Effects of Carriage and Expression of the Tn*10* Tetracycline-Resistance Operon on the Fitness of *Escherichia coli* K12[1]

Trinh N. M. Nguyen, Quang G. Phan,* Loan P. Duong,* Kevin P. Bertrand,† and Richard E. Lenski**

*Department of Ecology and Evolutionary Biology, University of California, Irvine; and †Biochemistry/Biophysics Program and Department of Microbiology, Washington State University

We have been examining the consequences of alternative modes of regulation of plasmid-borne, Tn*10*-encoded tetracycline resistance for the fitness of *Escherichia coli*. In a tetracycline-free environment, we measured the effects on fitness that were caused by (1) maximally induced expression of the resistance operon, (2) low-level constitutive expression of the resistance protein, (3) residual expression of the repressed resistance operon, (4) carriage of the resistance operon, (5) the remainder of the plasmid genome, and (6) hyperexpression of the repressor protein. We observed large reductions in fitness that were associated with induction and with constitutive expression of the tetracycline-resistance protein, but there was no discernible effect of hyperexpression of the repressor protein. We also observed a small reduction in fitness associated with the remainder of the plasmid genome. However, any reductions in fitness that were caused by residual expression and by carriage of the repressed operon were not more than 0.3%. We conclude that tight gene regulation has eliminated antagonistic pleiotropic effects of the resistance gene on fitness, so that possession of an inducible Tn*10*-encoded tetracycline-resistance operon imposes essentially no burden in the absence of antibiotic.

Introduction

A great deal of evolutionary theory is predicated on the assumption that there are genetically determined trade-offs between components of fitness (e.g., fecundity and longevity) or between measures of fitness across different environmental conditions (e.g., with and without pathogens). Pleiotropy, whereby a particular gene substitution influences multiple phenotypic traits, is generally regarded as the primary cause of these antagonistic effects. A number of experimental studies have clearly demonstrated trade-offs consistent with antagonistic pleiotropy (see, e.g., Rose 1984; Lenski and Levin 1985). In some other studies, however, trade-offs have been looked for but not observed (see, e.g., Luckinbill 1984; Simms and Rausher 1987). In a few studies, the effects of antagonistic pleiotropy have been observed to be diminished by subsequent evolution, presumably through the effects of mutations at modifier loci (McKenzie et al. 1982; Bouma and Lenski 1988; Lenski 1988).

It is of interest to know how readily trade-offs can be diminished and how this

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Address for correspondence and reprints: Dr. Richard E. Lenski, Department of Ecology and Evolutionary Biology, University of California, Irvine, CA 92717.

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is accomplished in terms of organismal function. A number of authors have suggested that the regulation of gene expression can play an important role in reducing burdens caused by possession of certain traits (see, e.g., Hall 1983; Diamond 1986; Uyenoyama 1986; Simms and Rausher 1987). Indeed, several studies have shown that constitutive expression of the lactose operon reduces fitness when cells are grown on other limiting carbon sources (Andrews and Hegeman 1976; Dykhuizen and Davies 1980; Koch 1983). One can also quantify the burden, if any, associated with the repressed function relative to the absence of function. In this regard, other studies have demonstrated significant fitness reductions associated with repressed tryptophan operons (Zamenhof and Eichhorn 1967; Dykhuizen 1978). Thus, there are two distinct but related questions that can be addressed: (1) How large is the fitness reduction caused by gene expression in the absence of environmental conditions that favor that gene's function? (2) Are there any detectable effects on fitness that are associated with carriage of a repressed gene function, as might arise from synthesizing the extra genetic material, from residual expression of the repressed gene, or from some aspect of gene regulation itself?

In this study, we examine the effects on fitness of *Escherichia coli* that are caused by carriage and by expression of a tetracycline-resistance operon derived from the transposon Tn*10*. [Chao et al. (1983) and Chao and McBroom (1985) have examined fitness effects associated with the mutator activities of Tn*10*.] The Tn*10 tet* operon encodes two proteins: a repressor, TetR, and the resistance function, TetA (Nguyen et al. 1983; Postle et al. 1984). The mechanism of resistance involves an active efflux of tetracycline by TetA (McMurry et al. 1980). TetR inhibits transcription of both *tetR* and *tetA* by binding to two operator sites that overlap the *tetR* and *tetA* promoters. Tetracycline induces transcription of *tetA* and *tetR* by binding to TetR, thereby reducing that repressor's affinity for the operators (Hillen et al. 1984).

Anhydrotetracycline is a tetracycline analogue that induces expression of the tetracycline-resistance operon (Moyed et al. 1983). Low concentrations of anhydrotetracycline inhibit the growth of cells carrying the *tet* operon on high-copy-number plasmids, but they have no discernible toxic effects on tetracycline-sensitive cells (Moyed et al. 1983). Moyed and Bertrand (1983) were unable to construct a high-copy-number plasmid that expressed tetracycline resistance constitutively, unless the plasmid had a much weaker *tetA* promoter. They interpreted these results as arising from some harmful pleiotropic effect associated with expression of the TetA resistance protein. This effect might be caused by the protein's efflux activity, or it may result from the protein's interference with the functioning of the cytoplasmic membrane (Moyed et al. 1983).

In this study, we used anhydrotetracycline to quantify the effect of induction of the resistance operon on fitness, where the net growth rate (i.e., growth minus death) of one strain relative to that of another is a measure of fitness in a specific environment. We also determined whether there were any effects on fitness that were associated with carriage or with residual expression of the repressed resistance operon. To that end, we compared the fitnesses of strains carrying three different plasmids. One has an inducible tetracycline-resistance operon, with the strong, wild-type *tet* promoters. The second plasmid is identical, except that the tetracycline-resistance operon is nonfunctional because of a deletion mutation in the *tet* promoter region. The third plasmid lacks the resistance operon altogether. We also measured the effects on fitness that were caused by hyperexpression of the repressor protein. An effect might occur, for example, if the repressor inappropriately binds to heterologous operator sites. To test

Table 1
Plasmids Used in Study

Designation	Description	Source
pBT107	Ap^r Km^r Tc^r; has strong *tetA* promoter and functional repressor (inducible)	Moyed et al. 1983
pBT1071S	Ap^r Km^r Tc^r; has weak *tetA* promoter and functional repressor (inducible)	Moyed and Bertrand 1983
pBT1071	Ap^r Km^r Tc^r; has weak *tetA* promoter and nonfunctional repressor (constitutive)	Moyed and Bertrand 1983
pBT7234	Ap^r Km^r Tc^s; has nonfunctional *tetA* and *tetR* promoters	Moyed and Bertrand 1983
pACYC177	Ap^r Km^r; lacks *tet* operon	Chang and Cohen 1978
pBT402	Ap^s Km^r; expresses functional repressor constitutively from strong promoter	Postle et al. 1984
pBT4022	Ap^s Km^r; expresses nonfunctional repressor constitutively from strong promoter	Postle et al. 1984

this, we compared the fitnesses of strains that constitutively express high levels of either functional or nonfunctional repressor protein.

Material and Methods

Bacterial Strains and Plasmids

Escherichia coli K12 strain JA221 (F^- *lacY1 leuB6 thi-1 tonA2 supE44 ΔtrpE5 recA1 hsr;* Moyed et al. 1983) is unable to utilize lactose (Lac^-). We isolated a spontaneous lactose-utilizing (Lac^+) revertant of JA221. JA221 and the revertant, designated Lac^- and Lac^+ respectively, in this paper, can be distinguished on the basis of their colony color on tetrazolium lactose (TL) indicator agar. The lactose marker has no detectable effect on fitness in the medium used in this study.

Plasmids pBT107, pBT1071S, pBT1071, pBT7234, pACYC177, pBT402, and pBT4022 were transformed into one or both of these bacterial strains, when the basic procedures described by Maniatis et al. (1982) were followed. All seven plasmids confer resistance to kanamycin. All except pBT402 and pBT4022 also confer resistance to ampicillin. Plasmids pBT107, pBT1071S, and pBT1071 also confer resistance to tetracycline.

Table 1 summarizes key properties of these plasmids; details of their construction can be found in the references cited therein. None of the plasmids carry the IS*10* insertion sequences derived from Tn*10*. None of the various manipulations affect the plasmids' origin of replication. Previous work indicates that similar manipulations have little or no effect on plasmid copy number, which is 15–30 copies/cell (Daniels and Bertrand 1985). All of the plasmids are nonconjugative.

Media and Culture Conditions

Strains were grown and competed in LB medium (Miller 1972), which contains, per liter deionized water, 10 g tryptone, 5 g yeast extract, and 10 g sodium chloride. We used LB because this was the medium in which Bertrand and colleagues had characterized various properties of the host/plasmid associations used in this study. TL plates were used to determine the ratio of competing strains; TL plates contain, per liter deionized water, 10 g tryptone, 1 g yeast extract, 5 g sodium chloride, 16 g

agar, 10 g lactose, 50 mg triphenyltetrazolium chloride, and 0.05 ml antifoam B emulsion (Sigma). TL + Tc plates and TL + Kan plates are identical, except that they are supplemented with 10 mg tetracycline/liter and 25 mg kanamycin/liter, respectively.

Plates were incubated for 24 h at 37°C. Cultures were grown for 24 h in 10 ml medium in 50-ml Erlenmeyer flasks at 37°C and were aerated by shaking at 120 rpm.

General Experimental Procedures

Strains were stored in a glycerol-based medium at −80°C immediately after their isolation, in order to maintain isogenicity. Prior to a competition experiment, the two competing strains went through three preparation steps. First, both strains were inoculated into LB and were allowed to grow to saturation. Then, plasmid-free strains were plated on TL agar, while plasmid-bearing strains were plated on TL + Kan agar to ensure that they retained their plasmid-encoded resistance. Finally, to ensure that both strains were in a similar physiological condition at the start of a competition experiment, single colonies of each were reinoculated into LB and were grown to saturation.

Experimental Design and Analysis for Determining the Effect on Fitness of Anhydrotetracycline Induction

Lac^+ was competed against Lac^- or against Lac^-/pBT107 in LB supplemented with each of six per-liter concentrations of anhydrotetracycline: 0.32, 0.16, 0.08, 0.04, 0.02, and 0.01 mg. There were three sets of competition experiments for Lac^+ versus Lac^-, and there were six sets for Lac^+ versus Lac^-/pBT107. Each set consisted of one experiment at each of the six concentrations. The same cultures were used to initiate the experiments within a set, so that the data obtained from a single set were not statistically independent. However, independent cultures (i.e., inoculated from separate colonies) were used for each set of competition experiments, thereby ensuring the statistical independence of the sets. Our analysis of these experiments was conservative; each set was treated as a single observation that provided only one degree of freedom for hypothesis testing.

To start a set of experiments, two competitors were mixed in a 1:1 ratio and an initial sample was obtained. The mixture was then diluted 10^6 into the appropriate concentration of anhydrotetracycline. Final samples were taken after 24 h. Samples were plated on nonselective (TL) agar; when Lac^-/pBT107 was at low density on nonselective agar, however, the samples were also plated at higher concentrations on selective (TL + Tc) agar in order to accurately estimate its abundance.

The log of the ratio of Lac^- colonies to Lac^+ colonies was determined for both samples. Their respective difference was obtained and divided by the log of the dilution factor (10^6) to calculate the corresponding selection differential. The relative fitness of the two competitors was expressed by adding one to this selection differential. Thus calculated, a relative fitness of one indicates that two strains had identical net growth rates (i.e., growth minus death). A relative fitness of zero would be obtained if one competitor simply failed to replicate while the other increased by the dilution factor.

The relative fitnesses of the two pairs of competitors were then regressed against the concentration of anhydrotetracycline in order to determine the effects of induction. Means and standard deviations for the slopes and intercepts were calculated from the independent sets of experiments, as defined above.

Design and Analysis for All Other Competition Experiments

In all other experiments, relative fitnesses were estimated by competing two strains, one Lac^- and the other Lac^+, for 5 d in LB (supplemented as indicated). Competing strains were mixed in a 1:1 ratio, then were diluted 10^6 to initiate the experiment. After each day, the saturated old culture was sampled, then diluted 10^6 into fresh LB. Independent cultures (i.e., inoculated from separate colonies) were grown up to initiate each replicate of these competition experiments, thereby ensuring their statistical independence.

Samples were plated on TL (if one of the competitors was plasmid free) or on TL + Kan (if both strains were plasmid bearing). These samples were scored the following day by colony color. Putative plasmid-bearing colonies were checked on TL + Kan plates; plasmid-free segregants never became a large fraction of the total during the competition experiments. Any slight increase in the frequency of segregants weakens statistical comparisons between plasmid-bearing and plasmid-free strains, so that our conclusions are conservative. From each sample, we obtained the log of the ratio of the number of colonies of one strain to the number of colonies of its competitor. The net growth rate of one strain relative to that of the other was obtained by linear regression of these values against time (Dykhuizen and Hartl 1983). The resulting slope was divided by the log of the daily dilution factor (10^6) to yield the selective differential between the two strains. The selective differential was converted to a relative fitness by adding one. As noted above, a relative fitness of one indicates that two strains had identical net growth rates, whereas a relative fitness of zero would result if one strain failed to replicate.

To determine whether the fitness of one strain relative to another was significantly different from one, 95% confidence intervals were constructed using the t distribution with $N - 1$ degrees of freedom, where N = the number of independent replicates of the competition experiment.

Results

1. Effect of Anhydrotetracycline Induction on Fitness

There was a pronounced reduction in the fitness of Lac^-/pBT107 relative to that of Lac^+ as the concentration of anhydrotetracycline, [At], was increased (fig. 1). This indicates an increasing burden to the plasmid-bearing strain with an increasing level of induction of the tetracycline-resistance operon. Fitness of the plasmid-bearing strain declined by 2%–3% for each 0.01 mg/liter increment of concentration (table 2); replication of the plasmid-bearing strain was almost completely inhibited at 0.32 mg anhydrotetracycline/ml. Note the striking linearity of the relationship between anhydrotetracycline concentration and fitness; even at very low levels of inducer, the reduction in the fitness of the plasmid-bearing strain was clearly evident. Extrapolation of the intercept indicates that the fitness of Lac^-/pBT107 relative to that of the plasmid-free Lac^+ was less than one in the absence of anhydrotetracycline (table 2), suggesting that the plasmid-bearing strain has some fitness disadvantage even without induction of the tetracycline-resistance operon.

In contrast, there was no evidence of a reduction in the fitness of Lac^- relative to that of Lac^+ in the control, at any concentration of anhydrotetracycline (fig. 1). Neither the slope nor the intercept was different from the null hypotheses of zero and one, respectively (table 2).

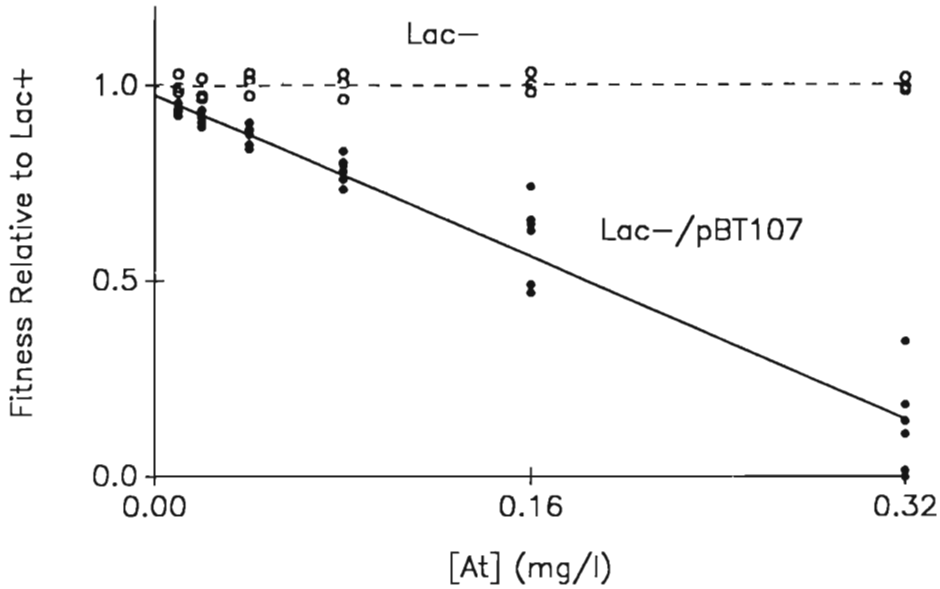

FIG. 1.—Effect of anhydrotetracycline concentration, [At], on the fitness of cells with (black dots) and without (open circles) the plasmid pBT107, with a control for the effect of the lactose marker. Regression lines are based on mean intercepts and slopes that are calculated from replicate sets of experiments, each set extending over the entire range of concentrations (see table 2).

2. Effect of Constitutive Expression of TetA on Fitness

The wild-type *tetA* promoter is quite strong (Daniels and Bertrand 1985). As shown above, full induction of the *tet* operon on pBT107 can completely inhibit net growth. In keeping with this result, Moyed and Bertrand (1983) found that cells carrying the *tet* operon on this multicopy plasmid could not tolerate constitutive expression of the TetA protein, unless there was also some reduction in the strength of the *tetA* promoter. Plasmid pBT1071 has two mutations: one, in the *tetA* promoter, that reduces

Table 2
Summary Statistics for Effect of Anhydrotetracycline Concentration on Cells With and Without the Plasmid pBT107, with a Control for the Effect of the Lactose Marker

	Mean	SD	95% CI
Control: Lac^- vs. Lac^+ ($N = 3$):			
Intercept	0.9980	0.0289	0.9262 to 1.0698
Slope	+0.0001	0.0003	−0.0006 to +0.0008
Experimental: Lac^-/pBT107 vs. Lac^+ ($N = 6$):			
Intercept	0.9751	0.0214	0.9526 to 0.9976
Slope	−0.0259	0.0043	−0.0304 to −0.0214

NOTE.—The intercept represents the fitness of the first strain relative to the second when there is no anhydrotetracycline present. The slope represents the change in fitness per 0.01 mg/liter increment in anhydrotetracycline concentration. The means of the intercept and the slope were obtained from N replicate sets of experiments, with each replicate set extending over the entire range of concentrations shown in fig. 1. SD = sample standard deviation; 95% CI = confidence interval based on the t distribution, with $N - 1$ degrees of freedom and $P = 0.05$.

Table 3
Effects on Fitness, in the Absence of Antibiotic, That Arise from Carriage and Expression of the Tn*10* Tetracycline-Resistance Operon on a Multicopy Plasmid

Type of Effect and Competing Strains	Relative Fitness[a]			
	Mean	*N*	SD	95% CI
Constitutive expression of TetA:				
Lac⁻/pBT1071S, Lac⁺/pBT1071S	1.0014	6	0.0017	0.9996 to 1.0032
Lac⁻/pBT1071, Lac⁺/pBT1071S ..	0.9695	9	0.0135	0.9591 to 0.9799
Lac⁻/pBT1071, Lac⁺/pBT1071S ..	1.0120[b]	3	0.0062	0.9966 to 1.0274
Residual expression and carriage of the repressed operon:				
Lac⁻/pBT107, Lac⁺/pBT107	1.0002	6	0.0058	0.9941 to 1.0063
Lac⁻/pBT7234, Lac⁺/pBT107	0.9966	6	0.0055	0.9908 to 1.0024
Lac⁻/pACYC177, Lac⁺/pBT107...	0.9978	6	0.0051	0.9925 to 1.0031
Remainder of the plasmid genome:				
Lac⁻, Lac⁺	0.9993	12	0.0035	0.9971 to 1.0015
Lac⁻/pACYC177, Lac⁺	0.9839	6	0.0040	0.9797 to 0.9881
Lac⁺/pACYC177, Lac⁻	0.9860	6	0.0024	0.9834 to 0.9886
Hyperexpression of TetR:				
Lac⁻/pBT402, Lac⁺	0.9829	6	0.0045	0.9782 to 0.9876
Lac⁻/pBT4022, Lac⁺	0.9797	6	0.0044	0.9751 to 0.9843

NOTE.—Abbreviations are as in table 2.

[a] Fitness of the first strain relative to that of the second, where a value of 1 indicates equal fitness. Means were obtained from *N* independent assays.

[b] In the presence of 2.5 mg anhydrotetraycline/liter.

the level of mRNA synthesis to ~0.5% of that of the wild type (Daniels and Bertrand 1985), and another, in *tetR,* that renders the repressor protein nonfunctional so that expression of the resistance protein, TetA, is constitutive (Moyed and Bertrand 1983). Plasmid pBT1071S has the weaker *tetA* promoter from pBT1071, but it has the functional repressor encoded by pBT107. Hence, we can compare directly the fitnesses of isogenic strains that express TetA either constitutively or inducibly.

The fitness of Lac⁻/pBT1071S relative to that of Lac⁺/pBT1071S was not significantly different from one (table 3, row 1). This is consistent with the hypothesis that the lactose marker is selectively neutral in LB. The fitness of Lac⁻/pBT1071 (constitutive) relative to that of Lac⁺/pBT1071S (inducible) was estimated to be 0.96–0.98 (table 3, row 2). This indicates that constitutive expression, in the absence of antibiotic selection, imposed a significant burden, despite pBT1071's weak *tetA* promoter.

As an additional control, we estimated the fitness of Lac⁻/pBT1071 (constitutive) relative to that of Lac⁺/pBT1071S (inducible) in medium supplemented with 2.5 mg anhydrotetracycline/liter. This treatment should fully induce the resistance function in pBT1071S and thereby was expected to cause its advantage relative to pBT1071 to disappear. Indeed, the relative fitness was found not to differ significantly from one (table 3, row 3). Together, these results indicate that plasmids pBT1071 (constitutive) and pBT1071S (inducible) have comparable effects on fitness when tetracycline resistance is expressed but that pBT1071S is less disadvantageous when tetracycline resistance is repressed.

3. Effect of Residual Expression of the Repressed Operon on Fitness

Plasmid pBT7234 carries a tetracycline-resistance operon that is nonfunctional owing to a deletion in the promoter region. The levels of mRNA transcribed from the *tetA* and *tetR* genes are <0.1% and <2%, respectively, of those of the wild type (Daniels and Bertrand 1985). If a strain that carries pBT7234 is significantly more fit than a strain that carries pBT107, then we can conclude that the difference in fitness is indicative of the burden associated with residual expression of the repressed operon in pBT107.

The fitness of Lac^-/pBT107 relative to that of Lac^+/pBT107 was not significantly different from one (table 3, row 4), which is again consistent with the presumed neutrality of the lactose marker. The fitness of Lac^-/pBT7234 (nonfunctional) relative to that of Lac^+/pBT107 (functional) was also not significantly different from one (table 3, row 5). Thus, we cannot detect any burden associated with residual expression of the repressed tetracycline-resistance operon. Indeed, we can infer from the 95% confidence interval that any reduction in fitness caused by residual expression was *at most* ~0.2%. It should be emphasized that this result was obtained with pBT107, which carries the strong wild-type *tetA* promoter and for which full induction completely inhibited bacterial growth (see subsection 1 above). Therefore, repression of the tetracycline-resistance function must be extremely tight.

4. Effect of Carriage of the Resistance Determinant on Fitness

Plasmid pBT107 was constructed by ligation of the Tn*10* *tet* operon into pACYC177. If a strain that carries pACYC177 is significantly more fit than a strain that carries pBT107, then we can conclude that the difference in fitness represents the burden of carriage of the additional DNA in the resistance determinant, plus the burden of any residual expression of the repressed tetracycline-resistance gene (which, as noted above, was insignificant).

We use the same marker control as reported above (table 3, row 4). The mean relative fitness of Lac^-/pACYC177 (without *tet*) relative to that of Lac^+/pBT107 (with *tet*) was not significantly different from one (table 3, row 6). From the 95% confidence interval, we can infer that the fitness disadvantage due to carriage of the resistance determinant (plus that due to any residual expression of the repressed operon) was *at most* ~0.3%.

5. Effect of the Remainder of the Plasmid Genome on Fitness

In subsection 1 above we noted that statistical extrapolation suggested some fitness reduction associated with carriage of pBT107, even without anhydrotetracycline induction of the tetracycline-resistance operon. However, as reported in subsections 3 and 4 above, there were no significant burdens associated with residual expression or with carriage of the repressed operon. Thus, we sought to determine whether there were any significant effects on fitness that could have resulted from carriage and expression of the remainder of the plasmid genome. As indicated above, plasmid pACYC177 lacks the Tn*10*-encoded tetracycline-resistance operon but is otherwise identical to pBT107.

The estimated fitness of Lac^- relative to that of Lac^+ was not significantly different from one, again consistent with the selective neutrality of the lactose marker (table 3, row 7). The fitness of Lac^-/pACYC177 relative to that of the plasmid-free Lac^+ strain was estimated to be 0.98–0.99 (table 3, row 8), which indicates a significant burden

associated with carriage of pACYC177. Similarly, the fitness of Lac$^+$/pACYC177 relative to that of the plasmid-free Lac$^-$ strain was estimated to be 0.98–0.99 (table 3, row 9), which also represents a significant burden. These analyses together demonstrate a small ($\sim$1.5%) but significant fitness reduction associated with carriage of the remainder of the plasmid genome (i.e., exclusive of the tetracycline-resistance operon).

6. Effect of Hyperexpression of TetR on Fitness

The repressor protein, TetR, represses its own synthesis, as well as that of the resistance protein, TetA. Induction of the tetracycline-resistance operon has been shown to increase the level of mRNA transcribed from *tetR* by about 50-fold (Daniels and Bertrand 1985). We sought to determine whether there would be any fitness cost associated with hyperexpression of the repressor protein. Such an effect might occur, for example, if excess repressor inappropriately binds to heterologous operators, thereby interfering with the proper regulation of other genes.

To test this possibility, we examined the fitness effects associated with two other plasmids, pBT402 and pBT4022. Both lack complete tetracycline-resistance operons. Instead, only the *tetR* gene was ligated into pACYC177; the repressor protein is expressed constitutively, and at a high level, from the promoter of the ampicillin-resistance gene (Postle et al. 1984). pBT402 and pBT4022 differ in that the latter contains a deletion in *tetR*, rendering the repressor protein nonfunctional (Postle et al. 1984). For this study, we confirmed the difference in repressor function between pBT402 and pBT4022 in the following manner: Carriage of pBT402 by a bacterial strain encoding Tn*10* chromosomally (i.e., Lac$^-$::Tn*10*) completely inhibited growth after a challenge with 50 mg tetracycline/liter, whereas carriage of pBT4022 did not impede the same strain's response to that challenge (fig. 2).

The fitness of Lac$^-$/pBT402 (functional) relative to that of Lac$^+$ was 0.98–0.99 (table 3, row 10). The fitness of Lac$^-$/pBT4022 (nonfunctional) relative to that of Lac$^+$ was $\sim$0.98 (table 3, row 11). There was no significant difference in fitness effects associated with carriage of these two plasmids; in fact, these fitnesses are quite similar to those presented in subsection 5 above for strains carrying pACYC177 (table 3, rows 8 and 9). Thus, we conclude that there is no detectable reduction in fitness associated with hyperexpression of the tetracycline-repressor protein.

Discussion

It is commonly assumed that possession of unnecessary functions reduces fitness (Diamond 1986; Lenski and Nguyen 1988). Many previous studies of microbial populations have documented reductions in fitness that are associated with plasmid carriage in the absence of selection for plasmid-encoded functions such as antibiotic resistance (Godwin and Slater 1979; Levin 1980; Zund and Lebek 1980; Helling et al. 1981; Noack et al. 1981; Cooper et al. 1987; Lenski and Bouma 1987). However, few of these studies have examined the contribution of particular gene functions to these reductions in fitness. One exception is the study by Lee and Edlin (1985), who demonstrated that a fitness disadvantage associated with carriage of plasmid pBR322 by *Escherichia coli* was due, in large part, to its tetracycline-resistance determinant. That particular resistance determinant lacks a repressor, and no effort was made to determine the burden, if any, associated with the repressed gene function. Our paper builds upon the work of Lee and Edlin (1985) and of Bertrand and colleagues (Moyed and Bertrand 1983; Moyed et al. 1983; Nguyen et al. 1983; Postle et al. 1984; Daniels and Bertrand

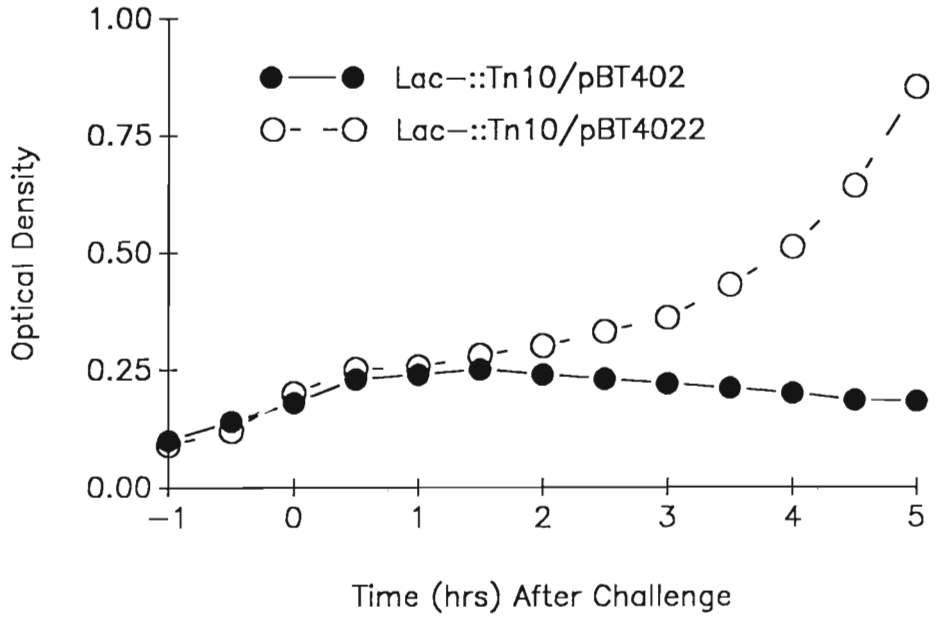

FIG. 2.—Assay of TetR repressor function in plasmids pBT402 (black dots) and pBT4022 (open circles). Bacterial strains carrying Tn*10* chromosomally were grown overnight in LB, then diluted into fresh medium. One hour later, they were challenged with 50 mg tetracycline/liter. The functional repressor encoded by pBT402 inhibited response to the challenge, whereas the nonfunctional repressor encoded by pBT4022 did not.

1985), who have studied the structure and regulation of the Tn*10*-encoded tetracycline-resistance operon.

In this study, we estimated relative fitnesses by comparing the net growth rates of competing strains; observed differences in fitness may have arisen from differences in lag phase, growth rate, or survival at saturation density. We observed, relative to plasmid-free cells, a strikingly linear relationship between increasing levels of the inducer anhydrotetracycline and the corresponding fitness of cells carrying pBT107 (fig. 1). Even very low levels of induction caused discernible reductions in fitness. At high concentrations of anhydrotetracycline, there was virtually no net growth of the plasmid-bearing cells, whereas plasmid-free cells continued to proliferate. Thus, the fitness of *E. coli* is extremely sensitive to induction of the wild-type *tet* operon on a multicopy plasmid. We also demonstrated that cells carrying pBT1071 (which has a very weak promoter and a nonfunctional repressor) were less fit than isogenic cells carrying pBT1071S (which also has a very weak promoter but possesses the functional repressor). The 2%–4% fitness disadvantage associated with constitutive expression occurs even though *tetA* and *tetR* mRNA comprise <0.1% of the total RNA in these cells (Daniels and Bertrand 1985). This implies that the costs of expression of the *tet* operon are not strictly energetic. The difference in fitness disappeared when the two strains competed in medium containing anhydrotetracycline, demonstrating that it was the constitutive expression of pBT1071 that caused its reduced fitness relative to that of the inducible pBT1071S. Our results thus provide direct evidence that there is a fitness reduction associated with even low-level expression of the tetracycline-resistance operon.

However, in the absence of induction or constitutive expression of the tetracycline-

resistance protein, possession of the tetracycline-resistance operon per se had no detectable effect on fitness. In fact, the *upper* limit of the 95% confidence interval for the fitness reduction associated with residual expression and with carriage of the repressed operon was only 0.3%, and this was for pBT107, which has the strong, wild-type promoter (table 3, row 6). We conclude that repression of the tetracycline-resistance function is extremely tight. We suspect that this is an adaptation to avoid the highly deleterious effects of the TetA resistance protein.

In natural isolates, Tn*10* is often present on low-copy-number plasmids (e.g., R100) or as a single copy in the chromosome. Thus, the large fitness reductions associated with induction of the wild-type Tn*10*-encoded tetracycline-resistance operon when present on the high-copy-number plasmid pBT107 are somewhat artificial. But, by the same token, the indiscernibly small effects on fitness that are associated with the repressed operon were also obtained on this high-copy-number plasmid—and hence are all the more remarkable.

We also sought to determine whether there was any fitness reduction associated with hyperexpression of the TetR repressor protein. Such an effect might occur, for example, if excess repressor inappropriately binds to heterologous operators, thereby interfering with the proper regulation of other genes. However, we could not detect any effect on fitness of high-level constitutive expression of TetR. We did, however, detect a modest (1%–2%) but significant fitness reduction associated with possession of the pACYC177 cloning vector, which does not carry any of the *tet* operon.

More generally, our results demonstrate that there can be a large burden imposed on a cell by constitutive expression of a gene function, such as tetracycline resistance, that has disruptive effects on cell physiology. However, the burden associated with a comparable gene function that is inducible but otherwise tightly repressed can be negligible. Thus, in the *absence* of selection for a particular gene function, possession of an inducible determinant can be highly economical.

In order to understand fully the selective pressures acting on alternative modes of gene regulation, one must also know the relative efficacy of inducible and constitutive gene functions in the *presence* of the corresponding selective agent. To this end, we will report in another paper the relative fitnesses of strains possessing constitutive and inducible tetracycline-resistance functions in environments that contain tetracycline some or all of the time.

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BARRY G. HALL

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