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Increased Reproductive Fitness of *Escherichia coli* Lambda Lysogens

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Lambda lysogens of *Escherichia coli* reproduce more rapidly than nonlysogens during aerobic growth in glucose-limited chemostats. If the environment is changed to anaerobic growth, the situation is reversed, and the lysogen reproduces more slowly than the nonlysogen. Based on a tetrazolium dye assay, the increased fitness of the lambda lysogen during aerobic growth seems to result from a continued high metabolic rate as glucose becomes limiting, whereas the metabolic rate of the nonlysogen declines. The lambda *rex* gene is required for the growth advantage of lysogens since lack of *rex* function causes lambda lysogens to lose their reproductive advantage over nonlysogens.

When the bacterium *Escherichia coli* is infected by the bacteriophage lambda, the host and the phage are jointly faced with a developmental choice that can lead either to the reproduction of lambda phage and lyses of the host, or to lysogeny, in which the lambda phage DNA becomes integrated into the host chromosome and becomes genetically quiescent (8, 14). Lysogeny provides a useful system for studying phage-host interactions; it can be regarded as a prokaryotic model for the provirus hypothesis of Temin (16) and for the oncogene hypothesis of Huebner and Todaro (13), which provide models of virus-host interactions in vertebrate cells. An understanding of the molecular mechanisms of lysogeny in bacteria may provide the basis for a more complete understanding of virus-cell interactions in eukaryotic cells.

Many of the important questions concerning the role of the prophage in bacterial growth can be framed in terms of evolutionary relationships. As pointed out by Dove (7), one evolutionary advantage of lysogeny for the bacteriophage is to propagate itself without depleting the supply of hosts and to provide the opportunity for genetic variations to accumulate under nonselective conditions. In terms of the host cell, there is an advantage because of the immunity to infection by homologous phage that results from the presence of prophage. In addition, the prophage provides a means for increasing gene dispersal among similar bacteria by transduction. Thus, the growth, survival, and evolution of the temperate bacteriophage and its host are closely interrelated.

Natural selection acts at the level of reproductive fitness of individual organisms. If natural selection were the mechanism that resulted in lysogeny, it is reasonable to suppose that the reproductive fitness of the lysogenic bacterium would be greater than that of the nonlysogen. Previously we reported that lambda lysogens of *E. coli* reproduce more rapidly than nonlysogens when grown in chemostats under conditions of carbon source limitation (9). We report here on other conditions that affect the growth of a lambda lysogen as compared with that of a nonlysogen, and we give some evidence of a possible mechanism for the increased fitness.

MATERIALS AND METHODS

Bacteria and phage strains. *E. coli* AB 257 *met*$^-$ was lysogenized with temperature inducible lambda phage cI_{857} *ind*$^-$ *sus* J (9). A spontaneous streptomycin-resistant mutant of AB 257 was selected on L-broth plates containing streptomycin (10 μg/ml). A spontaneous *rex*$^-$ mutant of $\lambda c I_{857}$ was isolated as described (10, 12), and a *rex*$^-$ lambda lysogen of AB 257 was selected.

Chemostat cultures. New Brunswick chemostats were inoculated with bacteria grown overnight in M-9 minimal medium plus 40 μg of methionine per ml and 0.01% glucose. When the growth chamber became visibly turbid, the minimal medium was replaced at a rate that gave a generation time of 3 h. In some experiments, individual bacterial strains were grown separately in chemostats for several days before they were mixed together. Both procedures of mixing strains together gave similar results, i.e., by mixing in limiting glucose strains grown separately in batch culture, or by mixing separate strains adapted to chemostat growth conditions. All chemostats were maintained at 30°C and bubbled with air (aerobic growth) or with 95% N_2-5% CO_2 (anaerobic growth). The frequency of the lysogen and nonlysogen in a mixed population was de-

termined by streaking on plates a diluted sample and incubating the plates at either 30 or 42°C. All bacteria form colonies at 30°C, whereas only the nonlysogen will form colonies at 42°C. When the streptomycin-resistant strain was used, samples were also streaked on agar medium containing streptomycin (10 μg/ml) and incubated at 30 and 42°C.

Glucose uptake. The kinetics of glucose utilization were measured by the methods of Cohen and Monod (6) and Hoffee and Englesberg (11). Uptake was measured by incorporation of α-methyl-D-[^{14}C]glucopyranoside. Glucose utilization was measured by filtration of a 2-ml bacterial sample and determination of glucose in the supernatant with a glucostat (Worthington Biochemicals Corp.).

Tetrazolium dye assay. Bacterial samples (1 ml) were added to 20 μl of 2,3,5-triphenyl-tetrazolium chloride (58 mg/ml). These were incubated at 45°C for 5 min followed by addition of 20 μl of a freshly prepared lysozyme solution (10 mg/ml in 0.025 M Tris, pH 8.0). After 5 min of additional incubation, 100 μl of a 0.25 M EDTA-10% SDS solution was added to complete cell lysis. Dye development was measured at 490 nm with a Zeiss spectrophotometer (1).

RESULTS

Lambda lysogens of *E. coli* reproduce more rapidly than nonlysogens when growth is limited by any of several different carbon sources (9). This increased fitness was not observed when lysogenic and nonlysogenic bacteria were grown together in broth medium or in minimal medium containing excess glucose. When the nonlysogen or the lambda lysogen were grown separately in either limiting or excess glucose, the doubling time for each strain was precisely the same (60 min at 30°C; see Fig. 4).

Whatever the mechanism of the increased fitness of the lambda lysogen, it only became evident at the point of glucose exhaustion, a condition that was maintained in chemostats but not in batch cultures. To ensure that the increased fitness of the lambda lysogen did not result from a trivial mechanism, such as infection of the nonlysogen by lambda phage released from the lysogen, additional controls were done to supplement those reported originally (9). The lambda phage used in these experiments is genetically *ind*$^-$ *sus* J, and the prophage is carried by an amber restictive *E. coli* strain AB 257. The number of lambda phage released from this strain at 30°C was less than 1 per 5×10^8 bacteria or less than 1 per ml in the chemostat.

To be able to identify genetically the nonlysogenic strain (even though the strains were no longer strictly isogenic) a streptomycin-resistant derivative of strain AB 257 was selected. An interesting property of the streptomycin-resistant strain is that it grows more slowly than the parent strain in broth medium without streptomycin (it has a generation time of 50 min as compared with the parental generation time of 37 min at 37°C), but it grows more rapidly than the parent strain in a glucose-limited chemostat. Figure 1 shows the increase in frequency of the streptomycin-resistant strain grown with the streptomycin-sensitive parent in a glucose-limited chemostat. This was an interesting but unusual example of streptomycin resistance conferring decreased fitness to the bacterium in broth medium and increased fitness in a glucose-limited medium. We did not investigate the mechanism of this difference in fitness under the different growth conditions, but the streptomycin-resistant strain provided an additional genetic control during the chemostat growth of a lysogen and nonlysogen. Figure 2 shows that the lambda lysogen reproduced more rapidly than the streptomycin-resistant nonlysogen when both were grown together in a glucose-limited chemostat. Since the streptomycin-resistant bacteria can be identified by growth on streptomycin-containing plates, they can be tested to determine if any have become lysogenic. Fifty streptomycin-resistant colonies derived from the chemostat experiment in Fig. 2 were tested daily for lyso-

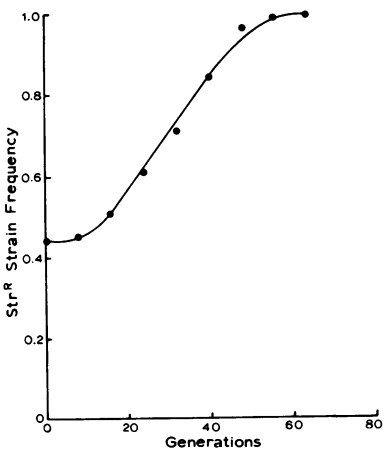

FIG. 1. *Competition of strain AB 257 met$^-$ versus strain AB 257 met$^-$str^R in a glucose-limited chemostat. Each strain was grown overnight in M-9 minimal medium with 0.01% glucose and 40 μg of methionine per ml. The chemostat was inoculated with an equal number of each bacterial strain, and growth was maintained at a 3-h generation time by the flow of fresh medium into the chemostat. Samples were streaked daily on LB agar plates and LB agar plates plus 10 μg of streptomycin per ml.*

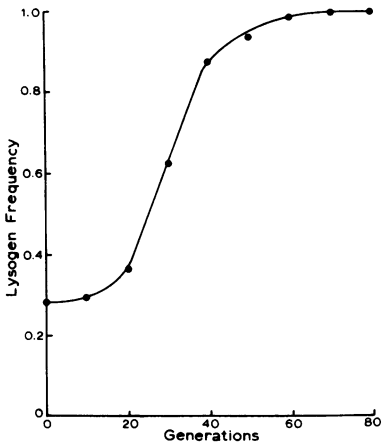

FIG. 2. *Competition of strain AB 257 met⁻str^R versus strain AB 257 met⁻ λI₈₅₇ sus J in a glucose-limited chemostat. The chemostat conditions were the same as those in Fig. 1. Samples were streaked on LB agar plates and incubated at 30 and 42°C.*

geny; no lambda lysogens were detected.

Having established that the lambda lysogen does reproduce more rapidly, we wanted to see if the chemostat environment could be altered so that the lysogen would become less fit than the nonlysogen. This was accomplished by changing conditions from aerobic to anerobic growth (Fig. 3). If the culture was saturated with a mixture of 95% N_2-5% CO_2, the nonlysogen reproduced more rapidly, and the frequency of the lysogen in the population dropped to 1% or less. If the culture was shifted back to aerobic growth, the frequency of the lysogen in the population rapidly increased. It should be noted that we have never observed the complete elimination of one strain from the chemostat culture in either aerobic or anaerobic growth. In some experiments the frequency of one strain dropped as low as 0.01%, but it was maintained in the population at this low level. Figure 3 demonstrates the environmental manipulation of fitness, in this case the reproductive rate of a bacterial population. As long as growth was limited by the supply of glucose, the lysogen was more fit in aerobic growth, whereas the nonlysogen was more fit during anaerobic growth.

At this point we wanted to determine the efficiency of glucose utilization by the lysogen and nonlysogen. Figure 4 shows the kinetics of glucose utilization by the lysogen and nonlysogen growing in 0.01% glucose minimal medium. During exponential growth both strains metabolized glucose with essentially identical kinetics. The incorporation of radioactive α-methyl-D-glucopyranoside by each culture growing under the same conditions was measured, and no difference was detected. These results support the idea that the difference in fitness between the lysogen and nonlysogen is manifested at the point of glucose exhaustion. To obtain a more sensitive measure of what the metabolic differences might be at this point, we utilized a tetrazolium dye assay (1). Tetrazo-

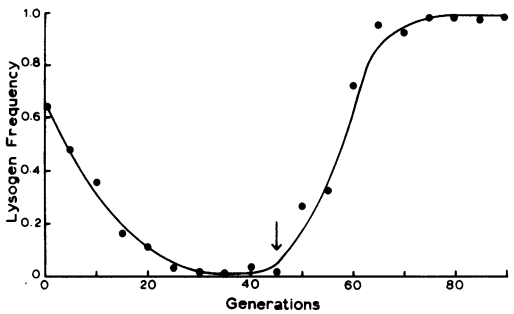

FIG. 3. *Competition of strain AB 257 met⁻ versus strain AB 257 met⁻ (λI₈₅₇ sus J) in a glucose-limited chemostat. The chemostat was operated anaerobically for 45 generations and then switched to aerobic growth (arrow indicates point of shift). Samples were streaked on plates as described in the legend of Fig. 2.*

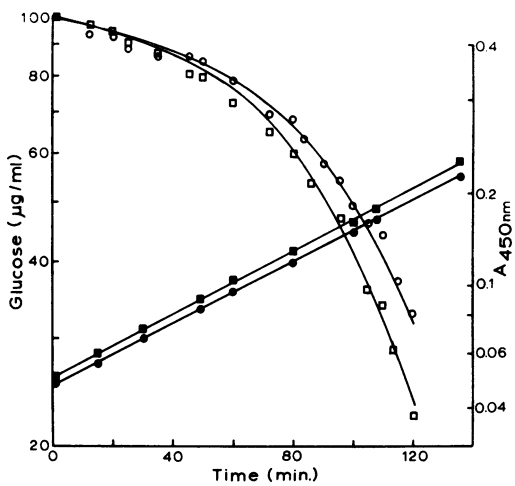

FIG. 4. *Kinetics of glucose utilization by the lambda lysogen and nonlysogen. E. coli strains described in the legend of Fig. 3 were grown in M-9 minimal medium plus 0.01% glucose and 40 μg of methionine per ml at 30°C in flasks that were aerated by shaking. The amount of glucose in the medium was measured at different times by filtering a sample and testing the cell-free medium with a glucostat. Symbols: □, AB 257 met⁻; ○, AB 257 met⁻ λcI₈₅₇ sus J. The shaded symbols show the growth of each strain measured at 450 nm. The initial concentration of bacteria was adjusted to give identical growth curves for both strains.*

lium dyes measure the transport of electrons from reduced nicotinamide adenine dinucleotide to tetrazolium salts and, generally, measure the overall metabolic activity of the cell. The higher the cellular metabolic rate, the more rapidly the dye changes color. The lysogen and nonlysogen were grown in 0.1% glucose minimal medium until the stationary phase of growth was reached. Figure 5 shows that while the bacteria were growing exponentially, the dye was converted rapidly. As the bacteria entered the stationary phase, the metabolic activity dropped abruptly. The interesting observation is that during aerobic growth the metabolic rate of the nonlysogen decreased markedly about 20 min before any change was noticed in the lysogen. However, during anaerobic growth the situation was reversed. In this case the metabolic activity of the lysogen decreased for about 10 min before any change was observed for the nonlysogen. These results are consistent with the chemostat results shown in Fig. 3. During anaerobic growth the lysogen was less fit (confirmed by the earlier metabolic shutdown when glucose was exhausted), whereas during aerobic growth the lysogen was more fit (and in this instance the metabolism of the lysogen remains high for a longer period than that of the nonlysogen).

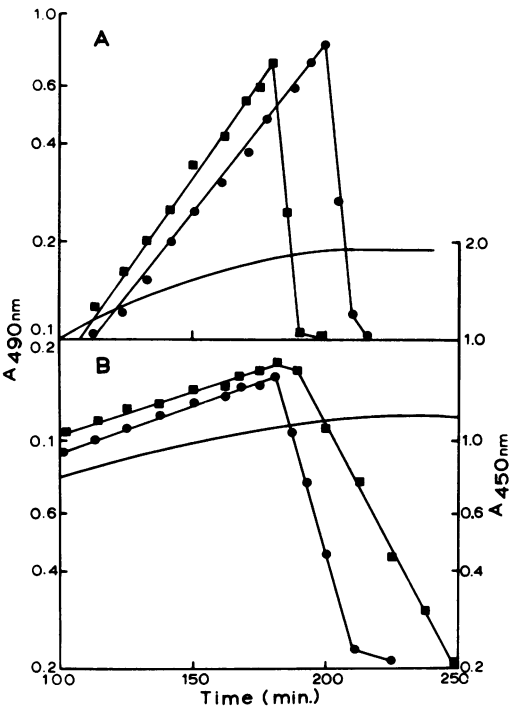

FIG. 5. *Tetrazolium dye assay of the lysogen and nonlysogen grown aerobically or anaerobically. Each strain was grown overnight in M-9 minimal medium plus 0.1% glucose and 40 μg of methionine per ml. The next day each culture was diluted 1:10 into fresh medium and, when growing exponentially, adjusted to the same cell density. Growth was measured by turbidity at 450 nm. The experimental points for the growth of both cultures fit this curve exactly and are not shown. The metabolic activity of the lysogen and nonlysogen was measured by the rate of dye color changed as described by Allen (1). (A) Metabolic activity of the aerobic cultures; (B) metabolic activity of the anaerobic cultures. Symbols: ■, AB 257 met⁻; ●, AB 257 met⁻ λI_{857} sus J.*

A second approach to understanding the mechanism of increased fitness of the lambda lysogen during aerobic growth is to assume that expression of one or more lambda genes affects the metabolism of the cell during glucose limitation. Two functions are known to be expressed by the prophage in the repressed state: *c*I, the repressor protein which maintains lysogeny, and the *rex* protein, whose function is not understood (10, 12). The lambda *rex* function can be identified phenotypically since T4rII mutants can grow in *rex*⁻ lambda lysogens, whereas they cannot grow in *rex*⁺ lambda lysogens. A spontaneous *rex*⁻ mutant of λcI_{857} *sus* J was isolated, and a lysogen of AB 257 was selected. The *rex*⁻ lysogen and nonlysogen were grown together in a glucose-limited chemostat. Figure 6 shows the results of two separate chemostat experiments. In one case, the frequency of the lysogen decreased slightly, and in the other it increased slightly; however, neither experiment showed any evidence for increased

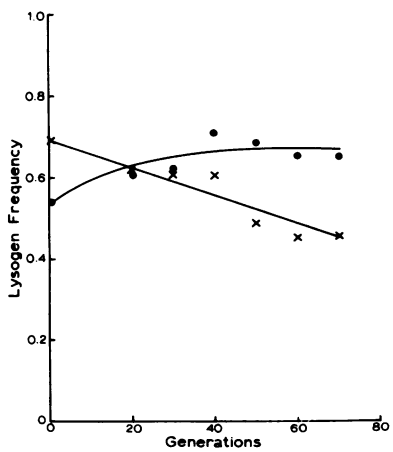

FIG. 6. *Competition of strain AB 257 met⁻ versus strain AB 257 met⁻ λI_{857} sus J rex⁻ in a glucose-limited chemostat. The chemostat growth conditions were the same as those described in the legend of Fig. 1, and the samples were streaked on LB agar plates and incubated at 30 and 40°C. Symbols: ●, chemostat no. 1; ×, chemostat no. 2.*

fitness of either the lysogen or the nonlysogen. Thus, without the *rex* protein, the lysogen showed no increased fitness for at least 70 generations, which is in marked contrast to the fitness shown by the lysogen in Fig. 2 and 3. We are testing additional *rex*$^-$ and other lambda mutants to confirm the role of lambda prophage genes in affecting the reproductive rate of lysogenic bacteria.

DISCUSSION

The relative reproductive rates of *E. coli* strains, which are a measure of fitness in terms of natural selection, are affected by the presence of a lambda prophage. The prophage can enhance the reproductive rate of the lysogen during aerobic growth in glucose-limited chemostats and retard the rate during anaerobic growth. These differences in fitness are observed under conditions of carbon source limitation (Fig. 2 and 3) but are not observed when mixed populations of the lysogen and nonlysogen are grown in broth medium or in minimal medium containing excess glucose (9).

These observations can be restated in terms of evolutionary biology. The lambda prophage is advantageous to the growth of *E. coli* under poor environmental growth conditions. Natural selection might have favored the reproduction of a lambda lysogen if bacterial growth had occurred in environments with limiting nutrients. Thus, in addition to protecting the bacterium from infection by homologous phage, the prophage serves an even more important evolutionary function by increasing the fitness of the bacterium in certain environments.

While clearly speculative, it is tempting to extrapolate these ideas to virus-host interactions in animal cells. As pointed out by Baltimore and others (2, 3), most, if not all, animal species have DNA related to one or more types of RNA viruses integrated into their chromosomes. This DNA is inherited in the same fashion as any chromosomal gene, and presumably it has survived as a result of natural selection. We propose that the presence of this viral DNA conferred an increased fitness to the host cell at some stage of evolution and might be doing so even in present day animals. From this viewpoint, cellular transformation caused by the induction of endogenous viruses might be an unfortunate side reaction to a genetic change of adaptive value, and might be considered analogous to the undesirable maintenance of sickle cell anemia (hemoglobin S), along with the mutation that conferred resistance to malaria (14).

Finally, a word about the mechanism of the increased fitness of lambda lysogens. Figure 4 shows that both the lysogen and nonlysogen utilize glucose with identical kinetics during exponential growth. Figure 5 shows that at the point where glucose becomes limiting and growth slows down, the lysogen that is growing aerobically continues to metabolize at a high rate. It seems likely that the prophage modifies the structure or function (or both) of the bacterial membrane. This suggestion is supported by the findings of Schnaitman and co-workers (15), who showed that the protein composition of the outer membrane of *E. coli* is altered by the presence of a lambdoid phage (PA-2). It may be that prophage alter the outer membranes of their host bacteria in such a way that they are better able to assimilate the limited nutrients so that their reproductive fitness under adverse environmental conditions is increased.

In the accompanying report we show that widely different temperate phages such as P1, P2, and Mu increase the reproductive rate of *E. coli* lysogens in limiting glucose. At present we are trying to understand the mechanisms that produce these changes in bacterial growth rate and fitness.

ACKNOWLEDGMENTS

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