell at 44 C prior to infection affect phage yield? Cells were grown for six to eight generations at 44 C and maintained at this temperature during infection. One sample was then diluted into broth at 37 C, another was maintained at 44 C, and final yields were determined. In two experiments, the 44 C/37 C yield

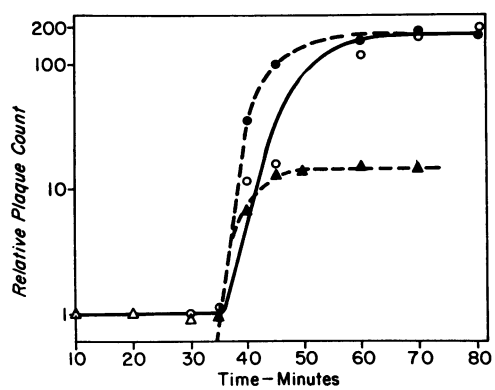

FIG. 6. *Effect on lambda-phage reproduction of a shift down to 37 C after 30 min at 44 C. Cells grown at 37 C were infected and maintained at 44 C for 30 min. A sample was then diluted into broth at 37 C. Throughout the experiment, infective centers were determined by direct assay of samples (44 C, △; 37 C, ○) and on chloroform lysates, (44 C, ▲; 37 C, ●). It will be noted that the 44-C curve is a composite of direct titration and chloroform-lysate values. At 44 C, the difference in the two types of determinations is not significant once intracellular phage has appeared.*

was 1.4 and 3%, respectively. These experiments, although adequate, suffered from the fact that phage adsorbed poorly to cells grown at 44 C. This indicated that surface alterations occurred during incubation at 44 C.

Lysogenic K-12 (λ) was employed in another test of the effect of temperature of cell growth on phage yield. Cells grown from six to eight generations at 44 C were maintained in TSY as close to 44 C as possible during induction by ultraviolet light, and were then diluted into TSY medium at 44 C. After 20 min, during which time the infective center count stabilized, a sample was diluted into broth at 37 C. In two experiments, the 44 C/37 C yields were 1 and 3.2%. Again, there was no indication that cells were conditioned in any way by a preinfection period at 44 C.

Does the medium influence the temperature effect? In limited tests of this question, cells were grown in TSY medium infected and then incubated at 37 and 44 C in a medium containing 1% Tryptone (Difco) and 0.5% NaCl. Similarly, cells were grown in the Tryptone medium, infected, and then incubated at the two temperatures in TSY medium. In both cases, the 44 C/37 C yields were in the range of 0.2 to 3.3%. Under these conditions, neither the growth nor incubation medium influenced the outcome.

DISCUSSION

The present study shows that alterations in temperature cause observable changes in the kinetics of lambda phage production and in the amount of phage produced. As the temperature is raised above 37 C, phage yield is progressively reduced. At 44 C, the yield is only 2 to 5% that at 37 C, despite the fact that the host bacterium grows normally at this temperature. For analysis of the effect of temperature on phage yield, the one-step growth cycle has been the major experimental tool. The validity of this approach is greatly enhanced by the observation that individual cells exhibit a rather uniform depression of phage yield at 44 C.

These data indicate that a temperature of 44 C has no significant effect on those processes concerned specifically with the replication and maturation of phage. The most revealing experiments are those in which infection began and was permitted to proceed at 44 C. The data show that, after a 10-min adsorption period, the infected cell count was consistent with expectation based on the disappearance of free phage. In addition, the infective center count remained steady during the latent period. Finally, when one transfers such cells to 37 C after as much as 20 to 30 min at 44 C, the time of appearance of intracellular phage and the temporal characteristics of the one-step growth curve are similar to what one would obtain if the entire infection had been carried out at 37 C. These observations show that there is no loss of infective centers during at least the first 30 min of exposure to 44 C. More specifically, they indicate strongly that adsorption-penetration does take place at 44 C, and that organizational and synthetic steps essential to phage reproduction are also being carried out during this period. Finally, the timing of events following shift-down suggests that the timing of steps carried out at 44 C is not much different than when the entire infection is carried out at 37 C.

It is clear from the shift-up experiments that maturation proceeds at 44 C, but it is not clear whether the rate of maturation is affected. Efforts have been made to compare maturation rates at 37 and 44 C following a shift-up at 10 min. The data obtained (Groman and Suzuki, *unpublished data*) reveal no difference; however, the data are limited because at 44 C the interval between the appearance of the first mature phage and the beginning of lysis is short. Once lysis begins, rate determinations become impossible, since the average number of phage-producing units present during any time interval is not known. Attempts to delay lysis in order to prolong the period of measurement failed.

It appears, therefore, that in this strain of λ, the lytic process is the only one which is obviously affected at 44 C, and an explanation for the depression of phage yield must largely concern itself with this phase of the cycle. When lambda-infected cells are transferred to 44 C after 10 min at 37 C, the latent period is shortened from 40 to 35 min and the rise period from 30 to 15 min (Groman and Suzuki, 1961). In effect, lysis starts sooner, relative to the time phage begins maturing, and thus the yield per infected cell is reduced. It is not possible from the present data to account quantitatively for the more than 95% depression of yield. The 50% reduction in the rise period sets a minimal value on the effect of a speeded-up lytic process, but there are ways in which even greater losses can be visualized within

the limits of this parameter. It is interesting to note that in the work of Maaløe and his associates with T4 bacteriophage the influence of temperature on the lytic period was also a key factor in the depression of phage yield. Maaløe (1950) observed that T4-infected cells held at 36 C began to lyse at 22 min, while infected cells held at 19 C began lysing at about 140 min and gave a yield which was reduced 40%. The longer cells were kept at 36 C prior to transfer to 19 C the sooner lysis began at the lower temperatures. In a subsequent paper (Weis-Bentzon et al., 1952), it was established that the drop to 19 C affected the rate of the lytic process to a lesser degree than it affected the rate of maturation, and as a result phage yield was reduced.

The basis for the speed-up in lysis observed in the present study is not known, but is under study. It could be dependent on the earlier appearance or more rapid rate of development of those materials or conditions required for lysis, or the result of temperature potentiation of lysis with or without an alteration in timing or quantity of such factors. Since endolysin is believed to be concerned with lysis in phage-infected *E. coli* (Jacob and Fuerst, 1958), a quantitative study of endolysin development is an obvious approach.

ACKNOWLEDGMENTS

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