ADAPTATIONS TO FITNESS COSTS OF STREPTOMYCIN RESISTANCE

Strain*	Evolutionary status	DNA sequence (amino acid) at codon 42†	Cost of resistance (% per generation, ± 1 s.e.)‡	Peptide chain elongation rate (amino acid per s, ± 1 s.e.)§
CAB281	Baseline	AAA (Lys)	–	18.26 ± 1.41
CAB281-1	Evolved	AAA (Lys)	–	20.48 ± 2.10
STR1	Baseline	ACA (Thr)	13.6 ± 0.57	10.60 ± 0.70
STR1-1	Evolved	ACA (Thr)	6.97 ± 0.60	17.54 ± 1.10
STR1-2	Evolved	ACA (Thr)	4.63 ± 0.55	17.93 ± 1.60
STR2	Baseline	AAC (Asn)	18.8 ± 0.79	8.74 ± 0.56
STR2-1	Evolved	AAC (Asn)	9.55 ± 1.3	18.45 ± 1.59
STR2-2	Evolved	AAC (Asn)	6.68 ± 0.51	18.47 ± 1.77

*CAB281, donated by C. A. Bloch, is an *E. coli* K1/K12 chimaera in which the K1 *kpsA* cluster has been lost. We selected spontaneous streptomycin-resistant mutants from a CAB281 population grown in the presence of 100 μg ml^{-1} streptomycin. We froze all strains in 15% glycerol immediately after isolation to prevent further genetic changes.

†We isolated chromosomal DNA from each strain and amplified it by PCR using primers external to the coding sequence of the *rpsL* gene. We sequenced both strands of this DNA fragment, either by double-strand cycle sequencing using external and internal primers, or by automated sequencing by the Molecular Genetics Instrumentation Facility of the University of Georgia (Athens) using the same external primers. We found strain differences only at codon 42.

‡Estimated by direct competition against an initially equal density of wild type[10].

§Estimated by measuring the time needed to synthesize a complete molecule of β-galactosidase, according to refs 4, 11.

per generation disadvantage, corresponding to different single-base substitutions in codon 42 of the *rpsL* gene (see table). The rates of peptide chain elongation of both mutants were also significantly lower than that of the Str^s parental strain.

To examine how bacteria adapt to the fitness costs associated with these *rpsL* mutations, we established 10 replicate, long-term serial transfer cultures[6] for each class of Str^r mutant in antibiotic-free minimal medium. All cultures were maintained for 135 generations. Because evolution occurred in the absence of antibiotics, higher-fitness Str^s wild-type revertants could have dominated these cultures. On the contrary, however, we found that of 200 randomly chosen colonies tested from each of the two series of 10, 135-generation cultures, all were resistant to high concentrations of streptomycin.

We randomly chose two 135-generation cultures of each Str^r mutant class, allowed these four cultures to evolve for an additional 45 generations of culture, and isolated one, presumably evolved, colony from each of these 180-generation cultures. The *rpsL* gene sequence in all four 180-generation strains is the same as the baseline sequence (see table). The cost of resistance in all four strains, estimated by direct competition experiments against the wild-type Str^s strain, is significantly lower than costs in their ancestors (table). One evolved mutant (strain STR2-1), however, has a higher cost of resistance than the other evolved strains, suggesting that compensation for the cost of resistance may have occurred by more than one mechanism.

The peptide chain elongation rates of all four evolved, higher-fitness Str^r mutants are significantly greater than those of the unevolved, parental Str^r strains, and not significantly different from that of the Str^s wild-type strain (table). To see whether increased elongation rates resulted simply from a general adaptation of the CAB281 genetic background to experimental culture conditions, we maintained two independent cultures of wild-type CAB281 for 180 generations. We then screened 30 single colonies from each evolved CAB281 culture for fitness improvements, by direct pairwise competition against the wild-type strain with an added neutral Nal^r marker. The clone (strain CAB281-1) with the largest fitness improvement, 6% relative to wild type, shows no significant increase in elongation rate relative to that of the original wild-type strain.

Thus, in the absence of streptomycin, the initially high cost of chromosomal resistance to this antibiotic is rapidly compensated for without a clinically significant reduction in the level of streptomycin resistance. This compensation is achieved by second-site, non-*rpsL* mutations in loci yet to be determined, and in all cases is associated with a return to wild-type (or near wild-type) rates of peptide chain elongation.

As well as demonstrating the physiological basis of a rapid adaptation, the present results, together with evidence that similar compensation may be possible for the fitness costs of plasmid carriage[7–9], suggest that a reduced incidence of antibiotics may not lead to decreases in the current frequency of resistant bacteria. We are not arguing against prudent antibiotic use, as the frequency of bacteria resistant to antimicrobial agents is proportional to the extent to which these agents are used for therapy and prophylaxis[2]. On the other hand, our results are a warning that prudence alone may not be sufficient to stem the tide of antibiotic resistance.

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Obligate anaerobe or not?

SIR — Silberman *et al.*[1] in Scientific Correspondence state that *Blastocystis hominis*, a parasite of the human intestine, is an obligate anaerobe. Many lower eukaryotes that inhabit regions where the O_2 supply is limited or episodic not only are capable of withstanding long exposure to low concentrations of O_2, but may even use it for energy generation, or in some cases even require it for growth and division[2].

At the mucosal lining of the upper intestine[3], O_2 reaches 60 μM, and another protozoal parasite, *Giardia lamblia*, has magnificently tuned mechanisms for adjusting its intracellular redox states and fermentative fluxes to accommodate this[4]. Even in the vagina, where less O_2 is available, *Trichomonas vaginalis*, another amitochondriate flagellate, benefits from O_2-enhanced electron transport[5]. Controlled culture conditions confirm higher growth rates and yields, and make *T. vaginalis* a microaerophile rather than merely an aerotolerant anaerobe.

It would be surprising if *B. hominis*, replete with mitochondria, is really an obligate anaerobe; as for so many 'anaerobic' metazoans, we do not yet know enough to be sure that O_2 is not essential in its metabolism or life cycle.

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