Selection of Laboratory Wild-Type Phenotype from Natural Isolates of \textit{Escherichia coli} in Chemostats$^1$

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We have followed, in glucose-limited chemostats, the evolution of natural isolates of \textit{Escherichia coli} possessing maximal growth rates of 0.48–1.43 doublings/h. Under these conditions a rapid-growth phenotype similar to that of standard laboratory wild-type strains was selected so that after 280 generations all of the cultures were characterized by bacteria with maximum growth rates close to 1.33 doublings/h. The growth yields of the natural isolates, on the other hand, were quite uniform and improved only slightly during the selection; it seems that the natural isolates are nearly maximally efficient at utilizing glucose. Some of the kinetic characteristics of ribosomes prepared from natural isolates vary markedly and in proportion to the growth rates of the original strains. After growth in glucose-limited chemostats, the ribosomes of all of the cultures become kinetically indistinguishable from those of laboratory wild-type bacteria. These observations confirm the interpretation that bacteria grown under normal laboratory conditions have been selected for maximum growth rates which demand maximum translation efficiency. In contrast, these characteristics do not seem to be strongly selected in the natural isolates.

\textbf{Introduction}

Bacteria in rich media that contain a high-energy carbon source such as glucose or glycerol as well as amino acids, small peptides, and yeast extract are dependent on an optimal translation system to support their rapid growth rates (Ehrenberg and Kurland 1984; Kurland and Ehrenberg 1987; Emilsson and Kurland 1990). The dominant role of the translation system in determining the growth performance of cells in rich media is a consequence of the fact that the translation system is the major mass investment of such cells (Maaloe 1979). In contrast, in a poor medium that contains only salts and some low-energy carbon source such as succinate or acetate, the importance of the translation system is reduced in proportion to its smaller mass fraction. This is illustrated by bacteria with mutant defects in their ribosomes. They are more able to compensate for their less-efficient performance in poor media than in rich media where the demands for translational efficiency are more stringent (Mikkola and Kurland 1988). This means that cells growing under poor conditions may be under less strong selective pressure to maintain optimal translation systems than are cells growing in rich media.

In nature the growth conditions for \textit{Escherichia coli} are normally much poorer and more transient than those used in most laboratories (Hartl and Dykhuisen 1984; Koch 1985; Morita 1988). Therefore, it should not have been as surprising as it was to discover that naturally occurring \textit{E. coli} strains are often slow growers in rich media and that they produce ribosomes that tend to be less efficient than those produced by

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standard laboratory strains (Mikkola and Kurland 1991). For these reasons we have suggested that domesticated wild-type strains are fast-growing variants selected in the rich culture conditions routinely used by experimentalists (Mikkola and Kurland 1991).

To test this notion we take advantage of earlier observations showing that mutations that appear in continuous cultures under substrate limitation may increase maximum growth rates as well as the ability to grow under substrate limitation (Adams et al. 1985; Helling et al. 1987; Hall 1988; Miller et al. 1988). We asked whether natural isolates can be converted to the fast-growing “laboratory phenotype” by continuous culture in glucose-limited chemostats. We observe that the maximum growth rates of continuously cultured natural isolates of E. coli increase and approach those of standard laboratory wild-type strains after only 280 generations. In addition, the ribosomes produced by the chemostat-selected strains are very similar to those produced by standard laboratory strains.

**Material and Methods**

**Chemicals**

Phenylalanine, phosphoenolpyruvate (PEP), GTP, putrescine, spermidine, myokinase (E.C.2.7.4.3), and pyruvate kinase (E.C.2.7.3.40) were purchased from Sigma (St. Louis). Poly(uridylic) acid (poly(U)) was obtained from Pharmacia (Uppsala, Sweden). Radioactive amino acids, (14C)-Phe and (3H)-Leu were obtained from Amersham International (Bucks, U.K.).

**Bacterial Strains**

The bacterial strains used in this study included the ECOR collection of natural isolates of E. coli (Ochman and Selander 1984). We also studied the natural isolate of E. coli, Bd1488b (Söderlind et al. 1988), as well as a laboratory wild type, O17 (Olsson and Isaksson 1979), and a streptomycin-resistant O17-SmR derivative, rpsL282 (Bohman et al. 1984). Except for the ribosomes, all of the components used in the in vitro translation studies were prepared from E. coli MRE 600 (Public Health Laboratory Service).

**Biochemical Preparations**

In all cases, ribosomes were prepared according to a method described by Jelenc (1980) and were stored at -80°C. All the other components were prepared, and their concentrations were determined according to a method described by Ehrenberg et al. (1990, and the references therein).

**In Vitro Assays**

Polymix buffer (Jelenc and Kurland 1979; Jelenc 1980) was used for all in vitro assays. The Michaelis constant (K_M), the catalytic rate constant (k_cat), and the second-order rate constant (R) were measured in standard assays according to a method described by Ehrenberg et al. (1990). The in vitro translational missense error rates were estimated by measuring the incorporation of leucine (Leu) from Leu-tRNA^{Leu} and comparing it to the phenylalanine (Phe) incorporation from Phe-tRNA^{Phe} in the poly(U)-directed polypeptide synthesis system described by Ehrenberg et al. (1990).

**Media and Growth Conditions**

The minimal M9 medium (Miller 1972) was used for all the chemostat experiments. Glucose was added at a concentration of 0.5 g/liter, which supports a population...
density close to $5 \times 10^8$ cells/ml. All of the growth experiments were carried out at 37°C in chemostats with a working volume of ~50 ml. The dilution rate ($D$) was ~0.3/h. The bacteria were pregrown in batch cultures before being used in chemostats.

Growth Yields and Maximal Growth Rates

Growth yields and maximal growth rates were measured after every 15 generations during the selection experiments. Yields were measured by filtrating 20 ml of the batch culture through a millipore filter, drying the filter at 100°C, and measuring its dry weight.

Maximal growth rates were measured in minimal M9 medium supplemented by 2.0 g glucose/liter, by following the turbidity at 540 nm. The same results are obtained also with the glucose concentration 0.5 g/liter.

Results

Growth Rates and Yields

We have selected for these experiments a group of natural isolates on the basis of their divergent growth characteristics and ribosome phenotypes (Mikkola and Kurland 1991). When cultures of the natural isolates were studied, the chemostats were used to maintain the doubling times at close to 3 h by limiting the glucose concentrations (see Material and Methods). The maximal growth rates and growth yields of the bacterial cultures were assayed every 15 generations during the chemostat selections. Each of the strains has a characteristic array of markers that include antibiotic resistance, phage resistance, utilizable carbon sources, and characteristic plasmids (R. Mikkola, unpublished results). Therefore, it was easy to demonstrate that the cultures had not been overgrown by contaminants at the end of each experiment. A summary of the growth characteristics of the bacteria in the initial cultures and after 280 generations is presented in table 1.

The laboratory wild-type strain O17 has a minimum doubling time close to 48 min, while that of the natural isolates is 43–124 min, with a mean value of 71 min and a variance ($S^2$) of 560 min$^2$. After 280 generations all cultures produced bacteria with minimum doubling times close to 50 min; the mean value was 49 min, the variance was 8.5 min$^2$, and the extremes were 44 and 52 min. Evidently, growth in glucose minimal medium for the short times of these experiments was sufficient to

<table>
<thead>
<tr>
<th>BACTERIA</th>
<th>MAXIMAL GROWTH RATE ($\mu$)</th>
<th>GROWTH YIELD</th>
<th>BEFORE</th>
<th>AFTER</th>
<th>BEFORE</th>
<th>AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(doublings/h)</td>
<td>(g glucose/mol)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOR2</td>
<td>.896</td>
<td>76.1</td>
<td>1.176</td>
<td>81.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOR11</td>
<td>1.034</td>
<td>88.5</td>
<td>1.304</td>
<td>85.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOR46</td>
<td>.968</td>
<td>56.7</td>
<td>1.154</td>
<td>83.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOR57</td>
<td>.923</td>
<td>84.2</td>
<td>1.277</td>
<td>84.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOR63</td>
<td>.789</td>
<td>65.3</td>
<td>1.225</td>
<td>79.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ECOR68</td>
<td>1.428</td>
<td>81.1</td>
<td>1.363</td>
<td>83.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bd1488b</td>
<td>.484</td>
<td>64.3</td>
<td>1.152</td>
<td>76.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>O17</td>
<td>1.249</td>
<td>72.3</td>
<td>1.298</td>
<td>76.4</td>
<td></td>
<td></td>
</tr>
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</table>
select a more rapid growth phenotype for all cultures. The selected growth rate approaches that of the laboratory wild types, and the terminal growth rates are independent of the different initial growth rates.

Figure 1 describes the time course for the evolution of the growth rates of bacteria in chemostats that initially contained some of the natural isolates. It is evident from these plots that after 200 generations all of the chemostat cultures have begun to grow at approximately the same rates and that by 280 generations the changes in the growth rate are negligible. We have continued to drive some of the chemostats for as long as 700 generations, with no appreciable improvements in the growth rates (data not shown).

For all the natural isolates, the growth yield with glucose is initially similar to that of the laboratory strain 017; the mean value was 74 g/mol glucose, with a variance of 119.7 g²/mol², compared with 72 g/mol glucose for 017 (see table 1). After the 280-generation selection in the chemostat, the yields had improved only slightly; the average increase was 11%, to a mean value of 82 g/mol glucose, with a variance of 8.6 g²/mol². These data suggest that, in contrast to their often low maximum growth rates, the natural isolates are initially relatively efficient at utilizing glucose. Further-

![Graph of growth rates](https://example.com/graph.png)

**FIG. 1.** — $\mu_{\text{max}}$ as function of number of generations during selections in glucose-limited chemostats (i.e., M9 minimal medium supplemented by 0.05% glucose) for ECOR2 (○), ECOR11 (■), ECOR46 (□), ECOR57 (●), ECOR63 (○), ECOR68 (△) and Bd1488b (●). Growth rates were measured in batch cultures in M9 minimal medium supplemented by 0.2% glucose by following the turbidity at 540 nm.
more, the growth-rate improvement during chemostat selection is in general not commensurate with the improvement of the yield for the glucose substrate.

Ribosome Characteristics

Ribosomes were prepared from the bacteria at the beginning and after 280 generations in the chemostats. We have previously shown that the natural isolates differ from one another with respect to their kinetics of interaction with the elongation factor Tu (EF-Tu) but that they are indistinguishable in their interactions with elongation factor G (Mikkola and Kurland 1991). Therefore, we have focused on titrations with EF-Tu in assays for polypeptide synthesis performed at fixed ribosome concentrations (see Material and Methods). The kinetic parameters calculated from these titrations are summarized in table 2.

Three kinds of numbers can be obtained from these titrations (table 2). One is the maximum turnover rate of the ribosomes in excess EF-Tu, the ribosomal $k_{\text{cat}}$. Another is $R$, which is the second-order rate constant that describes the rate of polypeptide synthesis for a given concentration of EF-Tu-aminocacyl-tRNA-GTP ternary complex (referred to as “ternary complex,” for short) and of ribosomes ready to bind ternary complex. Finally, there is the $K_M$, which describes the ternary-complex concentration at which the rate of polypeptide formation per ribosome is one-half the maximum rate. $R$ is given by the ratio $k_{\text{cat}} / K_M$.

The least variable kinetic parameter for the ribosomes from the natural isolates is the $k_{\text{cat}}$, which, with a mean value of 4.5/s ($S^2 = 0.65/s^2$), is close to half that of the laboratory strain 017 which has a $k_{\text{cat}}$ of 8.2/s. The $R$ factors of the ternary complex-ribosome interaction vary much more: they have values of 0.21–1.5 $\times$ 10$^7$ M/s, with a mean of 0.59 $\times$ 10$^7$ M/s ($S^2 = 0.161 \times 10^{14}$ M$^2$/s$^2$), compared with that of 1.34 $\times$ 10$^7$ M/s for the ribosomes of 017. Likewise, the $K_M$ for the interaction between ternary complex and ribosomes from the natural isolates is 3.5–26 $\times$ 10$^{-7}$ M, with a mean value of 10.5 $\times$ 10$^{-7}$ M ($S^2 = 44.3 \times 10^{-14}$ M$^2$), compared with 6.1 $\times$ 10$^{-7}$ M for the ribosomes of 017. After selection for 280 generations in the glucose-limited chemostats, the variations between the kinetic parameters of the ribosomes from the different cultures has been greatly reduced (table 2). For the ribosomes of the selected

### Table 2

Translational Kinetic Constants, Obtained from Titrations with Ternary Complex at Fixed Ribosome Concentrations

<table>
<thead>
<tr>
<th>BACTERIA STRAIN</th>
<th>BEFORE</th>
<th>AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_{\text{cat}}$</td>
<td>$R \times 10^{-7}$</td>
</tr>
<tr>
<td>ECOR2</td>
<td>3.5</td>
<td>.37</td>
</tr>
<tr>
<td>ECOR11</td>
<td>4.3</td>
<td>.55</td>
</tr>
<tr>
<td>ECOR46</td>
<td>3.5</td>
<td>.29</td>
</tr>
<tr>
<td>ECOR57</td>
<td>3.9</td>
<td>.57</td>
</tr>
<tr>
<td>ECOR63</td>
<td>5.4</td>
<td>.67</td>
</tr>
<tr>
<td>ECOR68</td>
<td>5.2</td>
<td>1.50</td>
</tr>
<tr>
<td>Bd1488b</td>
<td>5.4</td>
<td>.21</td>
</tr>
<tr>
<td>017</td>
<td>8.2</td>
<td>1.34</td>
</tr>
</tbody>
</table>

**Note.**—Ribosomes were prepared before and after chemostat selection for 280 generations. Kinetic measurements and calculations were done according to a method described by Ehrenberg et al. (1990).
cultures the mean value of the $k_{cat}$ is $7.9$ s ($S^2 = 0.064$ s$^2$); that for $R$ is $1.2 \times 10^7$ M/s ($S^2 = 0.020 \times 10^{14}$ M$^2$/s$^2$), and that for the $K_M$ is $6.5 \times 10^{-7}$ M ($S^2 = 0.59 \times 10^{-14}$ M$^2$). In other words, the interactions between ternary complex and ribosomes from the chemostat-selected bacteria are virtually indistinguishable from those of the ribosomes from the laboratory strain 017.

Finally, we have estimated the missense error rate of polypeptide synthesis by the different ribosomes in our in vitro system. Here we measure the incorporation of Leu into polypeptide and compare it with the Phe incorporation when Leu-tRNA$^{Leu}_e$ and Phe-tRNA$^{Phe}_e$ are present in the poly(U)-primed translation system (see Material and Methods). The data are summarized in table 3. There we see that several of the isolates produce ribosomes that translate with missense rates significantly higher than that of the laboratory strain 017, which has a Leu missense error rate of $3.3 \times 10^{-4}$. This may be compared with the mean value of $8.3 \times 10^{-4}$ ($S^2 = 4.3 \times 10^{-7}$) for the ribosomes of the natural isolates. After 280 generations in the chemostats, the different bacteria produce ribosomes that have a relatively uniform missense rate, which in all cases is close to the mean value of $3.8 \times 10^{-4}$ ($S^2 = 1.6 \times 10^{-9}$). In summary, the missense frequencies of ribosomes from each of the chemostat-selected bacteria are very similar to that of laboratory strain 017.

**Table 3**

<table>
<thead>
<tr>
<th>BACTERIA STRAIN</th>
<th>ERROR $\times 10^4$ Before</th>
<th>AFTER</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECOR2</td>
<td>2.21</td>
<td>3.42</td>
</tr>
<tr>
<td>ECOR11</td>
<td>3.40</td>
<td>3.45</td>
</tr>
<tr>
<td>ECOR46</td>
<td>4.78</td>
<td>3.23</td>
</tr>
<tr>
<td>ECOR57</td>
<td>5.22</td>
<td>4.21</td>
</tr>
<tr>
<td>ECOR63</td>
<td>10.30</td>
<td>4.26</td>
</tr>
<tr>
<td>ECOR68</td>
<td>22.80</td>
<td>3.94</td>
</tr>
<tr>
<td>Bd1488b</td>
<td>9.50</td>
<td>4.12</td>
</tr>
<tr>
<td>017</td>
<td>3.30</td>
<td>3.42</td>
</tr>
</tbody>
</table>

### Table 3

Missense Errors Measured In Vitro, as Incorporation of Leu from tRNA$^{Leu}_e$ instead of Phe from tRNA$^{Phe}_e$ in Poly(U)-directed Translation

**Discussion**

Initially we intended to characterize the ribosomes of the natural isolates by making measurements on the growing bacteria, as we have done elsewhere (Andersson and Kurland 1983; Bohman et al. 1984; Ruusala et al. 1984; Ruusala and Kurland 1984; Andersson et al. 1986). Several factors have contributed to our decision to carry out the ribosomal characterizations in vitro. First, in order to introduce the necessary plasmids and genetic markers to carry out such measurements in vivo, genetic manipulations and growth in laboratory media would have been required. In addition, it turned out to be unexpectedly difficult to do genetic manipulations with these strains, which means that strain construction with natural isolates would be complicated. Since extensive laboratory treatment of the bacteria would put in question...
their status as natural isolates, we chose to avoid this approach. The alternative in vitro approach was recommended by our previously published studies of known ribosomal mutants (Andersson and Kurland 1983; Bohman et al. 1984; Ruusala et al. 1984; Ruusala and Kurland 1984; Andersson et al. 1986). These studies show that our in vitro system faithfully reproduces the in vivo phenotype of all the mutants that we have studied. In particular, the kinetic characteristics of mutant ribosomes measured in vivo are quantitatively correlated with their in vitro kinetics in our system (Andersson et al. 1986).

Our observations suggest that both the maximum growth rates and the efficiency of the ternary complex–ribosome interaction are tightly coupled characteristics of the bacteria. Our previous results suggest that in natural isolates these characteristics are allowed to drift from their maximum values (Mikkola and Kurland 1991). In contrast, the present results suggest that by continuously providing the bacteria with a supply of nutrient it is possible to select fast-growing variants with characteristically efficient ribosomes. We take this as additional support for the view that the most rapid bacterial growth demands maximally efficient ribosomes (Ehrenberg and Kurland 1984).

The characteristics of the natural isolates before the chemostat selection are strikingly different from those seen after the chemostat selection. Initially they show a broad distribution of growth rates as well as of the performance characteristics of their ribosomes. After the selection, there is a remarkable coherence of these characteristics for the chemostat-selected variants as well as for laboratory wild-type strains. These patterns are consistent with two conclusions. The first conclusion is that the natural isolates have phenotypes for which maximized growth rates and ribosome performance may be of little relevance. Indeed, in some natural environments there might be selection against the fast-growing phenotype. This conjecture is supported by the observation that slow-growing natural isolates often survive better under conditions of starvation for carbon source than do fast-growing natural isolates or laboratory wild-type strains (authors' unpublished data). The other conclusion is that chemostat-selected variants as well as standard laboratory wild-type strains are under strong selective pressure to maximize both growth rate and ribosome performance in translation.

It is evident that faster-growing mutants either present in the original cultures or arising early in the chemostat selections could explain the rates at which the growth phenotypes of the natural isolates change during the growth-selection experiments. Initially the spontaneous-point-mutation rate is $10^{-9}$/bp/generation (Fersht and Knill-Jones 1983), and a 50-ml chemostat culture with a population density close to $5 \times 10^8$/ml would generate $\sim 25$ mutations/bp/generation. If we assume that under our selection conditions (0.3 generations/h) a mutant arises with a growth rate that is 0.1 doublings/h greater than that of the rest of the bacteria, then we can estimate how long it will take for this fast-growing variant to make up 99% of the culture containing $2.5 \times 10^{10}$ bacteria.

We may use the Monod equation to roughly estimate these times (Dykhuizen and Hartl 1983). The basic relationship is $(\mu_2 - \mu_1) = (\ln R_2/R_1)/(t_2 - t_1)$. Here $\mu_2$ and $\mu_1$ are the growth rates of the two variants, and $R_1$ and $R_2$ are their ratios at times $t_1$ and $t_2$. We find that roughly 285 h, or 86 generation times, would be sufficient to convert the initial cultures to the terminal ones after the appearance of one mutation with the assumed growth-rate advantage. This is obviously consistent with the behavior of some of the cultures described in figure 1. For others a more complex pattern is observed. For example, we observe stepwise changes of the growth rates for the culture
with the slowest initial growth rate; these changes suggest that as many as three mutations in succession have taken over this culture (see fig. 1). In summary, the behavior of the chemostat cultures suggests that only one to three mutations may be sufficient to account for the conversion of natural isolates to the laboratory phenotype.

Indeed, the ease of these selections is reminiscent of the ease of selecting (a) mutants with altered responses to streptomycin and (b) nonallelic mutations that suppress the antibiotic phenotypes. These mutations, which tend to arise primarily in the genes for ribosomal proteins S4, S5, and S12, modulate both the ribosomal interaction with ternary complex and the maximum growth rates of the bacteria (reviewed in Kurland and Ehrenberg 1987). Nevertheless, they have one idiosyncrasy that distinguishes them from those mutations responsible for the chemostat-selected mutants; this idiosyncrasy is that the chemostat selects variants that have more efficient ribosome kinetics and lower missense frequencies. In contrast, the conventional mutants of S4, S5, and S12 tend to have reciprocally related ribosome efficiency and accuracy of tRNA selection. This difference might reflect the novelty of the mutations that are selected in the chemostat. Alternatively, this difference may arise from a multiplicity of chemostat-selected mutations that may be able to produce more complex mechanistic changes in the ribosomes than can the single mutations that are responsible for the conventional antibiotic phenotypes. This issue can be decided once the mutations responsible for the chemostat-selected phenotypes have been identified.

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