Applied and Environmental Microbiology, Apr. 2006, p. 3032–3035 Vol. 72, No. 4
0099-2240/06/$08.00+0 doi:10.1128/AEM.72.4.3032–3035.2006
Copyright © 2006, American Society for Microbiology. All Rights Reserved.

# Laboratory-Dependent Bacterial Ecology: a Cautionary Tale

K. J. O'Keefe,[1]* N. M. Morales,[1] H. Ernstberger,[2] G. Benoit,[2] and P. E. Turner[1]

*Department of Ecology and Evolutionary Biology, Yale University, 165 Prospect Street, New Haven, Connecticut 06520,[1] and School of Forestry and Environmental Studies, Yale University, 370 Prospect Street, New Haven, Connecticut 06511[2]*

Received 9 September 2005/Accepted 31 December 2005

**Although laboratory dependence is an acknowledged problem in microbiology, it is seldom intensively studied or discussed. We demonstrate that laboratory dependence is real and quantifiable even in the popular model *Escherichia coli*. Here laboratory effects alter the equilibrium composition of a simple community composed of two strains of *E. coli*. Our data rule out changes in the bacterial strains, chemical batches, and human handling but implicate differences in growth medium, especially the water component.**

Laboratory dependence, or variable performance of organisms when they are cultured at different locations, is an acknowledged phenomenon in microbiology and has the potential to cause major problems for microbial science (and for laboratory-based biology in general). However, laboratory dependence is rarely studied in detail, or even discussed. Underreporting may stem from the perception that the observed laboratory dependence is uninteresting (detracting from the impact of other results) or unbelievable (blamed on unidentified differences in experimental technique). It is crucial that microbiologists begin to understand which organisms are susceptible to laboratory dependence (perhaps all are), what factors in the laboratory environment commonly underlie this phenomenon, and what types of changes in microbial growth are to be expected. We begin to address these issues with a simple community composed of two genotypes of *Escherichia coli*. We hope that our study motivates closer investigation of laboratory dependence in other systems and promotes discussion of this important topic within the microbial ecology community.

The strains used in this study, REL4397 (Lac$^+$) and REL4398 (Lac$^-$), are previously described recombinant genotypes of *E. coli* B and *E. coli* K-12 that differ in lactose utilization (15, 18). They evolved as a polymorphism in a single population propagated by 24-h serial culture. Under the conditions in which they evolved, the two strains coexisted stably at an equilibrium ratio of ~1:1 (18). The strains were isolated and stored at −80°C in the laboratory of R. E. Lenski at Michigan State University (lab I) in East Lansing, MI. Samples of the strains were shipped frozen to the laboratory of P. E. Turner at Yale University (lab II) in New Haven, CT.

The coexistence of the Lac$^-$ and Lac$^+$ strains in lab I was attributed to (i) the higher maximum growth rate (growth in abundant glucose) of the Lac$^-$ strain and (ii) the ability of the Lac$^+$ strain to scavenge glucose at the end of the growth cycle and also to increase in cell number and relative fitness late in the growth cycle, long after glucose should have been exhausted from the medium (18). This late-cycle growth occurred only when the Lac$^+$ strain was grown in the presence of the Lac$^-$ strain, which suggested a possible "cross-feeding" relationship (8, 14).

We cultured the *E. coli* Lac$^+$ and Lac$^-$ strains in lab II, where we observed a shift in the composition of this simple bacterial community in favor of the Lac$^+$ strain. Below we describe laboratory-specific differences in this ecological relationship and identify deionized water composition as the likely responsible factor within the laboratory environment. Our results show that laboratory dependence is real and quantifiable in a model system. Moreover, we demonstrate that laboratory dependence can have emergent consequences at the level of the microbial community, as community members react differently to changes in the laboratory environment (an effect called genotype-by-environment interaction).

**Culture methods and fitness assays.** Strains were cultured at 37°C in 10 ml of DM25 (Davis minimal medium [3]) supplemented with thiamine hydrochloride at $2 \times 10^{-3}$ μg/ml and 25 μg/ml glucose), which yields $5 \times 10^7$ cells/ml at stationary phase. Labs I and II followed identical medium recipes and obtained chemical ingredients from the same vendor. Media were prepared according to standard practice, using deionized water from the local tap, in both laboratories. We determined the relative fitness (population growth) of the Lac$^+$ and Lac$^-$ strains by allowing them to compete in culture for 24 h. Strain densities were estimated before and after the 24-h competition by growing diluted samples at 37°C on tetrazolium-lactose agar indicator plates, which distinguished the two genotypes by colony color: white for the Lac$^+$ strain and red for the Lac$^-$ strain (18). Relative fitness ($W$) was then calculated as the ratio of the Malthusian parameters (9); a $W$ value of 1.0 indicates that the two genotypes perform equally well. For consistency, fitness is reported as the Lac$^+$ strain relative to the Lac$^-$ strain.

**Statistical methods.** Mixed linear models (SAS mixed procedure) were employed to test the effect of predictive factors (laboratory, initial frequency, and their interaction) on the relative fitness of the Lac$^+$ strain. Time was also included in the model for the experiment that tested for late-cycle advantage. Nonsignificant terms were dropped to form best-fit models. The Satterthwaite approximation was used to estimate denominator degrees of freedom (10).

**Shift in equilibrium coexistence.** In a multiday serial culture experiment in lab I, the Lac$^+$ and Lac$^-$ strains were mixed at

* Corresponding author. Present address: Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138. Phone: (617) 496-8387. Fax: (617) 495-5667. E-mail: kara.okeefe@yale.edu.

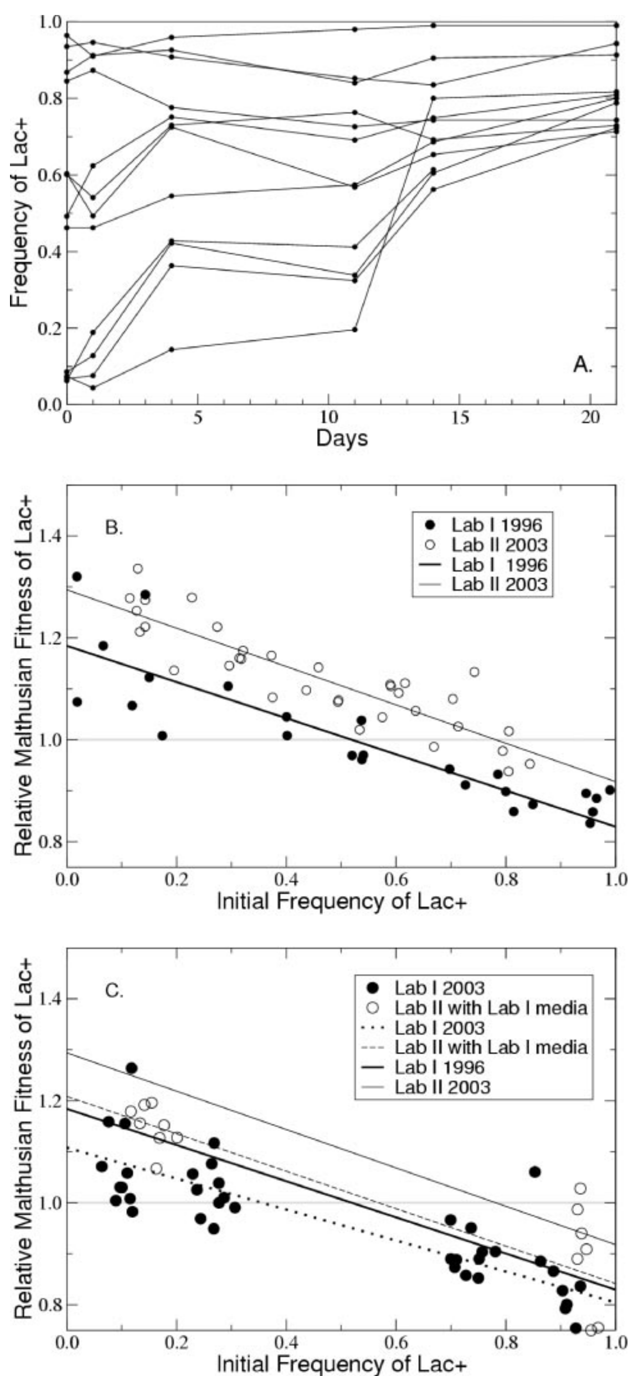


FIG. 1. (A) A 21-day serial culture experiment in lab II initiated cultures at three starting frequencies. The Lac$^+$ and Lac$^-$ strains approach a stable equilibrium where the Lac$^+$ strain dominates by at least 3:1 (compared with ratios of ~1:1 in lab I). (B) Malthusian fitness of *Escherichia coli* with the Lac$^+$ genotype relative to the Lac$^-$ genotype, estimated over 1-day growth cycles in lab I in 1996 and in lab II in 2003. Lines are least-squares regressions based on the full statistical model described in the text. Although both experiments show evidence for negatively frequency-dependent fitness (negative slope), the stable equilibrium frequency of the Lac$^+$ strain is predicted to be roughly 0.5 in lab I but about 0.8 in lab II. (C) Malthusian fitness of the Lac$^+$ relative to the Lac$^-$ strain in lab I in 2003 and in lab II in 2003 using media prepared in lab I. Neither experiment shows the behavior (high *y* intercept) normally observed in lab II. For comparison, regressions from panel B are replotted here.

a range of initial ratios, and every 24 h, 0.1 ml of stationary-phase (24-h-old) culture was transferred to fresh medium, resulting in 100-fold growth (or 6.6 generations) per transfer cycle. All populations converged on an equilibrium frequency of ~0.5 Lac$^+$ (18). We carried out a similar experiment in lab II with replicate cultures ($n = 4$), but in contrast to the behavior in lab I, the populations converged on a frequency of at least 0.75 Lac$^+$ (Fig. 1A).

**Frequency-dependent fitness.** In lab I, strains exhibited negatively frequency-dependent fitness (18). To test for frequency dependence in lab II, we conducted fitness assays at five different initial frequencies of the Lac$^+$ strain with sevenfold replication. The results indicated that there was no difference in the frequency dependence of fitness between the two laboratories (Fig. 1B) (df = 56; $F = 0.17$; $P = 0.68$). These assays also confirmed results of the multiday serial culture experiments by showing that the Lac$^+$ strain was more fit in lab II than in lab I, across all starting frequencies (Fig. 1B) (df = 57; $F = 46.75$; $P < 0.0001$).

The results of a 1-day fitness assay performed in lab I predicted that the Lac$^+$ strain should reach an equilibrium frequency of ~0.52 (Fig. 1B). Across several similar experiments in lab I (data not shown), predictions ranged between 0.4 and 0.6. In contrast, in lab II, results of four fitness assays predicted a higher frequency of the Lac$^+$ strain, between 0.78 and 1.0; data for the most conservative predicted equilibrium are shown in Fig. 1B.

**Eliminating confounding factors.** The altered equilibrium state in lab II might have resulted from a change in one or both strains, through physiological effects of freezer storage, unexpected mutation, or contamination. To examine this possibility, we shipped frozen lab II strains back to lab I, where they were assayed for relative fitness with replication ($n = 10$) at four initial frequencies in the laboratory where they were first isolated. The new lab I results resembled the original results from lab I (18) and were unlike the lab II data (Fig. 1C). Thus, we concluded that the unexpected behavior of the Lac$^+$ and Lac$^-$ strains in lab II was not due to contamination or another change in the strains. In the new lab I results, the fitness of the Lac$^+$ strain was actually lower than that in the original lab I study (df = 63; $F = 8.19$; $P = 0.0057$), and the predicted equilibrium frequency of the Lac$^+$ strain (~0.36) fell below the range previously observed in lab I (0.4 to 0.6); this small discrepancy is likely due to the inherent noisiness in 1-day fitness assays.

Fitness assays yielding similar results were performed by three of the authors (K.J.O., N.M.M., and P.E.T.) in lab II and by multiple people in lab I, including author P.E.T. Therefore, differences in experimental technique cannot explain the altered equilibrium state.

**Implicating the growth medium.** Differences in the growth medium provide the simplest explanation for altered coexistence. To investigate this possibility, DM25 prepared in lab I was shipped to lab II and used for fitness assays. Experiments were replicated ($n = 8$) at only low (0.1) and high (0.9) initial frequencies of the Lac$^+$ strain, since these extremes would be sufficient to test the hypothesis. The results (Fig. 1C) showed that use of the lab I medium in lab II restored the original (lab I) fitness relationship (18) (for the slope, df = 38, $F = 0.04$, $P = 0.8521$; for the intercept, df = 39, $F = 0.53$, $P = 0.4730$).

To test whether an aberrant batch of chemicals in lab II might have caused the equilibrium shift, we ran additional fitness assays in lab II using media prepared with newly purchased ingredients. Exchanging ingredients had no effect on the equilibrium (data not shown). These results suggested that the equilibrium shift was due to differences in deionized water, the only DM25 ingredient that was not exchanged.

Water may be an important component of laboratory effects in general, because its source is often local and because subtle changes in water chemistry (e.g., varying amounts of trace metals) can affect bacterial growth (12). Thus, one possible culprit was laboratory-specific differences in water composition, perhaps due to variation in the source water, deionization units (brand, quality, age), and/or pipe material used to transport the treated water to the laboratory tap (see further discussion below). We did not investigate the effects of other types of water because we were concerned with the microbial growth under the standard culture protocol in the two laboratories. It would be valuable to know whether laboratory effects might be eliminated if both laboratories prepared media using double-deionized water, ultrapurified (such as Milli-Q or Nanopure) water, or purchased bottled water. However, each of these types of water might have its own particular effect on microbial growth.

**Differences in deionized water across laboratories.** We investigated differences in the composition of water used in preparing the growth medium in lab I and lab II. Deionized water from both laboratories had immeasurably small amounts of phosphate (<0.5 μg/liter), nitrate (<0.02 mg/liter), chloride (<0.1 mg/liter), sulfate (<0.1 mg/liter), free chlorine (<0.03 mg/liter), and chloramines (<0.03 mg/liter) and traces of ammonia (~0.05 mg/liter). A qualitative analysis indicates that there was more Pb and Cu in the lab I water than in the lab II water, with a concentration of on the order of 1 μg/liter for both metals. Perhaps most importantly, the lab I water contained dissolved organic carbon (DOC) at 2.54 mg/liter compared to lab II's 0.22 mg/liter. DOC has been shown to influence bacterial growth by binding Fe (5), by functioning as an electron-shuttling agent (11), by serving as a carbon source (17), and through unspecified mechanisms (1, 16). DOC is also known to detoxify solutions by binding toxic metals and can interfere with nutrient availability by sequestering micronutrient metals such as Cu and Mn. These striking laboratory-specific differences in water composition are consistent with an important role of water in our laboratory observations. Future experimental work may identify the critical water component (whether DOC or another component) and a mechanism for the effect.

**Late-cycle and early-cycle advantage of the Lac$^+$ strain.** Previously in lab I, the Lac$^+$ strain was at a disadvantage in the first 12 h of growth and then increased in cell number between 12 and 24 h when in the presence of the Lac$^-$ strain (18). By contrast, we determined that in lab II, the Lac$^+$ strain was at a selective advantage both early and late in the growth cycle. We ran fitness assays at three initial frequencies of the Lac$^+$ strain, with replication ($n$ = 3), and sampled assay populations at both 12 and 24 h. The increase in fitness of the Lac$^+$ strain between 12 and 24 h (Fig. 2) was statistically significant (df = 40; $F$ = 43.34; $P$ < 0.0001) and greater in lab II than in lab I (df = 40; $F$ = 5.54; $P$ < 0.024), findings consistent with perhaps stronger cross-feeding interactions in lab II. In contrast with previous data from lab I (18), the Lac$^+$ strain also had an advantage over the Lac$^-$ strain early in the growth cycle: the 12-h fitness data generally exceeded 1.0 (by the Student one-sample $t$ test, df = 8, $t$ = 2.98, 0.005 < $P$ < 0.01). (Note that if the Lac$^+$ and Lac$^-$ strains were grown in 12-h serial culture, our data predict coexistence in lab I but extinction of the Lac$^-$ strain in lab II.)

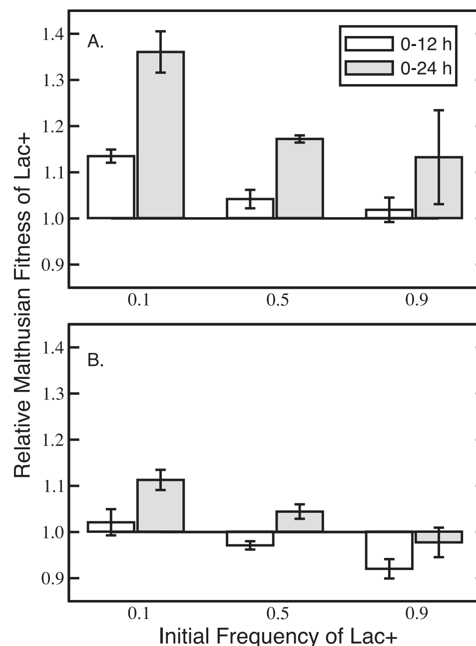


FIG. 2. Relative Malthusian fitness of the Lac$^+$ strain as measured at 12 and 24 h in lab II (A) and lab I (B) (data replotted from reference 18). In both laboratories, the Lac$^+$ strain gains fitness during 12 to 24 h. In lab II, in contrast to lab I, the Lac$^+$ strain is also at an advantage during 0 to 12 h. Error bars, standard errors of the means.

**Further anecdotal evidence.** For popular model systems such as the microbial workhorse *E. coli*, anecdotes of laboratory dependence abound. Since the inception of this study, informal discussions with colleagues have revealed numerous other likely examples of laboratory dependence. However, because the data either were not believed or were considered less important than other results, generally these cases were not confirmed experimentally. For example, in our own experience, the costs of carriage of an extrachromosomal plasmid were different for two laboratories growing *E. coli* in association with a plasmid (although we argued that this outcome may have resulted from freezer storage rather than being an actual laboratory effect [19]). In two other instances, the relative growth advantage of competing *E. coli* strains differed by as much as 10% between two laboratories (V. S. Cooper and F. Moore, personal communication). Fastidiousness about laboratory conditions is also not uncommon for microbes that are newly described and/or are particularly challenging to culture (2, 4, 6, 7, 13).

We acknowledge R. Woods for help with competition assays in lab I, R. Lenski for providing strains, N. Hajela for preparation of media

and shipment of material, S. K. Remold for statistical expertise and C. J. Marx for helpful comments.

National Science Foundation grant DEB-01-29089 partially supported K.J.O. and P.E.T.

## REFERENCES


1. **Arvola, L., and T. Tulonen.** 1998. Effects of allochthonous dissolved organic matter and inorganic nutrients on the growth of bacteria and algae from a highly humic lake. Environ. Int. **24:**509–520.
2. **Baird-Parker, A. C.** 1969. The use of Baird-Parker's medium for isolation and enumeration of *Staphylococcus aureus*, p. 1–6. *In* D. A. Sharpton and G. W. Gould (ed.), Isolation methods for microbiologists. Academic Press, London, United Kingdom.
3. **Carlton, B. C., and B. J. Brown.** 1981. Gene mutation, p. 222–242. *In* P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips (ed.), Manual of methods for general bacteriology. American Society for Microbiology, Washington, D.C.
4. **Castenholz, R. W.** 1988. Culturing methods for cyanobacteria. Methods Enzymol. **167:**68–92.
5. **Charest, M. H., C. J. Beauchamp, and H. Antoun.** 2005. Effects of the humic substances of de-inking paper sludge on the antagonism between two compost bacteria and *Pythium ultimum*. FEMS Microbiol. Ecol. **52:**219–227.
6. **Chenoweth, M. R., G. A. Somerville, D. C. Krause, K. L. O'Reilly, and F. C. Gherardini.** 2004. Growth characteristics of *Bartonella henselae* in a novel liquid medium: primary isolation, growth-phase-dependent phage induction, and metabolic studies. Appl. Environ. Microbiol. **70:**656–663.
7. **Childers, S. E., M. Vargas, and K. M. Noll.** 1992. Improved methods for cultivation of the extremely thermophilic bacterium *Thermotoga neapolitana*. Appl. Environ. Microbiol. **58:**3949–3953.
8. **Helling, R. B., C. N. Vargas, and J. Adams.** 1987. Evolution of *Escherichia coli* during growth in a constant environment. Genetics **116:**349–358.
9. **Lenski, R. E., M. R. Rose, S. C. Simpson, and S. C. Tadler.** 1991. Long-term experimental evolution in *Escherichia coli*. I. Adaptation and divergence during 2,000 generations. Am. Nat. **138:**1315–1341.
10. **Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger.** 1996. SAS system for mixed models. SAS Institute, Cary, N.C.
11. **Luu, Y. S., and J. A. Ramsay.** 2003. Microbial mechanisms of accessing insoluble Fe(III) as an energy source. World J. Microbiol. Biotechnol. **19:** 215–225.
12. **Prescott, L. M., J. P. Harley, and D. A. Klein.** 2001. Microbiology, 5th ed. McGraw Hill, New York, N.Y.
13. **Prufert-Bebout, L., H. Paerl, and C. Lassen.** 1993. Growth, nitrogen fixation, and spectral attenuation in cultivated *Trichodesmium* species. Appl. Environ. Microbiol. **59:**1367–1375.
14. **Rosenzweig, R. F., R. R. Sharp, D. S. Treves, and J. Adams.** 1994. Microbial evolution in a simple unstructured environment: genetic differentiation in *Escherichia coli*. Genetics **137:**903–917.
15. **Souza, V., P. E. Turner, and R. E. Lenski.** 1997. Long-term experimental evolution in *Escherichia coli*. V. Effects of recombination with immigrant genotypes on the rate of bacterial evolution. J. Evol. Biol. **10:**743–769.
16. **Stevenson, B. S., S. A. Eichorst, J. T. Wertz, T. M. Schmidt, and J. A. Breznak.** 2004. New strategies for cultivation and detection of previously uncultured microbes. Appl. Environ. Microbiol. **70:**4748–4755.
17. **Tulonen, T., P. Kankaala, L. Arvola, and A. Ojala.** 2000. Growth and production of bacterioplankton in a deep mesohumic boreal lake. Arch. Hydrobiol. **147:**311–325.
18. **Turner, P. E., V. Souza, and R. E. Lenski.** 1996. Tests of ecological mechanisms promoting the stable coexistence of two bacterial genotypes. Ecology **77:**2119–2129.
19. **Turner, P. E., V. S. Cooper, and R. E. Lenski.** 1998. Tradeoff between horizontal and vertical modes of transmission in bacterial plasmids. Evolution **52:**315–329.