Long-term survival during stationary phase: evolution and the GASP phenotype

Steven E. Finkel

Abstract | The traditional view of the stationary phase of the bacterial life cycle, obtained using standard laboratory culture practices, although useful, might not always provide us with the complete picture. Here, the traditional three phases of the bacterial life cycle are expanded to include two additional phases: death phase and long-term stationary phase. In many natural environments, bacteria probably exist in conditions more akin to those of long-term stationary-phase cultures, in which the expression of a wide variety of stress-response genes and alternative metabolic pathways is essential for survival. Furthermore, stressful environments can result in selection for mutants that express the growth advantage in stationary phase (GASP) phenotype.

In the seventeenth century, Hobbes described the life of man during periods of strife as "solitary, poor, nasty, brutish, and short" (Leviathan, chapter 13). In some respects, bacteria have been living similar lives for at least 2.7 billion years, although perhaps not as short as previously perceived. Many of our views of bacterial life come from laboratory studies of a small number of model organisms. In these experimental systems, bacteria are grown in various media that demonstrate the broad metabolic capacities of different species. A prevalent model of bacterial life is commonly referred to as the 'feast or famine' model. This is because bacteria can not only consume virtually all readily metabolizable nutrients in their environment, converting them to biomass[1,2], but they can also persist for long periods of time under starvation conditions once the available nutrients have been exhausted.

Clearly, the broad metabolic spectrum and prodigious reproductive capacity of many bacterial species in laboratory environments support the 'feast or famine' model. However, in natural environments the question becomes how much time a bacterium spends in each mode. How often does a microorganism experience an environment like the optimized growth conditions of Luria–Bertani (LB) medium or glycerol minimal medium in which carbon, phosphate and nitrogen are in abundance? Although there are undoubtedly particular environments and times when nutrients are abundant, overall, in the real world nutrient levels are usually limiting, and competition for those nutrients is intense. For example, although the mammalian gut provides nutrients in a protected environment, there is intense competition for these nutrients with the host organism as well as the hundreds of prokaryotic species that inhabit a typical gut. So, although it is clear that many microorganisms can 'make a good living' in the mammalian gut, some species might not be functioning at the peak of their metabolic capacity, frequently having to compete for nutrients just to survive. In other environments, it is easier to envision a life of nutrient deprivation. For example, in most aquatic environments there is very little free carbon; the dissolved carbon levels in sea water are typically 50 μM and nitrogen and phosphate levels are significantly lower[3]. Compare this to the 11 mM glucose levels (0.2% solution [w:v]) in some minimal media.

Furthermore, in many terrestrial environments, microorganisms are constantly subjected to variations in nutrient availability owing to the vagaries of water flow, wind, temperature, light and the activities of other organisms. Whereas at one moment nutrients might be flowing past a bacterial cell, at the next moment that flow might become a trickle or dry up. Yet, the bacteria persist. It is this ability of bacteria to survive through long periods of nutrient deprivation under conditions akin to those found during very-long-term stationary-phase batch-culture incubation in the laboratory that is the focus of this review.

Molecular and Computational Biology Programme, Department of Biological Sciences, University of Southern California, Los Angeles, California 90089-2910, USA.
e-mail: sfinkel@usc.edu
doi:10.1038/nrmicro1340

© 2006 Nature Publishing Group

2006. №4. P. 113-120

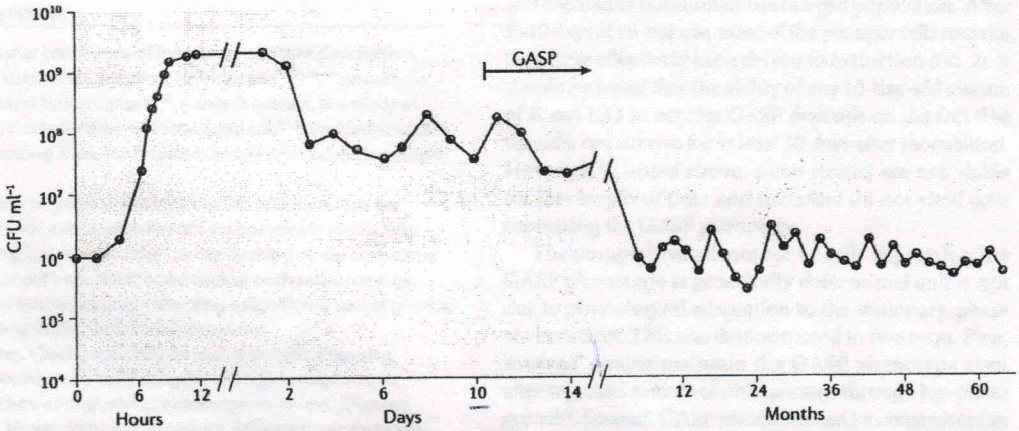

Figure 1 | **The five phases of the bacterial life cycle.** Once bacteria are inoculated into fresh medium, such as Luria–Bertani (LB) medium, there is an initial lag period followed by exponential-phase growth. After remaining at high density for 2 or 3 days, cells enter death phase. After ~99% of the cells die, the survivors can be maintained under long-term stationary-phase culture conditions for months or years. The arrow indicates the time after which cells expressing the growth advantage in stationary phase (GASP) phenotype are observed (usually day 10 in LB batch cultures).

The five phases of the bacterial life cycle

The standard textbook description of the bacterial life cycle (based on a completely unscientific survey of four textbooks) consists of three or four phases. However, in the laboratory there are actually five phases (FIG. 1): lag phase, exponential or logarithmic phase, stationary phase, death phase and extended or long-term stationary phase (a period referred to in one 1935 text[4] as the "period of prolonged decrease"). The first three phases are well described; here I focus on the fourth and fifth phases.

Death phase. Independent of the environmental conditions, cells incubated in batch culture eventually begin to lose viability, marking the transition from stationary phase into death phase. Formally, by death we are referring to the loss of viable counts (colony-forming units, CFU) using standard plating assays. However, during long-term incubation, the loss of viable counts is consistent with the observed numbers of cells[5,6]. Although the timing of death phase can vary from species to species (or even from strain to strain) in a given medium, both the timing of death phase and the degree of loss of viability are reproducible[5–8]. For example, for *Escherichia coli* K12 growing in LB medium, death phase usually occurs after 3 days and results in the loss of viability of ~99% of the cells.

The triggers for the transition from stationary phase to death phase and even the mechanism(s) of cell death are not well understood. The timing of death phase could be purely a stochastic event, in that a particular culture environment can only support a certain number of cells for a given period of time, after which most cells can no longer carry out repair and maintenance functions (leading to, for example, the accumulation of oxidatively damaged proteins and nucleic acids[9]) and begin to die. As cells die, cellular integrity is lost and the surviving cells can catabolize the detritus of their dead siblings, including amino acids from proteins, carbohydrates from the cell wall, lipids from cell-membrane material and even DNA[10].

A more intriguing mechanism of death-phase regulation is one in which high-density cell cultures experience a form of programmed cell death (bacterial apoptosis). In this model, cells sense that they are at high density and recognize that nutrients have become limited; perhaps this is a role for the quorum-sensing systems? Over evolutionary time, a mechanism might have evolved in which populations have 'learned' that if a proportion of the population commits suicide, the survivors can go on to reproduce. Therefore, after a long period at high density without abundant nutrients, most of the population enters 'death mode' and effectively commits an altruistic suicide. As it is not possible for all members of the population to act simultaneously, some cells will not die immediately. As siblings begin to die, another signal is released, perhaps the detrital nutrients themselves. Surviving cells can perceive this signal and 'exit' the death programme.

At present, there is little evidence to distinguish the 'stochastic' from 'programmed' cell-death mechanisms. There is, however, increasing interest in understanding the roles of toxin–antitoxin (TA) gene pairs that were first associated with the 'plasmid addiction' systems of bacteriophage P1 and other plasmids[11,12], seven pairs of which are present on the *E. coli* chromosome[13–18]. A role for these gene pairs in death phase remains an open question.

Long-term stationary phase. After death phase, *E. coli* can be maintained in batch culture for long periods of time without the addition of nutrients[5,6,19]. By regularly providing sterile distilled water to maintain the volume and osmolarity, aerobically grown cultures can be maintained at densities of ~10^6 CFU per ml for more than 5 years without the addition of nutrients[6] (FIG. 1). We call this period long-term stationary phase. Unlike early

Batch culture
A closed culture system in which all of the nutrient substrate is added at the beginning.

Quorum sensing
A system by which bacteria communicate. Signalling molecules — chemicals similar to pheromones that are produced by an individual bacterium — can affect the behaviour of surrounding bacteria.

Toxin–antitoxin
Paired loci found in the chromosomes of almost all free-living prokaryotes, and many plasmids and phage genomes, encoding a toxin and its antidote that have been proposed to function in bacterial programmed cell death or stress physiology.

© 2006 Nature Publishing Group

Box 1 | Long-term culture systems

Traditionally, to study bacteria under conditions of long-term nutrient deprivation, three culture systems have been used: chemostats or turbidostats[37,38,88–91], serial-passage regimes[60,92–96] and long-term batch culture[5–8]. A fourth system, the study of bacteria isolated from long-term storage 'stabs' of *Escherichia coli*[97,98] and *Salmonella* spp.[82,99–101], is also providing interesting data. Each system has distinct advantages and disadvantages.

In the chemostat and serial-passage systems, environmental conditions can be reproduced, frequently using minimal media with limited carbohydrate availability. However, cells are constantly being lost owing either to the flushing of the chemostat vessel or because only a fraction of cells are transferred during each serial passage. These cell losses result in constant bottlenecking, reflecting a significant loss of genetic diversity, in marked contrast to long-term batch-culture regimes.

In batch culture and stab cultures, there is virtually no loss of genetic diversity; however, environments are not constant. As cells adapt or change in response to environmental conditions, different waste products can accumulate and different compounds can be metabolized. These changes can make it difficult to recreate the environmental conditions under which mutants were initially selected. Therefore, in the study of long-term bacterial cultures, there is a trade-off between maintaining a constant or stereotypical environment with bottlenecking (as in chemostats and serial transfer) versus maintaining genetic diversity in ever-changing environments (as in batch culture and stabs).

stationary phase, in which there is little cell division, long-term stationary phase is a highly dynamic period in which the 'birth' and 'death' rates are balanced. That is, long-term batch cultures have an apparent carrying capacity that can only support a certain number of cells. As new cells are created, other cells must die. This dynamism is described in more detail below.

The GASP phenotype

In addition to the many physiological, morphological and gene-expression changes that occur as cells enter long-term stationary phase, potentially the most significant changes are associated with genetic alterations that occur in most (if not all) cells incubated during long-term stationary phase in batch culture (see BOX 1 for a discussion on long-term culture systems). The signal phenotype associated with these changes is the appearance of the growth advantage in stationary phase (GASP) phenotype[5–8,20]. GASP is defined by the ability of cells aged in long-term batch cultures to outcompete cells from younger cultures. The discovery of GASP and detailed studies of its genetic basis have been reviewed elsewhere[6,7,20].

The GASP phenotype is easily demonstrated by performing mixing experiments in which cells from cultures of different 'ages' are directly competed against one another. Virtually all LB batch cultures of *E. coli* will yield cells that express the GASP phenotype after 10 days of incubation; cells harvested from cultures before day 8 rarely express GASP (FIG. 1). For example, if a sample of *E. coli* incubated in LB medium for 10 days at 37°C with aeration is transferred into a culture of 1-day-old cells (usually a 1:1000 dilution [v:v]), within a few days the minority of aged cells will increase in number, with a concomitant reduction in the number of 'young' cells[5–8]. Cell populations of different 'ages' are distinguished by using chromosomally encoded antibiotic-resistance markers (see below). The initial minority of aged cells will eventually outnumber the unaged population. After 7–10 days of co-culture, none of the younger cells remain; they have effectively been driven to extinction (FIG. 2). It should be noted that the ability of any 10-day-old culture of *E. coli* K12 to express GASP depends on the fact that the cells can survive for at least 10 days after inoculation. However, as noted above, some strains are not viable for this length of time and therefore do not yield cells expressing the GASP phenotype.

The competitive advantage of cells expressing the GASP phenotype is genetically determined and is not due to physiological adaptation to the stationary-phase environment. This was demonstrated in two ways. First, 'evolved' strains maintain the GASP phenotype even after repeated rounds of serial passage through log-phase growth[8]. Second, GASP mutations can be constructed in unaged, naive strains, instantly transferring the GASP phenotype to cells which have never been aged[8].

To date, four GASP mutations have been identified in *E. coli* and three of the loci are well characterized[8,20–23]. The first GASP mutation identified was in *rpoS*[8], which encodes the alternative sigma (σ) factor RpoS or σ^S (this locus is further discussed below). Additional mutations have been mapped to *lrp*[20–22] and the *ybeJ–gltJKL* cluster[20,21,23], encoding the leucine-responsive protein and a high-affinity aspartate and glutamate transporter, respectively. Although each of these genes is involved in different processes, each GASP mutation results in an increased ability to catabolize one or more amino acids as a source of carbon and energy[20–23]. It is clear that this is an early and strong selective force in the evolution of GASP mutations.

Of particular note is the fact that, although these mutations all result in a similar phenotype, they differ considerably at the molecular level. In the best-studied aged lineage, the mutations consist of a 46-bp duplication in *rpoS*[8], a 3-bp deletion in *lrp*[20,22] and a transposon 'hop' coupled with a site-specific inversion in the *ybeJ–gltJKL* locus[20,23]. As discussed below, various mutations have been observed in *rpoS* in *E. coli*[8,24] and in *Salmonella* spp.[25]

Although *rpoS* is not essential for the evolution of the GASP phenotype in *E. coli* (*rpoS* null mutants still express the GASP phenotype when aged and competed against younger *rpoS* mutant strains; S.E.F., unpublished results), mutations in *rpoS* are the most common GASP mutations found in 10-day-old *E. coli* cultures[5,6,24,26], including transition and transversion point mutations, single- and multiple-base-pair deletions or insertions, and duplications.

In general, GASP mutations in *rpoS* lead to an attenuated phenotype, as opposed to a complete loss of function[7,8,26,27]. That is, these mutations reduce RpoS activity to 0.1–1% of normal levels[27]. The exact mechanistic basis of the competitive advantage conferred by GASP mutations of *rpoS* is not known. Part of the effect might be due to the misregulation of members of the RpoS regulon[28,29]. It appears that an alteration in the expression of genes of the RpoS and RpoD (encoding σ^{70}) regulons might also be important, as both σ factors compete for the core polymerase[9,30,31]. For example, several genes involved in the pH- and oxidative-stress responses are

Serial passage
An experimental evolution culture system in which a fraction of a culture is sampled and inoculated into a fresh culture of the same medium repeatedly. Over time, cells propagated in this way will show changes in genotype and phenotype associated with changes in relative fitness.

Alternative sigma (σ) factor
Alternative σ factors are produced under specific conditions and allow the RNA polymerase to transcribe a different set of genes than the housekeeping σ factor, σ^{70}.

Transition
A mutation between two pyrimidines (T–C) or two purines (A–G).

Transversion
A point mutation in which a purine base is substituted for a pyrimidine base and vice versa; for example, an AT to CG transversion.

© 2006 Nature Publishing Group

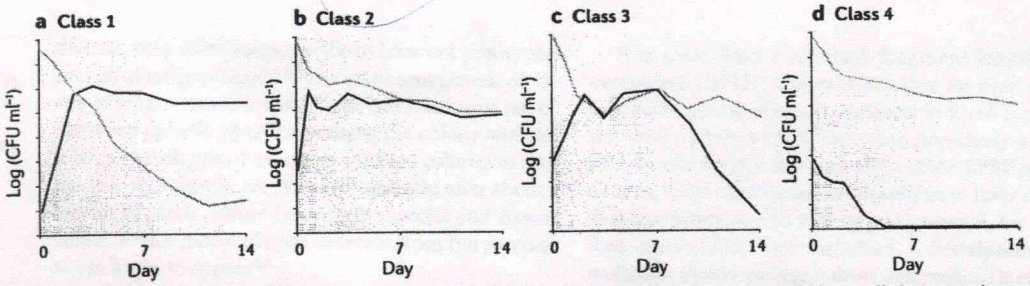

Figure 2 | **Four 'flavours' of growth advantage in stationary phase (GASP) phenotypes.** When cells from aged, long-term stationary-phase cultures (red lines) are competed initially as a minority with 1-day-old wild-type cells (green lines), the competitions can result in one of four phenotypes. In Class 1 and Class 2 competitions, aged cells express the GASP phenotype, increasing in frequency in the culture. In Class 1 competitions, younger cells are driven to extinction. In Class 3 competitions, aged minority cells initially appear to express GASP, but are eventually outcompeted by the younger cells. In Class 4 competitions, aged cells are unable to compete with younger strains.

part of the RpoS regulon[26,32,33]. The loss of expression of these genes is deleterious to the cell, as shown by the reduced competitive ability of null mutants of *rpoS* and their increased sensitivity to acid stress as compared to wild-type cells[26,32,34]. However, enough of these gene products are expressed when an attenuated GASP allele of RpoS is present, increasing fitness during stationary phase[26], but these effects are conditional. We have shown that, in environments buffered at pH 7, wild-type alleles are beneficial[26]. Schellhorn and co-workers have identified batch-culture conditions in which loss of RpoS function is under positive selection[35], Givskov's group has shown that *rpoS* might not confer a benefit in the intestine[36], and Ferenci and colleagues have shown that, in chemostats, *rpoS* null mutants can have a significant competitive advantage[31,37,38].

At this time, the best-characterized effect of GASP alleles of *rpoS* is an increase in the ability of the cell to catabolize certain amino acids. This altered ability confers a significant competitive advantage to the mutants. Zinser and colleagues have shown that GASP mutations in *rpoS*, as well as several other genes, can increase the ability to use alanine, arginine, aspartate, glutamate, glutamine, serine, threonine and proline as sole sources of carbon and energy[20–23]. Another intriguing role for attenuated alleles of *rpoS* could involve the regulation of error-prone DNA polymerases (see below).

Long-term stationary-phase population dynamics

After the initial appearance of GASP alleles of *rpoS*, novel GASP mutations continue to appear with continued incubation. This has been demonstrated in several ways. First, not only do cells from 10-day-old cultures outcompete 1-day-old cells, but cells from 20-day-old cultures outcompete 10-day-old cells, 30-day-old cells outcompete 20-day-old cells, and so on[5,6]. In fact, cells from cultures aged up to 60 days will outcompete cells from all younger cultures with varying degrees of relative fitness[39]. For example, 10–20-day-old cultures always express a strong GASP phenotype, driving majority cells to extinction (this is referred to as a Class 1 GASP phenotype[40]; FIG. 2). Cells from 30–60-day-old cultures will always express the GASP phenotype, but are as likely to coexist with formerly majority cells (Class 2) as they are to drive them to extinction (Class 1). This constant appearance of GASP mutations over time indicates that long-term stationary-phase cultures are not static, but are highly dynamic. After about 60 days of incubation, more Class 3 (mutants that attempt to develop the GASP phenotype, but fail) and Class 4 (mutants that have no increased competitive ability) phenotypes[40] are observed, indicating that the culture environment after 2 months of incubation is sufficiently different from overnight cultures to preclude aged cells from expressing a fitness advantage over unaged, 1-day-old cells.

When the GASP phenomenon was first observed, it was proposed that GASP mutations were accruing in the absence of significant cell turnover through a process of mutation and repair, and not due to population dynamics. This latter model was disproved using various competition experiments[5,6]. In these experiments, initially isogenic strains are aged independently for 30 days. Isogenic strains carry single point mutations conferring antibiotic resistance, usually a *gyrA* mutation conferring resistance to nalidixic acid and an *rpsL* mutation conferring streptomycin resistance. These mutations are neutral in terms of relative fitness in the absence of drug selection[5,6,8,26] and allow the identification of cells from 'young' versus 'old' cultures. After the cultures are aged and GASP mutants have appeared in the population, equal numbers of cells are mixed together. Subpopulations are then monitored through their drug-resistance phenotypes. As these mixed cultures are co-incubated, the total number of cells per culture does not change; however, the proportion of the two marked subpopulations will increase or decrease over time, frequently with one of the two groups eventually going extinct[5,6]. As the total number of cells does not change over time, these cultures are in a state of dynamic equilibrium in which new GASP mutants are constantly displacing less-fit siblings (FIG. 3).

Initial models of the GASP phenomenon described a system in which one GASP mutant would sweep through the population, displacing all other cells until it was itself displaced by the next mutant of increased fitness[7,8,39]. We know now that these cultures are, in fact, far more

Chemostat
A device that allows the continuous growth of a bacterial population on a growth-rate-limiting resource. The resource flows into the chemostat at a constant rate; depleted medium and cells are washed out at the same rate. The population grows and consumes the resource until the bacteria reach an equilibrium density at which their growth rate equals the flow rate through the vessel.

© 2006 Nature Publishing Group

Very short patch repair
A mismatch-correction system that corrects T:G mismatches to C:G in certain sequence contexts, independent of Dam methylation.

diverse, with many subpopulations (distinct genotypes) present simultaneously[5,6,26]. The enormous genetic diversity of long-term stationary-phase batch cultures can be observed directly by characterizing the colony and cellular morphologies of cells sampled from cultures as they age (for an example, see FIG. 4). Frequently, after about a month of batch culture (varying by species and strain) colonies with morphologies different from the parental strain begin to appear[6,20].

The mutation frequency during stationary phase

Is it surprising that virtually all 10-day-old cultures of *E. coli* have cells that express the GASP phenotype? It might be, until we revisit a few facts about the rates of mutation in bacterial cultures. Using a mutation frequency of $\sim 10^{-10}$ mutations per bp per generation[41,42], and with a genome size of $\sim 4.6 \times 10^6$ bp, we can assume that one in every 10,000 cells has a point mutation. However, using various experimental data, Drake has calculated an even-higher frequency of mutation of 1 cell in every 300–400 (REF. 42). This is an extraordinary amount of genetic variation upon which natural selection can act.

After death phase, populations initially level out at $\sim 5 \times 10^7$ CFU per ml (FIG. 1). Typically, when sampling cultures for a GASP competition experiment, 5 µl are removed from the culture, which is the equivalent of $\sim 5 \times 10^4$ cells. As we observe the GASP phenotype in virtually all 10-day-old cultures, this means that at least one cell in ~50,000 expresses GASP. However, we know that on average, of those 50,000 cells, up to 80% will express the GASP phenotype[26]. The fact that GASP mutants increase to a high frequency by 10 days of incubation and that independent, initially isogenic cultures yield different GASP mutations indicates that there is not only significant genotypic diversity in these cultures, but that the GASP alleles are under strong selection.

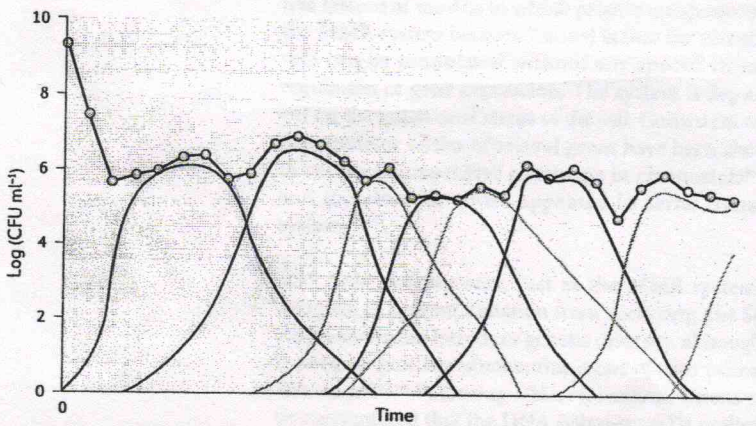

Figure 3 | **Population dynamics of long-term stationary-phase cultures.** After death phase, as cells continue to incubate under long-term stationary-phase conditions, the apparent number of colony-forming units (CFU) per ml remains relatively stable. However, these cultures are not static. There is a dynamic equilibrium between newly created growth advantage in stationary phase (GASP) mutants and less competitive cells. The birth rates and death rates within the population are balanced. Each coloured line represents a different GASP mutant that appears during long-term incubation. The black line represents the total population density.

It is clear from restriction fragment length polymorphism (RFLP) observations that an even greater amount of genetic diversity is present in *E. coli* long-term stationary-phase populations than previously appreciated. In one study, a determination of the RFLP patterns of three restriction enzymes allowed us to infer a mutation frequency of 1 in 600 bp per genome[5], far higher than other studies have indicated. A description of the molecular events leading to these observations is essential for our understanding of the mechanisms of survival in long-term stationary phase.

Mechanisms to generate genetic diversity

Just as important as identifying genes which, when mutated, result in the GASP phenotype is an understanding of the mechanisms that have a role in increasing genetic diversity. It is clear from what little we know about the genetic changes that take place during stationary phase that there is a considerable amount of genotypic diversity during long-term batch culture; some have suggested the existence of a (transient) hypermutable state[43,44]. Of the many mechanisms that could be important, I focus on two: the role of the methyl-directed mismatch repair system and the SOS-induced DNA polymerases. In both cases, the creation of genetic diversity is being achieved 'in house' through mutation, rather than by the acquisition of genes through horizontal transfer. Also of increasing interest is the role of insertion sequences and transposable elements. Although this is a fascinating and important area of inquiry, at this time, with one exception[23], there are little data available on the chromosomal structure of GASP mutants with regard to these kinds of mutation.

Methyl-directed mismatch repair. The methyl-directed mismatch repair (MMR) system, encoded in *E. coli* by the *mutS*, *mutL* and *mutH* genes, removes misincorporated bases in newly replicated DNA. MMR genes are conserved from bacteria to humans[45]. Following binding by MutS, which recognizes mismatches in double-stranded DNA, and MutL, the mismatch (as well as some adjacent DNA) is excised after cleavage by the MutH endonuclease. The methylation state of the DNA determines which strand is cleaved and excised. The system assumes that the unmethylated strand of a nascent, hemimethylated duplex DNA is the new strand and, therefore, the strand containing the mutation. However, during stationary phase, DNA should be fully methylated. So, what happens when DNA is damaged, resulting in an apparent mismatch? One model proposes that the MMR system, or perhaps the very short patch repair (*vsr*) system[46,47], will repair the lesion, but as it cannot determine which is the old strand and which the new strand, half of the time the wrong strand will be repaired, leading to the introduction of mutation[48,49].

Another model suggests that as cells enter stationary phase, the overall protein biosynthetic capacity is reduced, including the repair proteins. As MutL, unlike MutS and MutH, appears to be used stoichiometrically (once per MMR event), it can become limiting[50,51]. Other studies suggest that MutS levels can limit MMR

© 2006 Nature Publishing Group

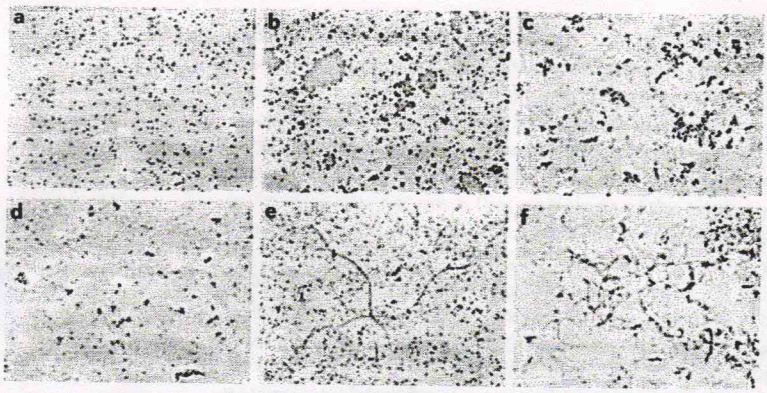

Figure 4 | **Morphologies of cells isolated from long-term stationary-phase batch cultures.** A Luria–Bertani (LB) culture was incubated for 150 days and sampled by spreading cells onto LB agar plates. In this example, five colony variants were observed. Phase-contrast micrographs of cells from each colony, as well as the unaged wild-type parent (**a**), reveal different cellular morphologies, including cells that aggregate (**b**), cells that are phase dark (**c**,**d**) and filamentous cells (**e**,**f**). The morphology of the colonies from which cells were isolated was as follows: **a**,**b**, colonies of normal, cream-coloured appearance; **c**, opaque colonies; **d**, mini-mucoid colonies; **e**, fried-egg colonies with ruffled edges and darker centres; **f**, nano colonies, yielding pinpoint-sized colonies after 24 hours.

activity[52,53]. Therefore, as cells complete rounds of replication during stationary phase, the MMR system is suppressed, leading to an increase in genetic diversity. One obvious result of an increase in mutation frequency is the increased possibility of mutating essential genes. However, it should be noted that it is likely that many cells in stationary-phase cultures have more than one chromosome. Akerlund *et al.* have shown that most cells incubated up to 5 days in batch culture have two, four or even eight chromosomes per cell[54]. This transient polyploidy might protect cells from the catastrophic loss of essential genes (see below). One attractive feature of models in which protein components of the MMR system become limited is that the mutation rate can be modulated without any special form of regulation of gene expression. The system is dependent on the nutritional status of the cell. Consistent with this, mutator alleles of several genes have been shown to confer a competitive advantage in chemostats[55–59], and mutators have also appeared in serial transfer systems[60–62].

SOS DNA polymerases. Just as the MMR system is designed to prevent mutation from occurring, the SOS polymerases can introduce genetic diversity, although it is an open question whether this is one of their primary functions[40,55,63–65]. During DNA replication, lesions can be encountered that the DNA polymerase III replicase cannot copy through. Frequently when this happens the SOS response is activated, inducing, among others, the genes encoding the alternative DNA polymerases PolII, PolIV and PolV[55,66]. These polymerases can replicate through various kinds of lesions, including abasic sites, photodimers and a wide variety of damaged bases. Typically, PolIV and PolV, encoded by *dinB* and *umuDC*, respectively, are considered error-prone polymerases, inserting one base preferentially over the other three. PolII, which is encoded by *polB*, is considered an error-free polymerase, but can still misincorporate bases at some lesions during repair polymerization[55]. We have shown that cells deficient in any of the SOS polymerases are at a competitive disadvantage compared with wild-type cells, as well as showing a defect in expression of the GASP phenotype[40]. The reduced ability to express GASP is probably the result of a combination of effects: a reduction in fitness owing to the altered physiology of cells that have trouble replicating past certain lesions and an alteration in the repertoire of genetic diversity from which novel GASP alleles can be selected. Several studies support a model in which the mutation spectrum of wild-type cells compared to SOS-polymerase mutants is different[40,55,63–67].

Foster and colleagues have shown that the expression of *dinB* is under the control of RpoS[68]. This raises the intriguing possibility that partially active, attenuated alleles of *rpoS* are required to help maintain minimal levels of PolIV activity to generate mutations that might confer a GASP phenotype.

Together, the data exploring the role of the MMR and SOS-polymerase systems allow one to propose models in which cells modulate replication fidelity as a stress response[69,70]. The notion of 'mutate or die' is not unreasonable, given the potentially hostile environments faced by bacteria coupled with the obvious genomic plasticity observed in laboratory, clinical and environmental samples at several loci[71–73], including *mut* genes and *rpoS*.

In addition to the point mutations or other small mutations created by the above-mentioned systems, another potentially significant contributor to genetic diversity during long-term stationary-phase incubation is the duplication or amplification of large regions of the chromosome. DNA amplifications have been observed in *E. coli* and *Salmonella* spp. under different culture conditions at different loci[74–81]. For example, using genomic array techniques, Porwollik and colleagues have observed an amplification of ~180 kb in cells harvested from an ~40-year-old stab culture[82]. Amplification has at least two potential benefits for the cell. First, it can increase the dosage of beneficial genes, either the parental version or GASP alleles, and second, by increasing gene copy number it increases the chances of getting a beneficial mutation at a particular locus. This is especially important for genes that are essential, where the additional gene copies complement the essential function while allowing for an exploration of 'genome space'. It is important that one understands that this does not suggest that the cell preferentially amplifies the genome at certain loci with the 'hope' of acquiring a beneficial mutation. What it does imply is that cells could have evolved systems that amplify large chromosomal regions because cells with this ability acquire beneficial mutations at a higher frequency than cells that do not. This same argument can be applied to the evolution of the activity or regulation of the MMR and SOS polymerase systems. The watchwords are 'mutate or die'.

SOS response
The bacterial response to DNA damage that is regulated by the LexA and RecA proteins and involves the expression of a network of >40 genes, including several DNA-repair enzymes.

© 2006 Nature Publishing Group

Future directions

The avalanche of information about the genomic composition of microbial organisms seems to generate more questions than answers. Even today we do not understand the function of about one-third (a conservative estimate) of the genes of *E. coli* K12. Further questions arise when one considers that organisms of the same species (or even the same strain) can show considerable genomic diversity. For example, consider the differences in the genomes of *E. coli* strains *E. coli* K-12, *E. coli* O157:H7 and others[83–87] which have only ~40% of their genes in common. Some of these questions can be addressed when we have information about the habitats or particular lifestyles of an organism. One overarching question is how did the strains or lineages become different? Are they the results of divergent evolution through horizontal gene transfer, vertical transmission after natural selection of new mutations, or some combination of the two? The most likely answer is 'all of the above'.

To understand these evolutionary processes, long-term batch culture serves as a valuable model system. By incubating cells under stressful conditions and observing the expression of the GASP phenotype, we can begin to understand the dynamics of population structure and the mechanisms that lead to the generation of genetic diversity. The molecular events that lead to the expression of GASP reflect a wide range, and perhaps only a subset, of the kinds of change that evolving populations can experience. The ability to observe evolution in a test tube in real time, coupled with old-fashioned genetics and cutting-edge array technology to analyse the products of evolution, will help to answer these fundamental questions surrounding the mechanisms of adaptation at the molecular level.

With their exceptional metabolic efficiencies, suggestions of the ability to organize and cooperate, and extreme longevity in some environments, perhaps Hobbes would have made bacteria an exception to his characterization of life under stressful conditions.

1. Morita, R. Y. Bioavailability of energy and its relationship to growth and starvation in nature. *Can. J. Microbiol.* **34**, 436–441 (1988).
2. Morita, R. Y. in *Starvation in Bacteria* (ed. Kjelleberg, S.) 1–23 (Plenum Press, New York, 1988).
3. Houghton, R. A. The contemporary carbon cycle. In *Treatise on Geochemistry* Vol. 8 (ed. Schlesinger, W. H.) 473–513 (Elsevier, Amsterdam, 2004).
4. Gay, F. P. Bacteria growth and reproduction. In *Agents of Disease and Host Resistance, Including Principles of Immunology, Bacteriology, Mycology, Protozoology, Parasitology, and Virus Disease* (eds Gay, F. P, Bachman, G. W., Benham, R. H. & Buchbinder, L.) 1–38 (Charles C. Thomas, Springfield, 1935).
5. Finkel, S. E. & Kolter, R. Evolution of microbial diversity during prolonged starvation. *Proc. Natl Acad. Sci. USA* **96**, 4023–4027 (1999).
Provided a direct demonstration of the dynamic nature of long-term stationary-phase batch cultures.
6. Finkel, S. E., Zinser, E. & Kolter, R. in *Bacterial Stress Responses* (eds Storz, G. & Hengge-Aronis, R.) 231–238 (ASM Press, Washington DC, 2000).
7. Zambrano, M. M. & Kolter, R. GASPing for life in stationary phase. *Cell* **86**, 181–184 (1996).
8. Zambrano, M. M., Siegele, D. A., Almirón, M., Tormo, A. & Kolter, R. Microbial competition: *E. coli* mutants that take over stationary phase cultures. *Science* **259**, 1757–1760 (1993).
First description of the appearance of GASP mutants in 10-day-old stationary-phase cultures.
9. Nystrom, T. Conditional senescence in bacteria: death of the immortals. *Mol. Microbiol.* **48**, 17–23 (2003).
10. Finkel, S. E. & Kolter, R. DNA as a nutrient: novel role for bacterial competence gene homologs. *J. Bacteriol.* **183**, 6288–6293 (2001).
11. Jensen, R. B. & Gerdes, K. Programmed cell death in bacteria: proteic plasmid stabilization systems. *Mol. Microbiol.* **17**, 205–210 (1995).
12. Lehnherr, M., Maguin, E., Jafri, S. & Yarmolinsky, M. B. Plasmid addiction genes of bacteriophage P1: *doc*, which causes cell death on curing of phage, and *phd*, which prevents host death when prophage is retained. *J. Mol. Biol.* **233**, 414–428 (1993).
13. Hayes, F. Toxins–antitoxins: plasmid maintenance, programmed cell death, and cell cycle arrest. *Science* **301**, 1496–1499 (2003).
14. Brown, J. M. & Shaw, K. J. A novel family of *Escherichia coli* toxin–antidote gene pairs. *J. Bacteriol.* **185**, 6600–6608 (2003).
15. Aizenmann, E., Engelberg-Kulka, H. & Glaser, G. An *Escherichia coli* chromosomal "additional module" regulated by guanosine 3′5′-bispyrophosphate: a model for programmed bacterial cell death. *Proc. Natl Acad. Sci. USA* **93**, 6059–6063 (1996).
16. Gotfredsen, M. & Gerdes, K. The *Escherichia coli relBE* genes belong to a new toxin–antitoxin gene family. *Mol. Microbiol.* **29**, 1065–1076.
17. Christensen, S. K. *et al.* Overproduction of the Lon protease triggers inhibition of translation in *Escherichia coli*: involvement of the *yefM–yoeB* toxin–antitoxin system. *Mol. Microbiol.* **51**, 1705–1717 (2004).
18. Gerdes, K., Christensen, S. K. & Lobner-Olsen, A. Prokaryotic toxin–antitoxin stress response loci. *Nature Rev. Microbiol.* **3**, 371–382 (2005).
Excellent review of the role of toxin–antitoxin pairs.
19. Steinhaus, E. A. & Birkeland, J. M. Studies on the life and death of bacteria. I. The senescent phase in aging cultures and the probable mechanisms involved. *J. Bacteriol.* **38**, 249–261 (1939).
An early paper demonstrating the ability of several bacteria to survive long periods of stationary-phase incubation.
20. Zinser, E. R. & Kolter, R. K. *E. coli* evolution during stationary phase. *Res. Microbiol.* **155**, 328–336 (2004).
Excellent review of the molecular characterization of several GASP mutants of *E. coli*.
21. Zinser, E. R. & Kolter, R. Mutations enhancing amino acid catabolism confer a growth advantage in stationary phase. *J. Bacteriol.* **181**, 5800–5807 (1999).
22. Zinser, E. R. & Kolter, R. Prolonged stationary phase incubation selects for *lrp* mutants in *E. coli* K-12. *J. Bacteriol.* **182**, 4361–4365 (2000).
23. Zinser, E. R., Schneider, D., Blot, M. & Kolter, R. Bacterial evolution through the selective loss of beneficial genes: trade-offs in expression involving two loci. *Genetics* **164**, 1271–1277 (2003).
24. Gupta, S. *Mutations That Confer a Competitive Advantage During Starvation*. Thesis, Harvard Univ. (1997).
25. Jordan, S. J., Dodd, C. E. & Stewart, G. S. Use of single-strand conformation polymorphism analysis to examine the variability of the *rpoS* sequence of environmental isolates of salmonellae. *Appl. Environ. Microbiol.* **65**, 3582–3587 (1999).
26. Farrell, M. J & Finkel, S. E. The growth advantage in stationary phase phenotype conferred by *rpoS* mutations is dependent on the pH and nutrient environment. *J. Bacteriol.* **185**, 7044–7052 (2003).
Demonstrates the wide diversity of GASP alleles of *rpoS* in stationary-phase populations.
27. Bohannon, D. E. *et al.* Stationary phase-inducible "gearbox" promoters: differential effects of *katF* mutations and role of σ^{70}. *J. Bacteriol.* **173**, 4482–4492 (1991).
28. Vijayakumar, S. R. V., Kirchhof, M. G., Patten, C. L. & Schellhorn, H. E. RpoS-regulated genes of *Escherichia coli* identified by random *lacZ* fusion mutagenesis. *J. Bacteriol.* **186**, 8499–8507 (2004).
29. Patten, C. L., Kirchhof, M. G., Schertzberg, M. R., Morton, R. A. & Schellhorn, H. E. Microarray analysis of RpoS-mediated gene expression in *Escherichia coli* K-12. *Mol. Gen. Genomics* **272**, 580–591 (2004).
30. Nystrom, T. Growth versus maintenance: a trade-off dicated by RNA polymerase availability and σ factor competition. *Mol. Microbiol.* **54**, 855–862 (2004).
31. Notley-McRobb, L., King, T. & Ferenci, T. *rpoS* mutations and loss of general stress resistance in *Escherichia coli* populations as a consequence of conflict between competing stress responses. *J. Bacteriol.* **184**, 806–811 (2002).
32. Richard, H. T. & Foster, J. W. Acid resistance in *Escherichia coli*. *Adv. Appl. Microbiol.* **52**, 167–186 (2003).
33. Vulic, M. & Kolter, R. Evolutionary cheating in *E. coli* stationary phase cultures. *Genetics* **158**, 519–526 (2001).
34. Vulic, M. & Kolter, R. Alcohol-induced delay of viability loss in stationary-phase cultures of *Escherichia coli*. *J. Bacteriol.* **184**, 2898–2905 (2002).
35. Chen, G., Patten, C. L. & Schellhorn, H. E. Positive selection for loss of RpoS function in *Escherichia coli*. *Mutat. Res.* **554**, 193–203 (2004).
36. Krogfelt, K. A., Hjulgaard, M., Sorensen, K., Cohen, P. S. & Givskov, M. *rpoS* gene function is a disadvantage for *Escherichia coli* BJ4 during competitive colonization of the mouse large intestine. *Infect. Immun.* **68**, 2518–2524 (2000).
37. Ferenci, T. What is driving the acquisition of *mutS* and *rpoS* polymorphisms in *Escherichia coli*? *Trends Microbiol.* **11**, 457–461 (2003).
Excellent review of the selective pressures that drive mutation in *rpoS* in nutrient-limited environments.
38. Notley-McRobb, L. & Ferenci, T. Experimental analysis of molecular events during mutational periodic selections in bacterial evolution. *Genetics* **156**, 1493–1501 (2000).
39. Finkel, S. E., Zinser, E. R., Gupta, S. & Kolter, R. Life and death in stationary phase. In *Molecular Microbiology* Vol. H 103 (eds Busby, S. J. W., Thomas, C. M. & Brown, N. L.) 3–16 (Springer-Verlag, Berlin, 1998).
40. Yeiser, B, Pepper, E. D., Goodman, M. F. & Finkel, S. E. SOS-induced DNA polymerases enhance long-term survival and evolutionary fitness. *Proc. Natl Acad. Sci. USA* **99**, 8737–8741 (2002).
41. Drake, J. W. A constant rate of spontaneous mutation in DNA-based microbes. *Proc. Natl Acad. Sci. USA* **88**, 7160–7164 (1991).
42. Drake, J. W., Charlesworth, B., Charlesworth, D. & Crow, J. F. Rates of spontaneous mutation. *Genetics* **148**, 1667–1686 (1998).
43. Bridges, B. A. Hypermutation in bacteria and other cellular systems. *Phil. Trans. R. Soc. Lond. B Biol. Sci.* **356**, 29–36 (2001).

© 2006 Nature Publishing Group

44. Torkelson, J. *et al.* Genome-wide hypermutation in a subpopulation of stationary-phase cells underlies recombination-dependent adaptive mutation. *EMBO J.* **16**, 3303–3311 (1997).
45. Schofield, M. J. & Hsieh, P. DNA mismatch repair: molecular mechanisms and biological function. *Annu. Rev. Microbiol.* **57**, 579–608 (2003).
46. Bhagwat, A. S. & Lieb, M. Cooperation and competition in mismatch repair: very short-patch repair and methyl-directed mismatch repair in *Escherichia coli*. *Mol. Microbiol.* **44**, 1421–1428 (2002).
47. Macintyre, G., Pitsikas, P. & Cupples, C. G. Growth phase-dependent regulation of Vsr endonuclease may contribute to 5-methylcytosine mutational hot spots in *Escherichia coli*. *J. Bacteriol.* **181**, 4435–4436 (1999).
48. Au, K. G, Welsh, K. & Modrich, P. Initiation of methyl-directed mismatch repair. *J. Biol. Chem.* **267**, 12142–12148 (1992).
49. Herman, G. E. & Modrich, P. *Escherichia coli* K-12 clones that overproduce *dam* methylase are hypermutable. *J. Bacteriol.* **145**, 644–646 (1981).
50. Harris, R. S., *et al.* Mismatch repair is diminished during stationary phase mutation. *Mutat. Res.* **437**, 51–60 (1999).
51. Harris, R. S. *et al.* Mismatch repair protein MutL becomes limiting during stationary-phase mutation. *Genes Dev.* **11**, 2426–2437 (1997).
52. Zhao, J. & Winkler, M. E. Reduction of GC→TA transversion mutation by overexpression of MutS in *Escherichia coli* K-12. *J. Bacteriol.* **182**, 5025–5028 (2000).
53. Bjedov, I. *et al.* Stress-induced mutagensis in bacteria. *Science* **300**, 1404–1409 (2003).
54. Akerlund, T., Nordstrom, K. & Bernander, R. Analysis of cell size and DNA content in exponentially growing and stationary phase batch cultures of *Escherichia coli*. *J. Bacteriol.* **177**, 6791–6797 (1995).
Demonstrates that long-term stationary-phase cells frequently have two, four and even eight chromosomes per cell.
55. Goodman, M. F. Error-prone repair DNA polymerases in prokaryotes and eukaryotes. *Annu. Rev. Biochem.* **71**, 17–50 (2002).
56. Trobner, W. & Piechocki, R. Competition between isogenic *mutS* and *mut*$^+$ populations of *Escherichia coli* K-12 in continuously growing cultures. *Mol. Gen. Genet.* **198**, 175–176 (1984).
57. Chao, L. and Cox, E. C. Competition between high and low mutating strains of *Escherichia coli*. *Evolution* **15**, 931–942 (1998).
58. Cox, E. C. & Gibson, T. C. Selection of high mutation rates in chemostats. *Genetics* **77**, 169–184 (1974).
59. Nestmann, E. R. & Hill, R. F. Mutagenesis by mutator gene *mutH I* in continuous cultures of *Escherichia coli*. *J. Bacteriol.* **119**, 33–35 (1974).
60. Sniegowski, P. D., Gerrish, P. J. & Lenski, R. E. Evolution of high mutation rates in experimental populations of *E. coli*. *Nature* **387**, 703–705 (1997).
61. Shaver, A. C. *et al.* Fitness evolution and the rise of mutator alleles in experimental *Escherichia coli* populations. *Genetics* **162**, 557–566 (2002).
62. Sniegowski, P. Evolution: bacterial mutation in stationary phase. *Curr. Biol.* **14**, R245–R246 (2004).
63. Tegova, R., Tover, A., Tarassova, K., Tark, M. & Kivisaar, M. Involvement of error-prone DNA polymerase IV in stationary-phase mutagenesis in *Pseudomonas putida*. *J. Bacteriol.* **186**, 2735–2744 (2004).
64. Wolff, E., Kim, M., Hu, K., Yang, H. & Miller, J. H. Polymerases leave fingerprints: analysis of the mutational spectrum in *Escherichia coli rpoB* to assess the role of polymerase IV in spontaneous mutation. *J. Bacteriol.* **186**, 2900–2905 (2004).
65. Rattray, A. J & Strathern, J. N. Error-prone DNA polymerases: when making a mistake is the only way to get ahead. *Annu. Rev. Genet.* **37**, 31–66 (2003).
66. Tippin, B., Pham, P. & Goodman, M. F. Error-prone replication for better or worse. *Trends Microbiol.* **12**, 288–295 (2004).
67. Foster, P. L. Stress responses and genetic variation in bacteria. *Mutation Res.* **569**, 3–11 (2005).
68. Layton, J. C. & Foster, P. L. Error-prone DNA polymerase IV is controlled by the stress-response σ factor, RpoS, in *Escherichia coli*. *Mol. Microbiol.* **50**, 549–561 (2003).
Demonstrated that PolIV is under the control of RpoS during stationary phase.
69. Matic, I., Taddei, F. & Radman, M. Survival versus maintenance of genetic stability: a conflict of priorities during stress. *Res. Microbiol.* **155**, 337–341 (2004).
70. Kivisaar, M. Stationary phase mutagenesis: mechanisms that accelerate adaptation of microbial populations under environmental stress. *Environ. Microbiol.* **5**, 814–827 (2003).
71. LeClerc, J. E., Li, B., Payne, W. L. & Cebula, T. A. High mutation frequencies among *Escherichia coli* and *Salmonella* pathogens. *Science* **274**, 1208–1211 (1996).
72. Kotewicz, M. L. *et al.* Evolution of multi-gene segments in the *mutS–rpoS* intergenic region of *Salmonella enterica* serovar Typhimurium LT2. *Microbiology* **148**, 2531–2540 (2002).
73. Li, B. *et al.* Molecular analysis of *mutS* expression and mutation in natural isolates of pathogenic *Escherichia coli*. *Microbiology* **149**, 1323–1331 (2003).
74. Albertini, A. M., Hofer, M., Calos, M. P., Tlsty, T. D. & Miller, J. H. Analysis of spontaneous deletions and gene amplification in the *lac* region of *E. coli*. *Cold Spring Harb. Symp. Quant. Biol.* **47**, 841–850 (1983).
75. Andersson, D. I., Slechta, E. S. & Roth, J. R. Evidence that gene amplification underlies adaptive mutability of the bacterial *lac* operon. *Science* **282**, 1133–1135 (1998).
76. Hendrickson, H., Slechta, E. S., Bergthorsson, U., Andersson, D. I. & Roth, J. R. Amplification-mutagenesis: evidence that "directed" adaptive mutation and general hypermutability result from growth with a selected gene amplification. *Proc. Natl Acad. Sci. USA* **99**, 2164–2169 (2002).
77. Tlsty, T. D., Albertini, A. M. & Miller, J. H. Gene amplifications in the *lac* region of *E. coli*. *Cell* **37**, 217–224 (1984).
78. Whoriskey, S. K., Nghiem, V. H., Leong, P. M., Masson, J. M. & Miller, J. H. Genetic rearrangements and gene amplification in *E. coli*: DNA sequences at the junctures of amplified gene fusions. *Genes Dev.* **1**, 227–237 (1987).
79. Anderson, R. P. & Roth, J. R. Tandem genetic duplications in phage and bacteria. *Annu. Rev. Microbiol.* **31**, 473–505 (1977).
80. Sonti, R. V. & Roth, J. Role of gene duplications in the adaptation of *Salmonella typhimurium* to growth on limiting carbon sources. *Genetics* **123**, 19–28 (1989).
81. Petit, M.-A., Dimpfl, J., Radman, M. & Echols, H. Control of large chromosomal duplications in *Escherichia coli* by the mismatch repair system. *Genetics* **129**, 327–332 (1991).
82. Porwollik, S. *et al.* DNA amplification and rearrangements in archival *Salmonella enterica* serovar *typhimurium* LT2 cultures. *J. Bacteriol.* **186**, 1678–1682 (2004).
Describes significant genomic changes, including deletions and duplications, in long-term stab cultures of *Salmonella*.
83. Kudva, I. T. *et al.* Strains of *Escherichia coli* O157:H7 differ primarily by insertions or deletions, no single-nucleotide polymorphisms. *J. Bacteriol.* **184**, 1873–1879 (2002).
84. Welch, R. A. *et al.* Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proc. Natl Acad. Sci. USA* **99**, 17020–17024 (2002).
A three-way genomic comparison of fully sequenced *E. coli* strains shows that only ~40% of genes are shared.
85. Grozdanov, L. *et al.* Analysis of the genome structure of the nonpathogenic probiotic *Escherichia coli* strain Nissle 1917. *J. Bacteriol.* **186**, 5432–5441 (2004).
86. Schneider, D. *et al.* Genomic comparisons among *Escherichia coli* strains B, K-12, and O157:H7 using IS elements as molecular markers. *BMC Microbiol.* **2**, 18 (2002).
87. Blattner, F. R. *et al.* The complete genome sequence of *Escherichia coli* K-12. *Science* **277**, 1453–1474 (1997).
88. Treves, D. S., Manning, S. & Adams, J. Repeated evolution of an acetate-crossfeeding polymorphism in long-term populations of *Escherichia coli*. *Mol. Biol. Evol.* **15**, 789–797 (1998).
89. Adams, J. Microbial evolution in laboratory environments. *Res. Microbiol.* **155**, 311–318 (2004).
90. Dykhuisen, D. E. Experimental studies of natural selection in bacteria. *Annu. Rev. Ecol. Syst.* **21**, 373–398 (1990).
91. Guttman D. S. & Dykhuisen, D. E. Clonal divergence in *E. coli* as a result of recombination, not mutation. *Science* **266**, 1380–1383 (1994).
92. Cooper, T. E., Rozen, D. E. & Lenski, R. E. Parallel changes in gene expression after 20,000 generations of evolution in *E. coli*. *Proc. Natl Acad. Sci. USA* **100**, 1072–1077 (2003).
93. Vasi, F. K. & Lenski, R. E. Ecological strategies and fitness tradeoffs in *E. coli* mutants adapted to prolonged starvation. *J. Genet.* **78**, 43–39 (1999).
94. Elena, S. F. & Lenski, R. E. Evolution experiments with microorganisms: the dynamics and genetic bases of adaptation. *Nature Rev. Genet.* **4**, 457–469 (2003).
95. Lenski, R. E. *et al.* Evolution of competitive fitness in experimental populations of *E. coli*: what makes one genotype a better competitor than another? *Antonie van Leeuwenhoek* **73**, 35–47 (1998).
96. Schneider, D. & Lenski, R. E. Dynamics of insertion sequence elements during experimental evolution of bacteria. *Res. Microbiol.* **155**, 319–327 (2004).
97. Faure, D. *et al.* Genomic changes arising in long-term stab cultures of *Escherichia coli*. *J. Bacteriol.* **186**, 6437–6442 (2004).
98. Naas, T., Blot, M., Fitch, W. M. & Arber, W. Insertion sequence-related genetic variation in resting *Escherichia coli* K-12. *Genetics* **136**, 721–730 (1994).
99. Edwards, K., Linetsky, I. Hueser, C. & Eisenstark, A. Genetic variability among archival cultures of *Salmonella typhimurium*. *FEMS Microbiol. Lett.* **199**, 215–219 (2001).
100. Sutton, A., Buencamino, R. & Eisenstark, A. *rpoS* mutants in archival cultures of *Salmonella enterica serovar* Typhimurium. *J. Bacteriol.* **182**, 4375–4379 (2000).
101. Tracy, B. S., Edwards, K. K. & Eisenstark, A. Carbon and nitrogen substrate utilization by archival *Salmonella typhimurium* LT2 cells. *BMC Evol. Biol.* **2**, 14 (2002).

Acknowledgements
The author is greatly indebted to R. Kolter, in whose laboratory his studies of long-term stationary phase were initiated, to S. Nair, G. O'Toole, V. Palchevskiy, E. Pepper, E. Zinser and three anonymous reviews for helpful comments and discussions, and to K. Sivaraman for assistance in the preparation of the manuscript. Work in the author's laboratory is supported in part by a grant from the W. M. Keck Foundation and a National Science Foundation CAREER award.

Competing interests statement
The author declares no competing financial interests.

DATABASES
The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
dinB | *gyrA* | *lrp* | *mutH* | *mutL* | *mutS* | *polB* | *rpoS* | *rpsL*
Entrez Genome: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genome
bacteriophage P1
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
Escherichia coli K12 | *E. coli* O157:H7
UniProtKB: http://us.expasy.org/uniprot
leucine-responsive protein | MutH | MutL | MutS | PolII | PolIV | PolV | RpoD | RpoS

FURTHER INFORMATION
Steven E. Finkel's homepage:
http://www.usc.edu/assets/biosci/faculty/mcb_23.html
Access to this interactive links box is free online.

© 2006 Nature Publishing Group