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Reproductive Fitness of P1, P2, and Mu Lysogens of *Escherichia coli*

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P1, P2, and Mu lysogens of *Escherichia coli* reproduce more rapidly than nonlysogens during aerobic growth in glucose-limited chemostats. Thus, prophage-containing stains of *E. coli* are reproductively more fit than the corresponding nonlysogens. If mixed populations are grown by serial dilution under conditions in which growth is not limited, both the lysogen and nonlysogen manifest identical growth rates. The increased fitness of the lysogens in glucose-limited chemostats correlates with a higher metabolic activity of the lysogen as compared with the nonlysogen during glucose exhaustion. We propose that P1, P2, Mu, and lambda prophage all confer an evolutionarily significant reproductive growth advantage to *E. coli* lysogenic strains.

Viruses are ubiquitous in nature, capable of infecting both prokaryotic and eukaryotic cells. Viruses cause profound metabolic changes in a cell even to the point of lysis and cell disintegration. However, the viral DNA can also be sequestered in the cell in such a way that it has no apparent effect on cellular growth or function. In the case of infection of bacteria by some temperate bacteriophage, the presence of inactive prophage DNA integrated into the bacterial chromosome is termed lysogeny (5, 11). Lysogenic bacteria reproduce and grow in a manner that is indistinguishable from that of nonlysogenic bacteria as long as the prophage remains repressed.

Escherichia coli can be infected by a variety of temperate phages. The best studied example of lysogeny is the phage lambda, in which the lambda DNA is covalently integrated into the bacterial DNA at a specific site. Other temperate phages that can be maintained in the *E. coli* chromosome or cytoplasm are P1, P2, and Mu. Each of these temperate phage associates by a different mechanism with the bacterium in a genetically transmissible fashion, and the bacteria have different properties. Phage P2 integrates its DNA into the bacterial chromosome at a number of different sites, and unlike lambda, cannot be induced by UV light (3). Phage P1 is maintained as an unintegrated plasmid in the cytoplasm with a single copy per cell (14). Phage Mu can integrate at virtually any site in the *E. coli* chromosome, and as pointed out by Razzaki and Bukhari, Mu lysogens have properties similar to those of eukaryotic cells infected by SV40 virus (12, 16).

Under many conditions bacteria and phage propagate in close association with one another. It seems reasonable to postulate that this association between bacterium and phage has been evolutionarily advantageous to both organisms. In fact, it has been pointed out by Campbell that "it is clear that a metabolically inert prophage would, in general, impart a selective disadvantage to its host, which should lead to the eventual elimination of both through competition with hosts which do not carry prophages. One therefore must look for possible means by which the phage might impart a selective advantage to its host" (4).

In a previous report we showed that lambda imparts an increased fitness to lambda lysogens of *E. coli* by increasing the reproductive rate under conditions of carbon source limitation (6). In the present report, evidence is presented that P1, P2, and Mu lysogens of *E. coli* also show a selective, reproductive advantage under conditions of carbon source limitation. Taken together, the evidence presented here and in the preceding report suggest that lysogeny was selected and maintained in nature due to the reproductive advantage enjoyed by lysogenic bacteria in environments with limiting nutrients. Whether the mechanism of the reproductive advantage is the same for all temperate phages or different for each remains to be determined.

MATERIALS AND METHODS

Bacteria and phage strains. *E. coli* AB 257 *met*$^-$ and AB 257 *met*$^-$ *str*^{R} were described in the preceding report. Derivatives of AB 257 *met*$^-$ *str*^{R} that were

resistant to infection by P1, P2, or Mu were isolated by picking bacteria from the center of a phage plaque on a nutrient agar plate that had been incubated at 42°C. Since the P1, P2, and Mu phages used are all temperature inducible, no lysogenic colonies are able to form at 42°C. Resistant bacteria were selected from colonies that were capable of growth at 30 and 42°C and which were resistant to the phage to which they had been exposed.

The P1 phage used is P1Cm and contains a temperature-sensitive repressor which allows induction of the P1 phage and lysis of the bacteria at 42°C. This phage also confers chloramphenicol resistance to the lysogenic bacteria, and it was constructed by J. L. Rosner (13). The P2 phage used is one designated as 186p which is closely related to P2 (3), which also has a temperature-sensitive repressor, and which was obtained from R. Calendar (2). The thermoinducible Mu-cts 62 was obtained from B. Waggoner (12).

Chemostat growth conditions and tetrazolium dye assays are the same as those described in the accompanying report (10).

RESULTS

Previous experiments showed that lambda lysogens of *E. coli* reproduce more rapidly than nonlysogens under conditions of carbon source limitation (6, 10). We wished to know if lysogens of *E. coli* containing other temperate phages such as P1, P2, or Mu would show a similar reproductive fitness as compared to the corresponding nonlysogens. To this end we constructed lysogens of *E. coli* AB 257 with each of these temperate phages that have the additional property of being thermoinducible at 42°C. At 30°C, however, the bacterial lysogens are stable. When growing the lysogen and nonlysogen in a mixed population in the chemostat, it is convenient to measure the frequency of each type by spreading a sample on nutrient agar plates and incubating duplicate plates at 30 and 42°C. Both the lysogen and nonlysogen form colonies at 30°C, whereas only the nonlysogen forms colonies at 42°C.

Unfortunately the P1, P2, and Mu lysogens are not as stable as the lambda lysogen described previously (6). Cultures of these lysogens grown in chemostats liberate phage, and the culture contains phage titers of about 10^4 phage per ml. In a mixed population of the lysogen and nonlysogen growing together, the phage titer sometimes rises to 10^7 phage per ml. With this number of free phage in the medium it is probable that some of the nonlysogenic bacteria will be infected and either lyse, liberating more phage, or become lysogenic themselves. To obviate this difficulty, phage-resistant mutants of AB 257 str^R were isolated and used in the chemostat competition experiments. Since in these experiments the lysogenic and nonlysogenic strains were not strictly isogenic, it was first necessary to show that the phage-resistant derivatives of AB 257 str^R met^- reproduce as rapidly or more rapidly than the parent strain AB 257 met^-. In the accompanying report (10), we showed that strain AB 257 str^R met^- reproduces more rapidly than the parent strain AB 257 met^- and, consequently, is evolutionarily more fit. Figure 1 shows the result of chemostat competition experiments in which various phage-resistant strains were grown with the parent strain AB 257 met^-. In all cases, the phage-resistant, streptomycin-resistant strains grew as rapidly as the parent strain. The frequency of each phage-resistant and parent strain in the population remained essentially constant for at least 80 generations.

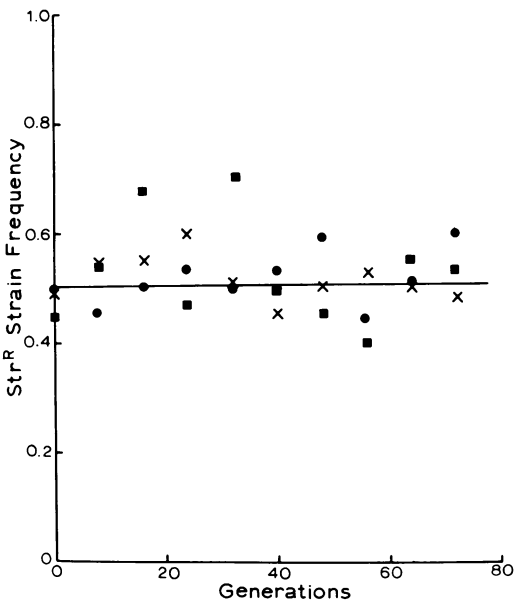

FIG. 1. *Growth of P1, P2, or Mu resistant strains with AB 257 met^- in glucose-limited chemostats. Each bacterial strain was grown separately in a chemostat containing M9 minimal medium plus 0.01% glucose and 20 μg of methionine per ml. After they were grown separately in chemostats for at least 48 h, equal numbers of bacteria were mixed together in a fresh chemostat, and the flow of medium was adjusted to give a 3-h generation time. Samples were streaked daily on nutrient agar plates and nutrient agar plates plus 10 μg of streptomycin per ml. The total number of bacteria were determined by the colony count on the nutrient agar plates, and the number of phage-resistant bacteria were determined by the colony count on streptomycin plates. Symbols: ●, AB 257 met^- $P1^R$ str^R versus AB 257 met^-; ■, AB 257 met^- $P2^R$ str^R versus AB 257 met^-; ×, AB 257 Met^- Mu^R str^R versus AB 257 met^-.*

Since the phage-resistant strains reproduced as rapidly as the original parent strain AB 257 *met*$^-$, we can infer that if the lysogen reproduces more rapidly than the phage-resistant nonlysogen, it also must reproduce more rapidly than the isogenic parent strain AB 257 *met*$^-$.

In fact, this is the result obtained. Figure 2 shows the data from a glucose-limited chemostat containing the P1 lysogen of AB 257 *met*$^-$ along with the P1-resistant derivative of AB 257 *met*$^-$ *str*^{R}. The P1 lysogen reproduced more rapidly. This experiment was repeated several times with similar results. In this experiment the starting ratio is 10:1 (nonlysogen-lysogen) since the P1 lysogen takes over even more rapidly than shown in Fig. 2 when the strains are mixed in equal numbers. The P1-resistant, streptomycin-resistant strain was continually monitored to determine if any P1 lysogens were being formed; no lysogens were detected. Thus, we are confident that the P1 phage are not being transferred from the lysogen to the nonlysogen in the mixed chemostat population.

Comparable experiments with the P2- and Mu-resistant strains versus the corresponding lysogens are shown in Fig. 3 and 4. In each instance the bacterial lysogens were more fit, and they became the most frequent type in the mixed population. In all of these chemostat experiments the minority type (the nonlysogen) was never completely eliminated from the population. However, due to the inevitable problem of bacteria sticking to the walls of the chemostat, it was difficult to draw any conclusions from the final frequency of the two strains in the chemostat population.

As a further control, to assure ourselves of the validity of the increased fitness of the different lysogens in glucose-limited chemostats, the lysogen and nonlysogen for each phage were grown together by daily serial dilution in minimal medium containing 0.01% glucose. This is a different growth condition since the glucose is

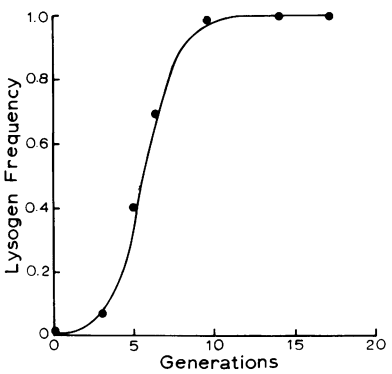

FIG. 2. *Growth of P1 lysogen and the P1-resistant nonlysogen in a glucose-limited chemostat. Each strain was grown separately in a chemostat containing M9 minimal medium plus 0.01% glucose and 20 μg of methionine per ml. After they grew separately for at least 48 h, equal numbers of bacteria were mixed together in a fresh chemostat, and the flow of medium was adjusted to give a 3-h generation time. Samples were streaked daily on nutrient agar plates and then incubated at 30 and 42°C. The total number of bacteria was measured at 30°C, and the frequency of the nonlysogen was measured by the colony count at 42°C. The frequency of the lysogen in the population was determined by the difference in colony count at 30 and 42°C and was usually confirmed by streaking on streptomycin-agar plates incubated at 30°C on which only the P1 resistant nonlysogen could grow. Symbols: ●, AB 257 met$^-$ (P1Cm) versus AB 257 met$^-$ P1^R str^R.*

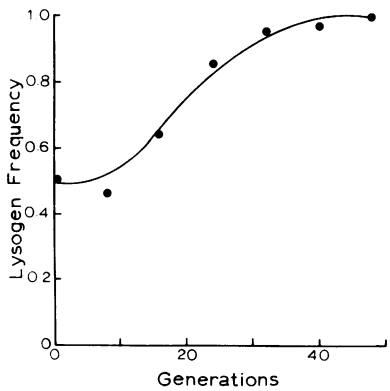

FIG. 3. *Growth of P2 (186p) lysogen and the P2-resistant nonlysogen in a glucose-limited chemostat. The experiment was performed as described in the legend of Fig. 2. Symbols: ●, AB 257 met$^-$ (186p) versus AB 257 met$^-$ P2^R str^R.*

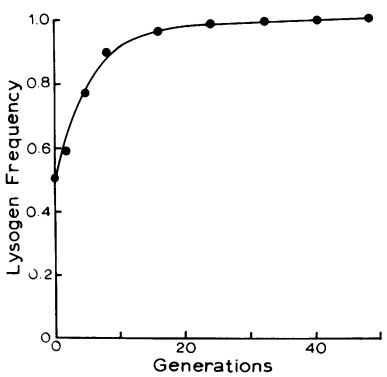

FIG. 4. *Growth of the Mu-cts lysogen and the Mu-resistant nonlysogen in a glucose-limited chemostat. The experiment was performed as described in the legend of Fig. 2. ●, AB 257 met$^-$ (Mu-cts 62) versus AB 257 met$^-$ Mu^R str^R.*

not limiting until the moment of depletion, whereas in the chemostat, the bacteria are continually deprived. In the 24-h period between dilutions, the culture is usually just able to deplete the supply of glucose. Figure 5 shows that under these conditions the lysogen and nonlysogen for phages P1, P2, and Mu reproduce at the same rates with some fluctations from day to day. Thus, the observation that lysogens of P1, P2, Mu, and lambda reproduce more rapidly than closely related nonlysogens in glucose-limited chemostats must reflect a biochemical difference between the bacterial lysogen and non-lysogen growing in the chemostat. As shown in Fig. 5, this difference is not manifested in medium containing the essential nutrients in excess.

As described in the accompanying report (10), the increased fitness of the lambda lysogen correlates with an increased metabolic activity during glucose depletion as measured by the tetrazolium dye assay (1). The P1, P2, and Mu lysogens were compared to the corresponding nonlysogenic phage-resistant strains for election transport capacity at various stages of growth by using the tetrazolium dye assay. Figure 6 shows the results for the P1 lysogen and nonlysogen. As the bacteria entered the stationary phase of growth, the metabolic activity of the nonlysogen began to decrease approximately 20 min before a decrease was observed in the lysogen. We believe that this extended metabolic activity was dictated by the prophage and was mainfested when growth became limited by carbon source availability. Although the data are not presented, virtually identical results to those shown in Fig. 6 were obtained for P2 and Mu lysogens as compared with those for the phage-resistant nonlysogens. Thus, in all cases where we found increased reproductive fitness of bacterial lysogens under conditions of carbon source limitation, we also found a more sustained metabolic activity of the lysogens under similar growth-limiting conditions.

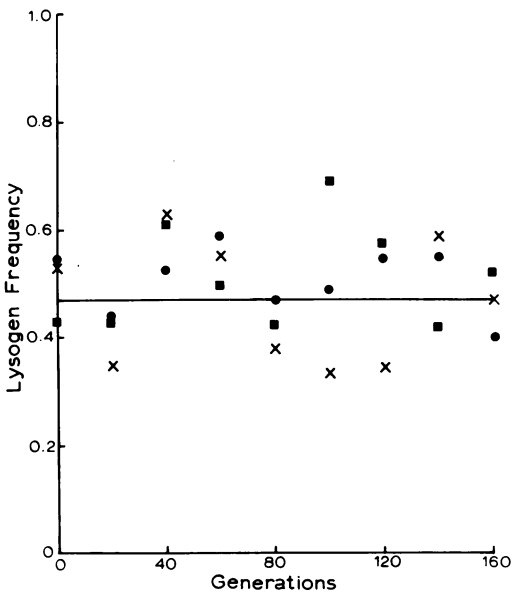

FIG. 5. *Growth of P1, P2, and Mu lysogens with phage-resistant nonlysogens by daily serial dilution into fresh medium. Each strain was grown separately in M9 minimal medium plus 0.01% glucose and 40 μg of methionine per ml in a bubbler tube aerated at 30°C. The next day equal numbers of bacteria were mixed together and then diluted 10^6, and growth continued in fresh medium. Samples were streaked daily on nutrient agar plates and nutrient agar plates plus 10 μg of streptomycin per ml and then incubated at 30°C. Colonies were picked from the streptomycin plate and restreaked on duplicate nutrient agar plates that were incubated at 30 and 42°C. No lysogenic colonies of the phage-resistant strain. were ever detected. Symbols: ●, AB 257 met− (p1Cm) versus AB 257 met⁻ $P1^R$ str^R; ■, AB 257 met⁻ (186p) versus AB 257 met⁻ $P2^R$ str^R; ×, AB 257 met⁻ (Mu cts 62) versus AB 257 met⁻ Mu^R str^R.*

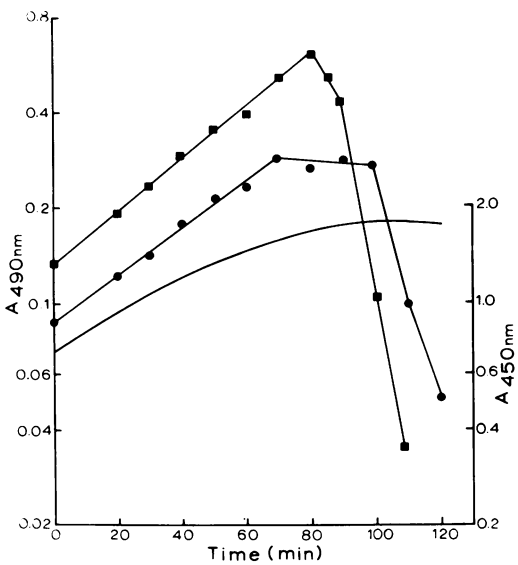

FIG. 6. *Tetrazolium dye assay (1) of the P1 lysogen AB 257 met⁻ (P1Cm) and the P1-resistant, streptomycin-resistant nonlysogen (AB 257 met⁻ $P1^R$ str^R) growing separately in M9 minimal medium plus 0.01% glucose and 40 μg of methionine per ml. The initial concentration of bacteria for each strain was adjusted to give identical values, and the growth rates of both strains were identical from that moment on. Thus, the metabolism of both strains can be directly compared. Symbols: ●, AB 257 met⁻ (P1Cm); ■, AB 257 met⁻ $P1^R$ str^R.*

DISCUSSION

It is rather surprising that four different temperate phages of *E. coli* can increase the reproductive fitness of *E. coli* under conditions of glucose limitation. This novel finding would appear to have considerable evolutionary significance. The accompanying report indicates that the *rex* gene of lambda may be involved in regulation of the increased fitness of the lambda bacterial lysogen. However, it is also apparent from the anaerobic chemostat experiment described in the previous report that the environmental growth conditions can alter the relative fitness of the lysogen versus the nonlysogen.

P1, P2, and Mu phages are not involved in the exclusion of T4rII mutants since *E. coli* lysogens of these phages can grow T4rII phage. Lambda is the only phage of the group possessing a Rex function. It is unlikely that the expression of a *rex* gene in the P1, P2, or Mu lysogens can account for the increased fitness of the lysogens. Growth in the chemostat is always limited by the instantaneous supply of glucose, so it seems reasonable to ascribe the increased fitness of the lysogens in the chemostat to their enhanced metabolic rate as compared with the nonlysogen. The results do not provide any insight into the molecular mechanism of the increased fitness, except that they show a correlation with tetrazolium dye conversion that implies increased metabolic activity. Since the increased fitness must reside ultimately in more efficient utilization of the available glucose, it seems reasonable to suppose that the presence of a prophage modifies the bacterial envelope in some manner that permits more efficient use of glucose. However, other interpretations are possible, and particular prophages may alter different cellular structures or functions.

A number of observations in animal cells fit in nicely with the idea of prophage modification of the bacterial envelope. Chicken embryo cells, transformed with Rous sarcoma virus, showed enhanced rates of sugar transport and glycolysis (15) as well as modification of the plasma membrane. Similar results were obtained with transformed rat embryo cells (7-9). All of these experiments indicate that animal cells, transformed by tumor viruses, acquire altered membranes as well as exhibit altered glucose utilization.

The experiments reported here and in the accompanying report (10) show that different prophages or plasmids can increase the fitness of *E. coli* strains grown in chemostats under glucose-limiting conditions. We propose that this reproductive growth advantage has played a role in the natural selection of prophage- and plasmid-containing bacteria in nature. We speculate that the presence of oncogenes in animal cells and their vertical transmission reflects a growth advantage enjoyed by these cells at some stage of evolutionary history. Further investigation of the mechanism of the increased fitness of bacterial lysogens should provide a better understanding of the role viruses play in animal cell growth and development, as well as the role of temperate phage in bacterial evolution.

ACKNOWLEDGMENTS

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