of the American Society for Virology, Lexington, KY, 20 to 24 July 2002.
32. Abbreviations for amino acids: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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35. We thank all the staff at the BCCA Genome Sciences Centre for helping to facilitate the rapid sequencing of the SARS-CoV genome; R. Tellier (Hospital for Sick Children) for information on primer sequences that amplify a 216–base pair region of the Pol gene; I. Sadowski (Department of Biochemistry and Molecular Biology) and J. Hobbs and his staff (Nucleic Acid and Protein Services Unit) of the University of British Columbia for rapid synthesis of PCR primers; F. Ouellette (University of British Columbia Bioinformatics Centre) for advice and assistance; the staff at the National Center for Biotechnology Information for rapidly processing and making available our sequence data; and anonymous reviewers for their useful suggestions. The BCCA Genome Sciences Centre is supported by the British Columbia Cancer Foundation, Genome Canada/Genome British Columbia, Western Economic Diversification, Canada Foundation for Innovation, British Columbia Knowledge Development Fund, Canadian Institutes of Health Research, Michael Smith Foundation for Health Research, and Natural Sciences and Engineering Research Council of Canada. Clones derived from the SARS virus are available from the Genome Sciences Centre (www.bcgsc.bc.ca).

**Supporting Online Material**
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# Stress-Induced Mutagenesis in Bacteria

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**The evolutionary significance of stress-induced mutagenesis was evaluated by studying mutagenesis in aging colonies (MAC) of *Escherichia coli* natural isolates. A large fraction of isolates exhibited a strong MAC, and the high MAC variability reflected the diversity of selective pressures in ecological niches. MAC depends on starvation, oxygen, and RpoS and adenosine 3′,5′-monophosphate regulons; thus it may be a by-product of genetic strategies for improving survival under stress. MAC could also be selected through beneficial mutations that it generates, as shown by computer modeling and the patterns of stress-inducible and constitutive mutagenesis. We suggest that irrespective of the causes of their emergence, stress-induced mutations participate in adaptive evolution.**

Bacteria are champions of evolutionary success; they grow in practically all ecological niches. Evolutionary success depends on phenotypic selection, which in turn depends on available genetic variability. The genetic variability is produced primarily by mutagenesis and secondarily by recombination, which shuffles preexisting mutations. Molecular mechanisms controlling mutation rates are themselves indirectly subject to natural selection through genetic modifications they produce (second-order selection) (*1*, *2*). The linkage between selected mutations and the alleles responsible for their generation is particularly high in bacteria because their gene-transfer and recombination rates are generally low. Consequently, when adaptation is limited by the supply of mutations, selection was shown to favor strains having constitutively increased mutation rates. Such strains display high mutation rates owing to the loss of genetic fidelity functions, e.g., mutational inactivation of the mismatch repair system results in a $10^2$- to $10^3$-fold increase in mutagenesis (*3*). The selection of constitutive mutators and their role in adaptive evolution of bacteria has been supported by in vivo and in vitro experimental evolution, computer modeling, molecular evolution, and studies of natural isolates (*1*, *2*).

Mutation rates in bacteria can also be increased by stress-induced reversible activation of some gene functions, which results in a transient mutator phenotype, the SOS response being a paradigm of such a process (*4*). However, the evolutionary significance of stress-inducible mutagenesis in bacterial evolution remains a subject of intense debate (*5, 6*). While some argue that it is a consequence of a genetically programmed evolutionary strategy which, by increasing mutagenesis, increases the probability of generation of adaptive variants, others argue that mutations arise in stressed bacteria only as an accidental consequence of accumulation and/or processing of DNA lesions. However, these hypotheses are based on results obtained with laboratory strains. It is difficult to assess the evolutionary significance of any phenomenon without knowing its frequency and ecological distribution in natural populations, as well as their physiological and genetic determinants. With this premise, we have studied stress-induced mutagenesis phenotypes among 787 worldwide natural isolates of *Escherichia coli* from diverse ecological niches: commensal and pathogenic isolates from a variety of hosts and isolates from air, water, and sediments (*7*). To mimic stress conditions commonly encountered by bacteria in natural environments, we used progressive starvation following an exponential growth phase occurring in colonies. We chose colonies, instead of liquid cultures, because the primary natural *E. coli* habitat is the gut of warm-blooded animals, where it can be found in tightly packed communities. In secondary environments, like soil and water, bacterial cells also tend to aggregate and form (micro)colonies and biofilms.

**Diversity of constitutive and colony-aging induced mutation rates among natural isolates of *E. coli*.** To estimate mutagenesis in aging colonies (MAC) of natural isolates of *E. coli*, we measured the frequency of mutations conferring resistance to rifampicin ($Rif^R$) in 1-day- (D1) and 7-day- (D7) old colonies (*7*). For all strains, the median values of the frequency of $Rif^R$ mutations were $5.8 \times 10^{-9}$ on D1 and $4.03 \times 10^{-8}$ on D7 (Fig. 1, A and B). Thus, the frequency of mutations increased on average sevenfold between D1 and D7 [(Fig. 1C) Mann-Whitney: $P < 0.0001$], while the median number of colony-forming units (CFU) increased 1.2-fold. The *E. coli* K12 MG1655 laboratory strain showed a 5.5-fold increase in frequency of $Rif^R$ mutagenesis and a 1.7-fold increase in CFU. The increase in CFU from D1 to D7 was not correlated with the increase in the D7/D1 ratio of mutation frequency.

Strains having D1 mutation frequencies >10-fold or >100-fold higher than the median D1 mutation frequency of all the strains represented 3.3 and 1.4% of isolates, respectively, which corresponds to previous reports on the frequency of constitutive mutators in natural *E. coli* populations (*8–10*). The D7/D1 mutation frequency ratio showed that 40% of strains had more than a 10-fold, and 13% more than a

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100-fold, increase in mutagenesis over 7 days (Fig. 1C). Interestingly, constitutive mutagenesis and MAC showed a negative correlation [Fig. 1D (Spearman's correlation −0.347, $P < 0.0001$)]. Hence, strains with a lower D1 mutagenesis show significantly higher MAC than those with a higher D1 mutagenesis and vice versa (Fig. 1E). This was true even when strong constitutive mutators (>10-fold the median value of D1 mutagenesis for all strains) were excluded from the analysis (*11*).

Because resistance to rifampicin is conferred by mutations in only one gene, *rpoB*, we have also measured mutagenesis at several other genomic targets by using *lacI* papillation assay and by scoring mutations conferring resistance to other antibiotics in a subset of studied strains [see (*7*) and supporting online text]. The results showed that mutagenesis increased genome-wide, in a large fraction of natural isolates, as a consequence of the stresses they encounter in aging colonies.

**Bacterial phylogeny, ecological diversity, and variability of MAC phenotypes.** The response of bacteria to stress in aging colonies may depend on the frequency of such stresses encountered in the course of their evolutionary histories. Because no significant correlation was observed between MAC and phylogenetic groups of the 341 strains with established phylogenies (*11*), and because phylogeny reflects long-term evolutionary histories, we concluded that MAC variability reflects recent events. Strains belonging to the same phylogenetic group are found often in different ecological niches and, thus, face different selective pressures. Furthermore, strains belonging to different phylogenetic groups can share the same ecological niches, for example, the human commensals used in our study (*12*).

To determine whether MAC variability reflects differences in the ecological niche, we measured the MAC for commensal strains isolated from different hosts with different diets. The results indicated that the host's nutrition might explain some of the variation of MAC (Fig. 2, A and B). For example, strains isolated from different classes of carnivores [mammals ($n = 47$), birds ($n = 7$), and reptiles ($n = 9$)] do not show any significant difference between their MACs. However, these strains are significantly different from most strains isolated from noncarnivorous hosts within the same host class. Furthermore, strains isolated from humans do not have significantly different MAC from isolates found in other omnivores.

We also compared MAC phenotypes of commensal strains isolated from feces of healthy volunteers and pathogenic isolates from patients with two main types of pathologies caused by *E. coli*, diarrhea and urinary tract infections (Fig. 2, C and D). The results show that both groups of pathogens from our collection have similar D7/D1 mutation ratios and these are significantly lower than those observed for commensal strains (Mann- Whitney: $P < 0.0001$). The pathogens not only showed significantly lower MAC, but also had significantly higher constitutive mutation rates than commensals (Mann-Whitney: $P = 0.026$). Hence, the causes and consequences of the MAC phenotype may be relevant for our understanding of infectious diseases.

The differences in recent evolutionary selective pressures experienced by bacteria in different habitats may be responsible for the variability of MAC phenotypes. Other parameters (e.g., migration, population size of the bacteria or of the host, and composition of gut microflora), which were not taken in account, may also play an important role in shaping MAC phenotypes.

**Physiological requirements for MAC.** Ten randomly chosen isolates with strong MAC phenotype were cultivated under different experimental conditions, and the mutagenesis generating $Rif^R$ phenotype was measured (Table 1). We found that only enrichment of 869 plates with a carbon source, e.g., galactose (or arabinose; not shown) significantly reduced MAC. The addition of amino acids, nitrogen, or phosphate sources had no effect. No significant increase in the D7/D1 mutagenesis ratio was observed when bacteria were kept in liquid 869 medium. Therefore, carbon-source starvation is necessary but not sufficient for the increase in MAC.

An important difference between structured (colony) and nonstructured (liquid culture) environments is changing gradients of various chemical and physical parameters. Bacterial colonies are nearly completely anaerobic during the first days of growth because of intense metabolic activity of the bacteria on the surface of the colony. When nutrients are exhausted, metabolic activity slows, and oxygen penetrates the colony (*13*). Under anaerobic conditions, the D7/D1 ratio of mutagenesis to $Rif^R$ was only 1.9 (Table 1), suggesting that oxidative metabolism is required for MAC. Other works suggest that

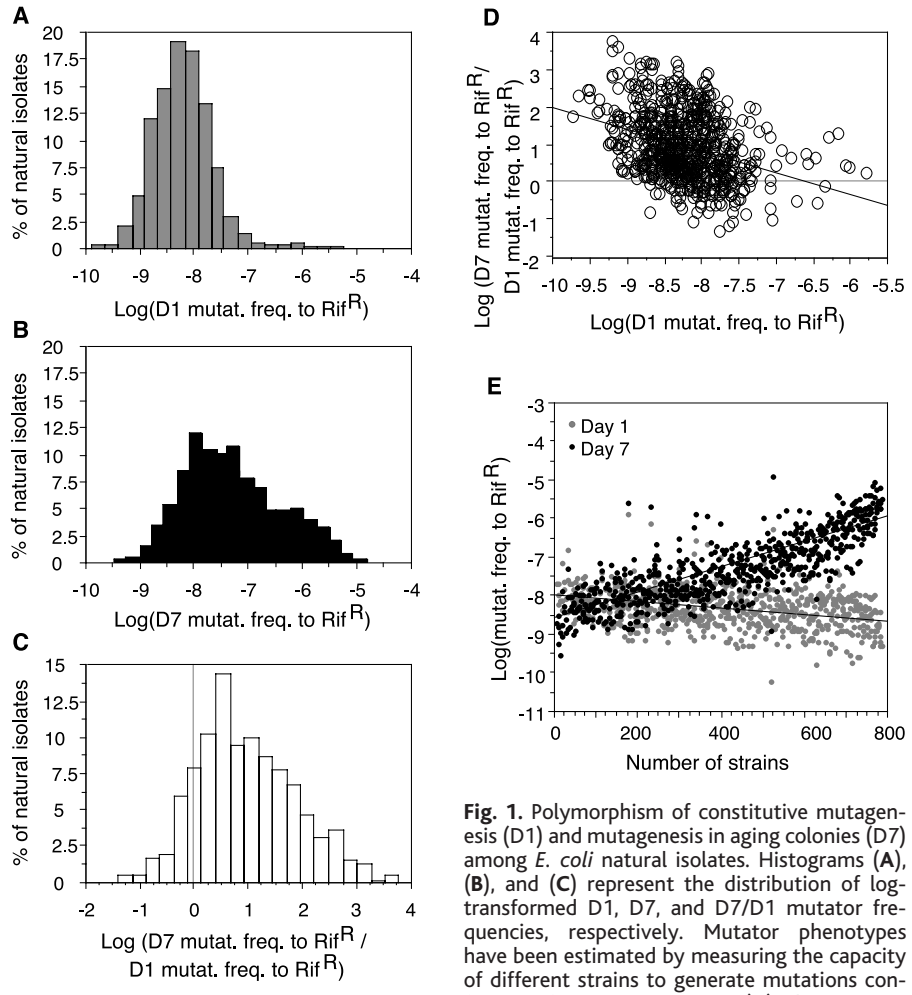


**Fig. 1.** Polymorphism of constitutive mutagenesis (D1) and mutagenesis in aging colonies (D7) among *E. coli* natural isolates. Histograms (**A**), (**B**), and (**C**) represent the distribution of log-transformed D1, D7, and D7/D1 mutator frequencies, respectively. Mutator phenotypes have been estimated by measuring the capacity of different strains to generate mutations conferring rifampicin resistance. (**D**) The negative correlation between log-transformed D7/D1 ratios and log-transformed D1 mutator phenotype (Spearman's correlation −0.347, $P < 0.0001$). Each open circle represents one strain. (**E**) Median values of D1 (filled gray circles) and of D7 (filled black circles) mutagenesis are represented for each strain. The strains were ordered according to ascending D7/D1 mutation ratios for each strain. This illustrates the origin of the negative correlation shown in (D), and reveals an apparent mutual exclusion of high constitutive and high MAC mutation phenotypes.

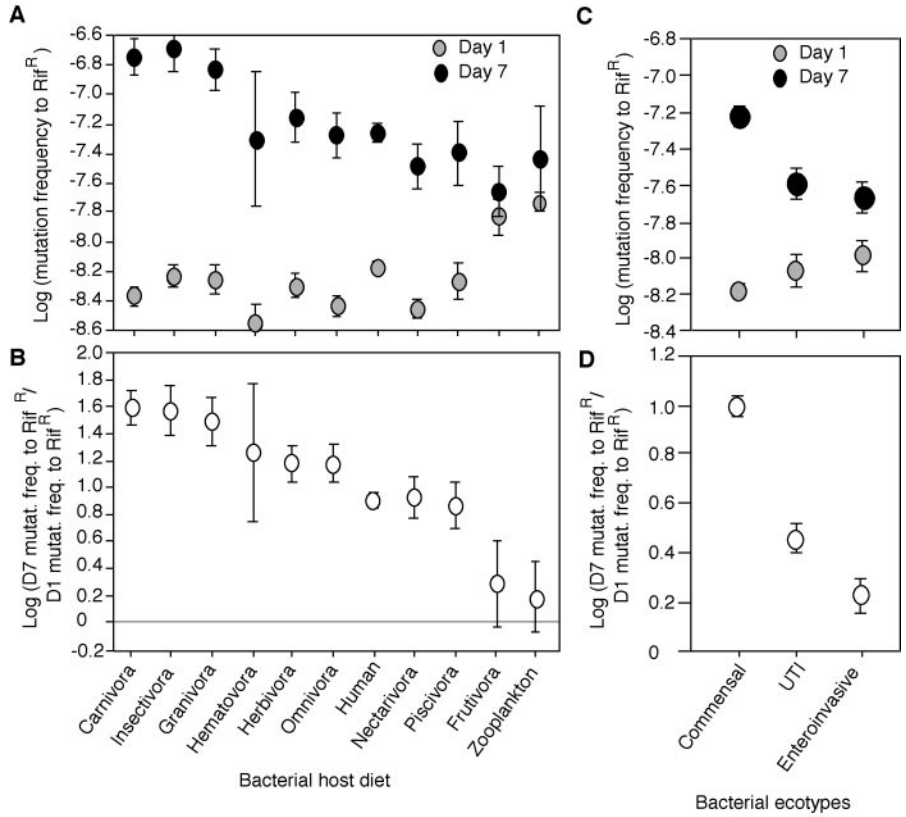


**Fig. 2.** Ecological diversity and the variability of mutator phenotypes among *E. coli* natural isolates. (**A** and **B**) Fecal isolates were classified according to their host diet; carnivora- ($n = 63$), insectivora- ($n = 39$), granivora- ($n = 33$), hematovora- ($n = 4$), herbivora- ($n = 38$), omnivora- ($n = 32$), human commensals– ($n = 217$), nectarivora-, ($n = 47$), piscivora- ($n = 21$), frutivora- ($n = 9$), and zooplankton-eating animals ($n = 3$). (**C** and **D**) The role of bacterial life-style. Human strains are classified according to their commensal or pathogenic origin: commensal ($n = 217$), enteroinvasive [*Shigella* ($n = 73$), and enteroinvasive *E. coli* ($n = 13$)], and urinary tract infections (UTI; $n = 91$) strains. (A) and (C) represent log-transformed constitutive mutagenesis (D1; filled gray circles) and mutagenesis in aging colonies (D7; filled black circles), whereas (B) and (D) show log-transformed D7/D1 mutagenesis ratio (open circles). Mutator phenotypes have been estimated by measuring the capacity of different strains to generate mutations conferring rifampicin resistance. Data points represent the pooled mean values (±SE) for a given group of isolates.

"adaptive" mutagenesis in starving *E. coli* K12 is suppressed under anaerobic conditions, or by addition of oxygen scavengers, but increased in a superoxide dismutase mutant (*14*). Together these observations and our data suggest that oxidative stress is a major contributor to MAC.

**Genetics of MAC: C4750, a case study.** To study the molecular mechanisms of the MAC phenomenon, we chose one natural isolate with a strong MAC phenotype as a model (Tables 2 and 3). Although one strain cannot represent the genetic diversity of the species, this strain responded just as the other nine strains did when it was exposed to different experimental conditions described in Table 1. Mutational spectra for MAC of C4750 were established in the (i) *rpoB* and (ii) *lacZ* genes, and its mutant derivatives were constructed to study the role of (iii) RpoS and adenosine 3′,5′-monophosphate (cAMP)/cAMP receptor protein (CRP) regulons, (iv) mismatch repair, and (v) SOS response in MAC.

(i) The sequence of the *rpoB* genes from C4750 $Rif^R$ mutants, isolated on D1 ($n = 40$) and D7 ($n = 38$), revealed that all possible base substitutions had occurred (*7, 11*). The majority of the D1 and D7 mutations (50 and 74%, respectively) were G:C→A:T transitions. The increase in the frequency of these mutations between D1 and D7 is significant ($\chi^2$ test: $P = 0.03$). In addition, we sequenced *rpoB* genes from 90 $Rif^R$ mutants belonging to 23 different natural isolates having a strong MAC and found that, similar to C4750, G:C→A:T transitions represent the majority of substitutions (57 and 82% on D1 and D7, respectively; $\chi^2$ test: $P = 0.009$). However, unlike C4750, those strains also showed a significant increase of G:C→T:A transversions (0 and 11% on D1 and D7, respectively; $\chi^2$: $P = 0.018$).

**Table 1.** The physiological requirements of mutagenesis in aging colonies. Mutagenesis to rifampicin resistance in aging colonies of 10 randomly chosen bacterial strains was measured under different experimental conditions. Pooled values of Day 1 and Day 7 mutation frequency obtained for each exprimental condition for 10 strains are presented. ↑ Mutation frequency significantly higher relative to mutation frequency on 869 plate. ↓ Mutation frequency significantly lower relative to mutation frequency on 869 plate.

| Growth conditions | Frequency of mutagenesis to $Rif^R$ on day 1 | | | Frequency of mutagenesis to $Rif^R$ on day 7 | | |
|---|---|---|---|---|---|---|
| | Mean (±SE) | Change of median value relative to 869 plate | *P* value of Mann-Whitney test* (relative to 869 plate) | Mean (±SE) | Change of median value relative to 869 plate | *P* value of Mann-Whitney test* (relative to 869 plate) |
| 869 plate | $3.0 \pm 1 \times 10^{-8}$ | 1 | – | $3.2 \pm 0.7 \times 10^{-6}$ | 1 | – |
| Anaerobic 869 plate | $2.2 \pm 0.8 \times 10^{-7}$ | 7.1 | <0.0001 ↑ | $4.3 \pm 2.2 \times 10^{-7}$ | 0.08 | <0.0001 ↓ |
| 869 plate 1% galactose | $3.6 \pm 1.3 \times 10^{-8}$ | 1.2 | 0.46 | $9.2 \pm 3.3 \times 10^{-8}$ | 0.02 | <0.0001 ↓ |
| 869 plate 1% casamino acids | $9.8 \pm 1.7 \times 10^{-9}$ | 0.3 | 0.42 | $4.4 \pm 1.4 \times 10^{-6}$ | 2.1 | 0.19 |
| 869 plate 100 mM $NH_4Cl$ | $1.7 \pm 1 \times 10^{-8}$ | 0.5 | 0.36 | $1.4 \pm 0.3 \times 10^{-6}$ | 0.6 | 0.52 |
| 869 plate 10 mM $K_2HPO_4$ | $1.6 \pm 0.2 \times 10^{-9}$ | 0.5 | 0.29 | $3.2 \pm 0.5 \times 10^{-6}$ | 1.8 | 0.13 |
| 869 liquid | $5.8 \pm 2.1 \times 10^{-8}$ | 3.7 | <0.0001 ↑ | $2.0 \pm 0.6 \times 10^{-7}$ | 0.03 | <0.0001 ↓ |

*Significance threshold: $P = 0.05$.

Because the G:C→A:T transitions represent the majority of mutations on D1 and D7 in the C4750 strain, we used competition experiments to test whether *rpoB* Rif$^R$ alleles carrying these mutations confer a growth advantage in old colonies, which would bias the observed mutation frequency. The results suggested that there is no growth advantage and that the increase in the frequency of G:C→A:T mutations in *rpoB* is caused by increased mutagenesis (see supporting online text).

(ii) We measured mutagenesis in *lacZ* alleles in colonies incubated 7 days on agarose plates (*7*). On agarose, C4750 showed a 3.8-fold increase in frequency of Rif$^R$ mutagenesis (Mann-Whitney: $P < 0001$) and a 3-fold decrease in CFU. Four reversions in the *lacZ* gene showed a significant increase over 7 days: G:C→A:T transitions (9.9-fold; Mann-Whitney: $P = 0.04$) and −1G (10-fold; Mann-Whitney: $P = 0.01$), −2CG (681-fold; Mann-Whitney: $P = 0.02$), and −1A frameshifts (97-fold; Mann-Whitney: $P = 0.02$).

The most frequent MAC substitution mutations in *rpoB* and *lacZ* genes are G:C→A:T transitions that are increased in many *E. coli* DNA-repair mutants [e.g., *ung*$^-$, *nth*$^-$, *nei*$^-$, *ada*$^-$ and *ogt*$^-$, *mutS*$^-$ (*3*)], so it was not possible to assign a specific mutagenic pathway for MAC. However, a coincidently high increase of frameshift mutations in the *lacZ* gene suggests the involvement of a mismatch-repair deficiency or insufficiency (*15*) [see (iv) below].

(iii) Because in C4750 strain, MAC is associated with carbon-source starvation and oxidative shock, we investigated different pathways contributing to bacterial survival under such stresses. The σ$^s$ subunit of RNA polymerase is the regulator of expression of genes involved in responses to diverse stresses including stationary phase, starvation, osmotic, acid, heat, and oxidative shocks (*16*). The inactivation of either of *rpoS* or *hfq*, which encode transcription factor σ$^s$ and the protein controlling *rpoS* mRNA translation, respectively (*16*), abolished the increase in MAC on D7 (Table 2). The inactivation of *rssB*, resulting in high constitutive levels of σ$^s$ (*16*), led to significantly increased MAC over the parental strain level, further confirming positive control of MAC by the RpoS regulon (Table 2).

We examined the role of the *cyaA* and *crp* genes (encoding adenylate cyclase and cAMP receptor protein, respectively) in MAC because MAC can be attenuated by adding more sugar to the plates and because carbon-source starvation results in activation of the cAMP/CRP regulatory network (*17*). It appears that MAC requires the activity of both genes (Table 2).

(iv) The spectra of mutations in the *rpoB* and *lacZ* genes implies the involvement of the

**Table 2.** Genetic analysis of mutagenesis in aging colonies of the *E. coli* C4750 natural isolate. For the change of number of viable cells in aging colonies of different C4750 derivatives see supporting online text. ↑ Mutant strain mutation frequency significantly higher relative to mutation frequency of parental strain. ↓ Mutant strain mutation frequency significantly lower relative to mutation frequency of parental strain.

| Genotype | Frequency of mutagenesis to Rif$^R$ on day 1 | | | Frequency of mutagenesis to Rif$^R$ on day 7 | | |
|---|---|---|---|---|---|---|
| | Mean (±SE) | Ratio of median values mutant/ parental strain | *P* value of Mann-Whitney test* Mutant versus parental strain | Mean (±SE) | Ratio of median values mutant/ parental strain | *P* value of Mann-Whitney test* Mutant versus parental strain |
| Parental strain | $3.5 \pm 0.9 \times 10^{-8}$ | 1 | – | $2.7 \pm 0.6 \times 10^{-6}$ | 1 | – |
| *rpoS* | $8.6 \pm 4 \times 10^{-9}$ | 0.2 | 0.028 ↓ | $3.2 \pm 2 \times 10^{-8}$ | 0.005 | <0.0001 ↓ |
| *hfq* | $1.2 \pm 0.6 \times 10^{-8}$ | 0.4 | 0.16 | $9.3 \pm 6 \times 10^{-8}$ | 0.004 | 0.0007 ↓ |
| *rssB* | $1.3 \pm 0.4 \times 10^{-8}$ | 0.7 | 0.24 | $6.8 \pm 1 \times 10^{-6}$ | 5.5 | 0.0012 ↑ |
| *cyaA* | $1.2 \pm 0.5 \times 10^{-8}$ | 0.4 | 0.10 | $3.4 \pm 2 \times 10^{-8}$ | 0.009 | <0.0001 ↓ |
| *crp* | $2.5 \pm 1 \times 10^{-8}$ | 0.7 | 0.71 | $7.7 \pm 1 \times 10^{-8}$ | 0.06 | 0.007 ↓ |
| *recA* | $1.6 \pm 0.4 \times 10^{-8}$ | 0.6 | 0.29 | $2.1 \pm 0.6 \times 10^{-7}$ | 0.05 | <0.0001 ↓ |
| *lexA1* | $2.4 \pm 0.6 \times 10^{-8}$ | 1.4 | 0.47 | $1.8 \pm 0.5 \times 10^{-6}$ | 0.9 | 0.82 |
| *polB* | $3.4 \pm 0.9 \times 10^{-8}$ | 1.3 | 0.28 | $6.0 \pm 2 \times 10^{-7}$ | 0.3 | 0.0007 ↓ |
| *uvrA* | $7.2 \pm 3 \times 10^{-8}$ | 2.9 | 0.12 | $2.9 \pm 1 \times 10^{-6}$ | 0.6 | 0.98 |
| *polA* | $1.5 \pm 0.8 \times 10^{-7}$ | 4.8 | 0.045 ↑ | $2.5 \pm 2 \times 10^{-5}$ | 4.1 | 0.12 |
| *recB* | $2.3 \pm 0.7 \times 10^{-8}$ | 0.9 | 0.99 | $3.4 \pm 1 \times 10^{-6}$ | 1.1 | 0.75 |
| *mutS* | $3.7 \pm 0.7 \times 10^{-6}$ | 195 | <0.0001 ↑ | $1.8 \pm 0.3 \times 10^{-6}$ | 1.25 | 0.96 |

*For this nonparametric test, all values obtained from at least three independent experiments (each with three independent cultures) for each genotype were used (significance threshold, $P = 0.05$).

**Table 3.** The role of MutL and MutS protein overproduction in mutagenesis in aging colonies of the *E. coli* C4750 natural isolate The strains designated pvector, p*mutL*$^+$, and p*mutS*$^+$ were C4750 derivaties carrying pACYC184, pMQ341, and pMQ339 plasmids, respectively. pvector was considered the parental strain and was used for comparison with the other two strains carrying plasmids. ↑ Mutant strain mutation frequency significantly higher relative to mutation frequency of parental strain. ↓ Mutant strain mutation frequency significantly lower relative to mutation frequency of parental strain.

| Genotype | Frequency of mutagenesis to Rif$^R$ on day 1 | | | Frequency of mutagenesis to Rif$^R$ on day 7 | | |
|---|---|---|---|---|---|---|
| | Mean (±SE) | Ratio of median values mutant/ parental strain | *P* value of Mann-Whitney test* Mutant versus parental strain | Mean (±SE) | Ratio of median values mutant/ parental strain | *P* value of Mann-Whitney test* Mutant versus parental strain |
| Pvector | $9.0 \pm 3 \times 10^{-9}$ | 1 | – | $1.3 \pm 0.3 \times 10^{-7}$ | 1 | – |
| P*mutL*$^+$ | $9.4 \pm 2 \times 10^{-9}$ | 1.39 | 0.84 | $8.5 \pm 0.8 \times 10^{-8}$ | 0.70 | 0.099 |
| P*mutS*$^+$ | $5.7 \pm 0.8 \times 10^{-9}$ | 0.85 | 0.81 | $2.2 \pm 0.5 \times 10^{-8}$ | 0.17 | 0.006 ↓ |

*For this nonparametric test, all values obtained from at least three independent experiments (each with three independent cultures) for each genotype were used (significance threshold, $P = 0.05$).

mismatch-repair system in MAC. The mismatch-repair system is a major contributor to DNA replication and recombination fidelity in growing *E. coli* K12 cells, but seems to be nonfunctional during stationary phase (*18*). During stationary phase the *mutS* gene transcript and the MutS [mismatch recognizing and binding (*4*)] protein decrease to undetectable levels through a RpoS- and Hfq-dependent mechanism (*19*). Because MAC in C4750 strain was also found to be *rpoS* and *hfq* dependent, we looked for the potential involvement of mismatch-repair down-regulation in MAC. The *mutS* mutant did not show any significant elevation of MAC (Table 2). By contrast, overproduction of the MutS protein significantly depressed MAC, whereas overproduction of the MutL protein had no effect (Table 3). Hence, down-regulation of mismatch repair seems to be responsible for the increase in MAC, and consequently, the majority of these mutations arise during DNA synthesis.

(v) It has been previously reported that *E. coli* K12 MAC and "adaptive" mutagenesis depend on the induction of the SOS system (*20*, *21*). The SOS regulon is composed of at least 40 genes (most of which encode DNA-repair functions) and is induced by a variety of stresses that damage DNA and/or block DNA replication (*22*). The RecA protein acts as a positive regulator, whereas LexA is the repressor of the SOS genes (*4*). Our data indicate that 95% of mutations that arise in aging colonies were RecA dependent and LexA independent (Table 2). We tested two other SOS genes, *uvrA* and *polB*, encoding the nucleotide excision and DNA polymerase II (Pol II) proteins, respectively. The inactivation of the *uvrA* gene had no significant effect, whereas 73% of mutations in aging colonies were *polB* dependent (Table 2). The error-prone activity of this polymerase has also been observed in other studies (*23*, *24*). Because Pol II has been shown to be involved in the response to oxidative damage (*25*), and because MAC is oxygen dependent (Table 1), it may be that this polymerase participates in the error-prone copying of oxidative lesions.

The inactivation of the *polA* gene (not a part of the SOS regulon), which encodes DNA polymerase I [involved in gap-filling steps during DNA replication and nucleotide excision repair (*4*)], did not modify the MAC phenotype (Table 2). The involvement in MAC of at least two SOS genes, *recA* and *polB*, suggests that either the basal levels of expression of these genes is sufficient for MAC or that some SOS genes may be regulated in a noncanonical (LexA-independent) manner in quiescent cells. SOS induction and "adaptive" mutagenesis in *E. coli* K12 quiescent cells has been shown to depend on the *recA* and *recB* genes (*26*, *27*), suggesting the involvement of double-strand breaks and recombination in that mutagenic pathway. However, *recB* gene function is not required for MAC of the C4750 strain (Table 2).

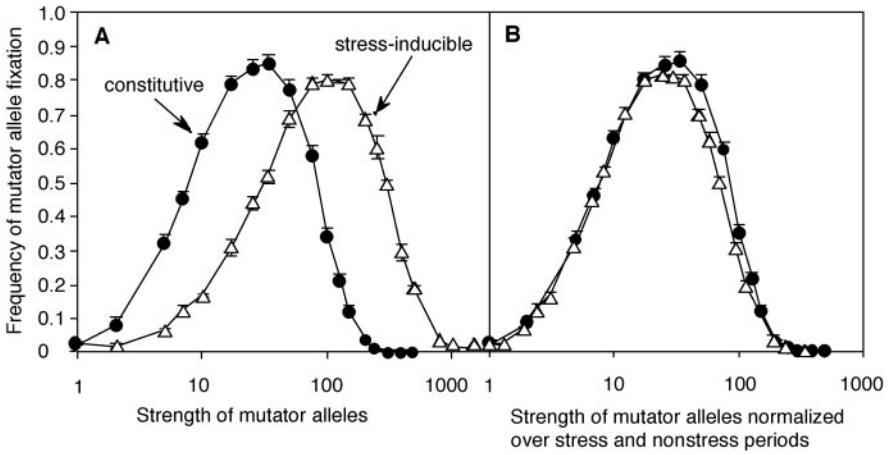


**Fig. 3.** Probability of fixation of mutator alleles as a function of their effect on mutation rate. The adaptation of a population of $10^9$ cells was simulated in environments in which stress phases occurred (on average) every 33 generations and lasted for (on average) 10 generations. In this environment, the overall fraction of time spent under stress (K) is 23%. (**A**) Constitutive mutator alleles (circles) had a permanent X-fold increase in mutation rate, whereas the stress-inducible mutators (triangles) had an X-fold increase in mutation rate only during stress phases. (**B**) The strength of the stress-inducible mutators has been normalized to reflect its mean effect on the generation of mutations over stress and nonstress periods [$X' = K \times X + (1 - K) \times 1$]. Each point is the fixation probability over 500 simulations. Error bars represent the standard error of the fixation frequency over 100 simulations.

**Simulation of the selection of stress-induced mutator alleles and their effects.** We used computer simulations previously developed to study the role of constitutive mutators in the adaptive evolution of bacteria (*7*, *28*). We assumed that stress-inducible mutator alleles cannot be selected for or against because of their pleiotropic effects. We did not take into account mutations that result in immediately improved survival to these stresses, but only those that have an impact on the longer-term adaptation of bacterial populations. The simulations showed that stress-induced mutator alleles can be selected almost as efficiently as constitutive mutators [(Fig. 3) and supporting online text], which is in contrast to the observed higher proportion of MAC mutators relative to constitutive mutators among natural isolates (for 10-fold mutators, 40 versus 3.3%, respectively). However, the wide distribution of MAC phenotypes among natural isolates (Fig. 1) and a variety of genetic mechanisms involved (*14*) suggest that the number of genes involved in the MAC phenotype is higher than the number of genes involved in the constitutive mutator phenotype. Consequently, the selection of MAC mutator genotypes in nature could be more efficient than the selection of constitutive ones under directional selection.

The selection of stress-inducible mutators in nature may be also due to pleiotropic effects of strongly selected functions, e.g., DNA repair or other survival-promoting systems. However, regardless of the nature of selection of stress-inducible alleles, mutations produced under stress could represent a large proportion of overall mutations and may have evolutionary consequences. Since the mean strength of MAC among natural isolates is about 10-fold, if phases of stress were to occur 25% of the time, the proportion of mutations produced under stress would be, on average, 46%. Because some of those mutations might be adaptive, stress-inducible mutagenesis would increase the speed of adaptation. Our simulations showed that a population with a 10-fold stress-inducible mutator phenotype will adapt up to 15% faster than a nonmutator population, whereas a population having a 100-fold stress-inducible mutator phenotype will adapt up to 38% faster.

Increase in adaptation rate due to stress-inducible mutagenesis may also limit the selection of constitutive mutator alleles. The fixation of an allele improving the rate of adaptation has been shown to decrease the selection for other alleles improving the rate of adaptation (*29*). Indeed, our simulations showed that the frequency of fixation of a constitutive mutator decreases as a function of increased stress-inducible mutagenesis (Fig. 4). The relative decrease in the fixation of a constitutive mutator allele was almost perfectly correlated with the relative improvement of the adaptation rate due to stress-inducible mutagenesis. This effect of MAC on adaptation rate could explain the negative correlation observed between constitutive mutagenesis and MAC (Fig. 1D).

**Concluding remarks.** Most natural isolates of *E. coli* exhibits increased mutation rates under stress encountered in aging colonies (Fig. 1). MAC is characteristic to each strain, varies greatly, and increases as a consequence of stress imposed by carbon-source starvation and oxidative shock (Table 1). Because this stress-inducible mutagenesis is genetically controlled (Table 2), and therefore subject to selective pressures, its high variability should reflect the diversity of selective pressures in different environments. Indeed, our simulations showed that the strength of

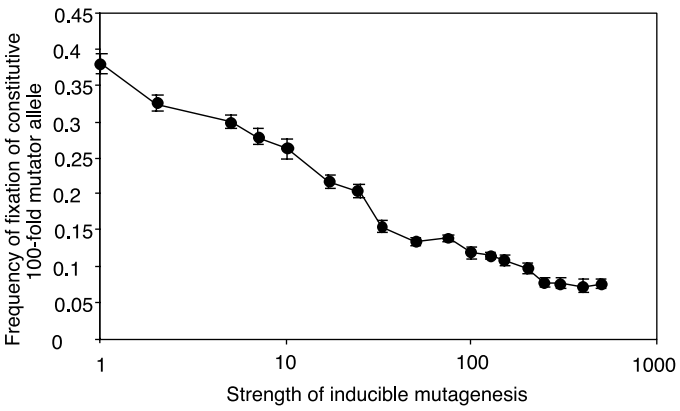


**Fig. 4.** Probability of fixation of a 100-fold constitutive mutator in populations with various strengths of stress-inducible mutagenesis. Populations evolved under the conditions described in Fig. 3. During stress periods the nonmutator population had an X-fold increased mutation rate (where X is the strength of stress-inducible mutagenesis), whereas the 100-fold constitutive mutator cells had the strongest increase in mutation rate, between 100- and X-fold. During nonstress periods only constitutive mutator individuals had a 100-fold increased mutation rate. Each point is the fixation probability over 1000 simulations; error bars represent the standard error of the fixation frequency over 100 simulations.

the selected stress-induced mutator alleles is positively correlated with the strength of selection and negatively with the frequency of such stresses (*11*). The fact that the ecological niche from which the strain was isolated appears to be the major determinant of mutator phenotype (Fig. 2), and the absence of correlation between bacterial phylogeny and mutator phenotype suggest that the evolution of stress-inducible mutation rates is rapid on an evolutionary time scale. This hypothesis is corroborated by our simulations where the selection of stress-induced mutator alleles occurred on a range of hundreds to thousands of generations.

What are the mechanisms responsible for these variable mutator phenotypes? Computer simulations, which took into account only the mutagenic effect, clearly show that second-order selection may drive the evolution of stress-inducible mutator alleles (Fig. 3). However, our results also agree with the possibility that stress-induced mutagenesis is a side effect of some stress-resistance mechanisms emerging from first-order selection. For example, we have observed that the down-regulation of mismatch repair seems to be involved in the increase of mutation rates in aging colonies (Table 3). Transient limitation of mismatch-repair activity was also reported to be involved in "adaptive" mutagenesis (*30*). Because such down-regulation was shown to be RpoS dependent (*19*), and because the RpoS-regulated network normally increases stress resistance (*16*), it may be that this down-regulation was selected for to avoid the energy cost incurred by the repair functions when food is limited. Moreover, potentially mutagenic SOS polymerases are known to increase survival of bacteria exposed to DNA-damaging agents, while increasing mutagenesis (*31, 32*). In aging colonies, DNA lesions may be processed by such error-prone repair mechanisms, as suggested by the implication of Pol II polymerase in MAC (Table 2) and Pol IV in "adaptive" mutagenesis (*33*). In both cases, the resulting production of deleterious mutations may have a less severe immediate effect on fitness than the direct cost imposed by efficient error-free survival mechanisms. It is plausible that different stress-resistance mechanisms have different pleiotropic effects, and thus different mutator phenotypes may be selected in different ecological niches. However, independent of the selective pressure that led to the emergence of MAC, genetic diversity produced during stress seems to be a substrate for natural selection. Consequently, MAC is expected to have an important impact on bacterial adaptive evolution.

It has been proposed that natural bacterial populations switch between constitutive mutator and nonmutator genotypes by mutations inactivating mismatch-repair genes and by horizontal gene transfer that restores their function (*34*). This study shows that bacteria switch phenotypically between high and low mutation rates depending on environmental conditions. This phenotypic switch seems to depend on a variety of molecular mechanisms, as suggested by the variability of MAC phenotypes among natural isolates and different genetic requirements for MAC in different *E. coli* strains (*14*, *26*, *35*). Because MAC-related mechanisms have been observed both in bacteria [e.g., *Mycobacterium smegmatis* (*36*) and *Pseudomonas putida* (*37*)] and yeast (*38*), we are aware of the possibility that the age-related increase in cancer incidence (*39*), somatic mutations (*39*), and germline mutations (*40*) could also be due to the accumulation of mutations in quiescent stem cells by mechanisms akin to MAC.

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