Mutations Enhancing Amino Acid Catabolism Confer a Growth Advantage in Stationary Phase

ERIK R. ZINSER AND ROBERTO KOLTER*

*Department of Microbiology and Molecular Genetics, Harvard Medical School,
Boston, Massachusetts 02115*

Starved cultures of *Escherichia coli* undergo successive rounds of population takeovers by mutants of increasing fitness. These mutants express the growth advantage in stationary phase (GASP) phenotype. Previous work identified the *rpoS819* allele as a GASP mutation allowing cells to take over stationary-phase cultures after growth in rich media (M. M. Zambrano, D. A. Siegele, M. A. Almirón, A. Tormo, and R. Kolter, Science 259:1757–1760, 1993). Here we have identified three new GASP loci from an aged *rpoS819* strain: *sgaA*, *sgaB*, and *sgaC*. Each locus is capable of conferring GASP on the *rpoS819* parent, and they can provide successively higher fitnesses for the bacteria in the starved cultures. All four GASP mutations isolated thus far allow for faster growth on both individual and mixtures of amino acids. Each mutation confers a growth advantage on a different subset of amino acids, and these mutations act in concert to increase the overall catabolic capacity of the cell. We present a model whereby this enhanced ability to catabolize amino acids is responsible for the fitness gain during carbon starvation, as it may allow GASP mutants to outcompete the parental cells when growing on the amino acids released by dying cells.

In the natural environment, chemoorganotrophs such as *Escherichia coli* obtain both their carbon and energy from organic matter released by other cells. The mechanisms of organic nutrient release are variable, ranging from regulated extrusion of metabolic end products to release as a result of death and lysis of donor cells (3, 21–23, 34). However, the actual bioavailability of carbon in nature is low due to intense competition (22, 23). As a result, natural microbial populations spend the majority of their lives under starvation stress, interspersed with sporadic and short-lived periods of growth as nutrients become available.

Our laboratory uses carbon-starved cultures of *E. coli* as an experimental model to understand the processes of survival and evolution in natural microbial populations. *E. coli* can survive extended periods of starvation. In aerated rich medium (Luria-Bertani [LB] broth), *E. coli* ceases growth due to carbon limitation (37). During the first several days of starvation, the population loses 90 to 99% of the viable counts (40). However, the viable counts nearly level off after these first few days, and populations can survive for several years in this spent LB medium aerated at 37°C without further addition of carbon (7, 8). As the cultures consume exogenous carbon during exponential growth, the biomass is the most likely source of carbon during extended survival, which becomes available when the cells die.

While the overall population of stationary-phase *E. coli* cultures may be considered starved in that there is no net increase in biomass, there are subpopulations that are clearly not starved, as they are able to grow as a subculture and take over the population (8, 38–40). These subpopulations consist of mutants with enhanced fitness during starvation. The ability to grow during starvation has been termed the growth advantage in stationary phase (GASP) phenotype (38). Studies on cultures starved for extended periods demonstrate that the GASP phenomenon is continuous: multiple rounds of population takeovers occur throughout the starvation period (7, 40). Interestingly, as the cultures age, they increase in diversity, as several genetically distinct subpopulations coexist (7).

The first mutation conferring the GASP phenotype after growth in rich media was identified as an allele of *rpoS* (40), a gene whose product, σ^S, is responsible for the regulation of many genes during starvation stress (12). Transduction of the GASP allele of *rpoS* (*rpoS819*) into an otherwise wild-type strain was sufficient to confer the GASP phenotype (40). The *rpoS819* allele is a 46-bp duplication at the 3′ end of the gene, which results in a replacement of the last four residues in σ^S with 39 new amino acids. Expression of two σ^S-dependent genes, *katE* (25) and *bolA* (4, 15), are both reduced in the *rpoS819* strain (40), indicating a reduction of function in this allele. The physiological basis for the fitness gain of the *rpoS819* mutation is not yet known.

The purpose of our investigation was to understand how GASP mutations alter cell physiology to provide fitness gains in stationary phase. To this end, we sought to identify and characterize new GASP mutations. ZK1141, an isolate from an aged culture of the *rpoS819* strain, was capable of outcompeting its *rpoS819* parent, indicating that additional GASP mutations accumulated in this strain (38). In this study, we have demonstrated that the ZK1141 strain has acquired three new GASP mutations, each of which can confer the GASP phenotype on the *rpoS819* parent. Each of these newly identified GASP alleles, as well as the *rpoS819* allele, increased starvation survival fitness in an additive manner. Each of these four GASP alleles also conferred growth advantages on amino acids as the sole sources of carbon and energy. Similar to the competitive fitnesses, these growth phenotypes were additive.

MATERIALS AND METHODS

Bacterial strains. The *E. coli* strains used in this study are listed in Table 1.

Media and growth conditions. All experiments were performed at 37°C, except where noted. The media used in this study have been previously described (19). M63 minimal medium was supplemented with 1 µg of thiamine per ml and 1 mM MgSO$_4$. All amino acids used in this study were of the L configuration. Where appropriate, LB plates were supplemented with streptomycin (25 µg/ml), nali-

* Corresponding author. Mailing address: Department of Microbiology and Molecular Genetics, Harvard Medical School, 200 Longwood Ave., Boston, MA 02115. Phone: (617) 432-1776. Fax: (617) 738-7664. E-mail: kolter@mbcrr.harvard.edu.

TABLE 1. *E. coli* strains used in this study

Strain	Genotype or phenotype	Reference or source
GASP strain		
ZK126	W3110 *tna2 ΔlacU169*; G_0	6
ZK819	ZK126 *rpoS819 rpsL* Sm^r; G_I	40
ZK820	ZK126 *rpoS819 gyrA* Nal^r; G_I	40
ZK1141	ZK819 *sgaA sgaB sgaC*; G_{II}	38
ZK2552	ZK819 *ilvGMEDA$^+$* (spontaneous suppressor) Val^r	This study
ZK2553	ZK819 *bgl$^+$* (spontaneous suppressor) Bgl$^+$	This study
ZK2554	ZK1141 *ilvGMEDA$^+$* (same allele as in ZK2552) Val^r	This study
ZK2555	ZK1141 *bgl$^+$* (spontaneous suppressor) Bgl$^+$	This study
ZK2557	ZK2553 *rpoS$^+$*	This study
ZK2559	ZK2553 *sgaB*	This study
ZK2561	ZK2553 *sgaA*	This study
ZK2563	ZK2553 *sgaC*	This study
ZK2564	ZK2553 *sgaA sgaB sgaC*	This study
ZK2618	ZK1141 *trpB*::Tn*10*Tc^r	This study
Other strains		
CAG12173	MG1655 *cysC95*::Tn*10*Tc^r	31
CAG18501	MG1655 *rbsD*::Tn*10*Tc^r	31
CAG18528	MG1655 *zbj-3110*::Tn*10*Kan^r	31
KER176	*rpsL lipA150*::Tn*1000*dKan^r	35
NU1107	VJS433 *serC*::mini-Mu*d*I194 Kan^r	14
ZK173	MB2 (Hfr *Δgal trpB*::Tn*10 proC*::Tn5 *lacU169*)	Lab stock
ZK1000	ZK126 *ΔrpoS*::Kan^r	4

dixic acid (20 µg/ml), tetracycline (15 µg/ml), kanamycin (50 µg/ml), or chloramphenicol (30 µg/ml). All chemicals were from Sigma. Optical density (OD) was monitored with a Spectronic 20D+ Spectrophotometer (Milton Roy).

Genetic techniques. Phage P1*vir* transduction and Hfr conjugation using the Singer et al. (31) strain collections were performed as described elsewhere (19). Insertional mutagenesis with mini-Tn*10* transposons from the vectors λNK1323 (Tc^r), λNK1316 (Kan^r), and λNK1324 (Cm^r) was performed as described previously (13).

Construction of GASP strains. Because incorporation of new genetic markers can alter the fitness of bacteria, we constructed our strains such that the final strains differed from the parental strains only by the allele(s) of the GASP loci. This was achieved by first bringing an auxotrophy mutation or the streptomycin-sensitive (Sm^s) allele of *rpsL* that mapped near the GASP loci (see Results) into the recipient by P1*vir* transduction. We could then cotransduce the GASP alleles with P1*vir* grown on ZK1141 or ZK126 into these strains by selecting for prototrophy or Sm^r, and then testing among those transductants for the cotransduction of the GASP allele, by assaying directly for the GASP phenotype or another physiological phenotype where appropriate (see below).

The *rpoS$^+$* strains were constructed by using the *cysC95*::Tn*10*Tc^r mutation from CAG12173 and the *ΔrpoS*::Kan^r mutation from ZK1000 (4). Strains carrying the *sgaA* allele of ZK1141 were constructed with the *lipA150*::Tn*1000*dKan^r marker from strain KER176 (35). Mutants with the *sgaA* GASP allele were identified by their larger colony sizes when grown on M63 glutamate (0.5%) plates (see Results). Strains carrying the *sgaB* allele of ZK1141 were constructed with the *serC*::mini-Mu*d*I194 allele from strain NU1107 (14). Mutants with the *sgaB* GASP allele were identified by their increased sensitivity to serine, determined by a filter disc technique and confirmed by assaying for mucoidy at 30°C (see Results). Strains carrying the *sgaC* GASP allele were constructed with the *rpsL$^+$* (Sm^s) allele of ZK126. Mutants with the *sgaC* GASP allele were identified by scoring for the *sgaC* GASP phenotype (see below).

Stationary-phase competitions. Competition experiments were adapted from those of Zambrano et al. (40). For competitions in LB, initial cultures were inoculated from frozen glycerol stocks into 3 ml of LB and grown overnight. These were then subcultured 1:100 into fresh LB and incubated for 24 h before being mixed for the competitions. The two populations were monitored by serial dilution in M63 medium and plating on minimal salicin and minimal glucose-plus-valine plates. We verified that the majority of the population remained prototrophic (and hence detectable on the selection media) by comparing the counts on the selection media with those on LB. In no case did we observe a difference in total viable counts on minimal and rich media. For competitions in M63-serine (0.5%)-isoleucine (0.03%)-valine (0.03%)-leucine (0.03%)-NaCl

(0.5%), colonies were inoculated from an LB plate into the defined medium, and the cultures were incubated until they reached stationary phase (1 to 4 days). Cultures of strain ZK2618 (*trpB*::Tn*10*) were also supplemented with 0.004% tryptophan to facilitate growth. The ZK1141 and ZK2618 cultures were washed in M63 medium before inoculating as a 1:10,000 minority into the ZK820 cultures. The two populations were monitored by serial dilution in M63 medium and plating on LB-streptomycin and LB-nalidixic acid.

Molecular techniques. The DNA flanking the mini-Tn*10*Cm^r transposons was determined by using an arbitrary PCR-based protocol (5), with the modifications described by Pratt and Kolter (28). PCR products were subjected to sequence analysis by the Micro Core Facility, Department of Microbiology and Molecular Genetics, Harvard Medical School, and the sequences were compared with the GenBank DNA database by using the BLAST program (1).

RESULTS

Isolation and mapping of three new GASP loci from an aged *rpoS819* strain. Our approach to investigate the physiology of the GASP phenomenon was to identify and characterize several mutations able to confer the GASP phenotype. That many rounds of GASP takeover occur in starved cultures of *E. coli* (7, 40) implies that the survivors have acquired multiple GASP mutations. As we were interested in exploring potential genetic interactions of accumulated GASP mutations, our strategy was to isolate and investigate the GASP mutations of a single mutant survivor (ZK1141) from a culture that had undergone several rounds of population takeover.

We have devised a nomenclature that describes the relationship of the GASP mutants within a single lineage (Table 2). G_n (short for GASP$_n$) denotes an isolate from an aged culture of strain G_{n-1} that is capable of outcompeting the G_{n-1} strain in a stationary-phase competition. In this study we analyzed the G_{II} strain ZK1141 (*rpoS819 sgaA sgaB sgaC*), which is descended from the G_I strain ZK819 (*rpoS819*), which is descended from the G_0 wild-type strain ZK126 (all G_n designations in this work refer to these strains).

ZK1141 was isolated as a Sm^r survivor of a mixed culture; a 10-day-old culture of ZK819 and a 1-day-old culture of the Sm^s nalidixic acid-resistant (Nal^r) version of ZK819 (ZK820) were grown together in fresh LB and then incubated for a week under starvation conditions (37). ZK1141 has the G_{II} phenotype: it is capable of completely taking over a 1-day-old population of G_I when inoculated as a 1-day-old minority (38).

The mutation responsible for the G_I GASP phenotype of ZK819 has been identified as an allele of *rpoS* called *rpoS819* (40). The G_{II} phenotype of ZK1141 was initially thought to be due to a single mutation, which was termed *sga*, for stationary-phase growth advantage (38). However, genetic analysis of ZK1141 (see below) has demonstrated that there are three GASP mutations that each contribute to the G_{II} GASP phenotype.

We identified the mutations responsible for the G_{II} phenotype of ZK1141 with a genetic selection technique adapted from Zambrano et al. (40): when introduced into the G_I strain, the G_{II} GASP alleles conferred a GASP phenotype versus the G_I parent. We made a pool of approximately 1,000 G_{II} mutants with randomly inserted mini-Tn*10*Tc^r or Kan^r transposons in

TABLE 2. GASP nomenclature

GASP mutant designation	Strain used in this study	Strain history	GASP mutations
G_0	ZK126	Initial strain	None (wild type)
G_I	ZK819	G_0 strain aged in LB	*rpoS819*
G_{II}	ZK1141	G_I strain aged in LB	*rpoS819 sgaA sgaB sgaC*

the chromosome. We then selected for linkage of the mini-Tn10 to the GASP alleles by making a P1vir lysate of the pool and infecting G$_I$ with this lysate to obtain a new pool of about 500 Tc^r or Kan^r transductants. These pools were then inoculated as a minority into 1-day-old ZK820 (the Nal^r Sm^s G$_I$ strain) cultures, and the cultures were allowed to further starve to select from the pool those G$_I$ transductants carrying G$_{II}$ alleles: these transductants could grow in the starved culture, whereas the G$_I$ transductants not carrying the G$_{II}$ GASP alleles could not. The G$_I$ transductants carrying the G$_{II}$ GASP alleles were isolated from the culture several days after the pool was inoculated by titering the culture onto LB-streptomycin Sm plates. We then confirmed that the mutants isolated on the LB-streptomycin titer plate had the G$_{II}$ GASP alleles by moving their mini-Tn10 alleles into a fresh G$_I$ background by P1vir transduction and testing among those transductants for cotransduction of the G$_{II}$ GASP phenotype by competition versus the Nal^r G$_I$. In this manner, we identified three distinct mutations harbored by G$_{II}$ that conferred a GASP phenotype to G$_I$. These mutations were designated $sgaA$, $sgaB$, and $sgaC$.

Mapping the G$_{II}$ GASP loci. During our investigations of strain ZK1141, we discovered that it has two phenotypes that its ZK819 parent lacks: mucoid growth on glucose at 30°C (but not at 37°C) and an enhanced sensitivity to the amino acid serine. Serine is a competitive inhibitor of homoserine dehydrogenase I, and high levels of intracellular serine result in isoleucine starvation (9, 10). We scored relative serine sensitivities by streaking the strains on an M63-glucose plate toward a filter disc soaked with 10% serine placed in the center of the plate and determining the relative sizes of the growth inhibition zones. Instrumental in the mapping of the $sgaB$ locus was the discovery that the $sgaB$ GASP allele is responsible for both the mucoidy and serine sensitivity phenotypes of ZK1141. The $sgaB$ mutation was mapped to min 20 by using the Hfr and P1 mapping sets (31); the $sgaB$ mutation was 95% linked to the zbj-3110::Tn10Kan^r marker of CAG18528, located at min 19.8 (2, 31).

The $sgaA$ and $sgaC$ mutations were each mapped by determining the location of random mini-Tn10Cm^r insertions linked to the initial mini-Tn10Tc^r or mini-Tn10Kan^r insertions used to isolate the GASP alleles (see above). These mini-Tn10Cm^r markers were mapped by the arbitrarily primed PCR technique. The $sgaA$-linked mini-Tn10Cm^r was found to be in $ybdN$ at min 13.7 and was 60% linked to the mini-Tn10Tc^r, which in turn was 10% linked to $sgaA$. The $sgaC$-linked mini-Tn10Cm^r was found to be in $gspA$ at min 74.4, which was 66% linked to the mini-Tn10Kan^r, which in turn was 50% linked to $sgaA$.

The GASP phenotypes of three new GASP loci: $sgaA$, $sgaB$, and $sgaC$. Having isolated and mapped three GASP alleles of strain G$_{II}$, we wanted to test whether each of these alleles alone could confer a selective advantage over the G$_I$ parent during stationary phase. We assayed for the GASP phenotype by mixing 24-h-old cultures of the two strains in question and monitoring changes in each population by viable count assay. The two populations were distinguished because they carry different neutral markers. Marker neutrality was confirmed empirically by switching the markers between the strains and performing all mixes reciprocally. Viable counts of each population are determined by titering the culture on the two relevant selection plates. Previous reports suggested that the $rpsL$ allele conferring Sm^r and the $gyrA$ allele conferring Nal^r are neutral in stationary-phase competitions in $E.$ $coli$ (7, 40). We confirmed that the Sm^r and Nal^r markers are neutral during extended starvation. However, during the first 4 days of competition of a 1:1 mix (see below), viable counts were consistently 2- to 10-fold higher for the Sm^r strain than for the Nal^r
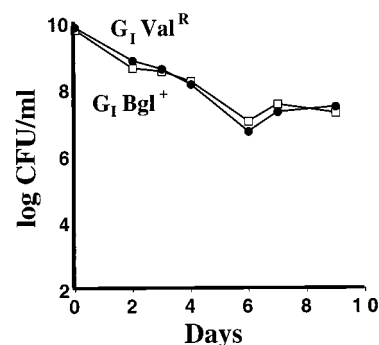

FIG. 1. The Val^r and Bgl$^+$ markers are selectively neutral in stationary phase. One-day-old cultures of the Val^r (ZK2552) (●) and Bgl$^+$ (ZK2553) (□) derivatives of the G$_I$ mutant (ZK819) were mixed 1:1 and cocultured. Viable counts were assayed on M63-glucose-valine or M63-salicin plates. Neither strain was outcompeted in four competitions.

strain, although the Sm^r counts eventually dropped to equal those of the Nal^r population. Since the first 4 days of the competitions in this study were critical, we differentially marked the ZK819 (Sm^r) strain with two new markers that remain neutral throughout the competition: valine-resistant growth on glucose (Val^r) and the ability to grow on β-glucosides (Bgl$^+$). The Val^r and Bgl$^+$ markers were isolated as spontaneous mutations conferring the ability to grow on M63-glucose-valine or M63-salicin plates, respectively. Unless otherwise noted, this pair of markers was used for all competitions described below.

The Val^r mutation mapped to the $ilvGMEDA$ operon. Since $E.$ $coli$ K-12 has a frameshift in $ilvG$, which prevents expression of the one Val^r isozyme of acetohydroxy acid synthase of $E.$ $coli$, encoded by $ilvGM$ (16), the Val^r mutation is most likely a suppressor of this frameshift. The Bgl$^+$ mutants arose at high frequency in our ZK819 and ZK1141 strains (about 1 in 10^7 plated cells), which prevented transduction of the same allele into either background. We therefore selected for Bgl$^+$ mutants in both ZK819 and ZK1141 backgrounds. Both Bgl$^+$ mutations mapped to the bgl operon and are likely to be insertions or point mutations in the $bglR$ regulatory locus (30). The neutrality of the Val^r and Bgl$^+$ mutations throughout the starvation period was demonstrated by competitions of the Val^r and Bgl$^+$ derivatives of the ZK819 and ZK1141 strains. Neither marker conferred a competitive advantage or disadvantage, as determined by 1:1 competitions (Fig. 1). Furthermore, neither strain grew as a 1:1,000 minority versus the other strain (data not shown). Finally, neither of the two mutations altered the fitness of the strains versus the Val^s Bgl$^-$ parent (data not shown). Each mix was performed at least four times.

The fact that we can isolate mutants with the GASP phenotype from starved bacterial cultures suggests that these GASP mutants, starting from a single mutant cell, are able to increase in number during the starvation period to establish themselves as the majority population. One assay for the GASP phenotype is thus the ability of the mutant, when placed as a minority population in a culture of the parent, to grow relative to the parent and eventually establish itself as the majority population. To demonstrate this aspect of GASP for each of the G$_{II}$ GASP alleles, we constructed the G$_I$ $sgaA$ (the G$_I$ strain that has the $sgaA$ allele of G$_{II}$), G$_I$ $sgaB$, and G$_I$ $sgaC$ strains with the Val^r or Bgl$^+$ selectable marker and competed them as a 1,000-fold minority with the G$_I$ parent that had the other selectable marker. Figure 2 demonstrates that each of the three GASP alleles confers the ability to grow when inoculated
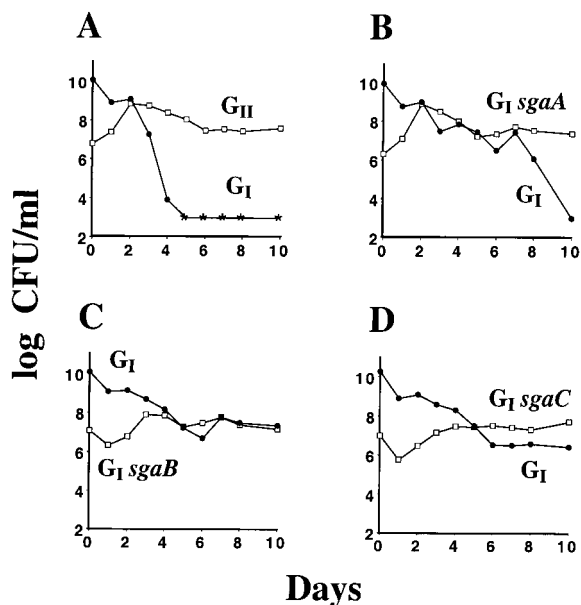
FIG. 2. The G_{II} alleles of ZK1141 confer the GASP phenotype. Into a 1-day-old culture of the G_I mutant (ZK2552) (●) was inoculated as a 1,000-fold minority of a 1-day-old culture of the G_{II} strain (ZK2555) (A), the G_I sgaA strain (ZK2561) (B), the G_I sgaB strain (ZK2559) (C), or the G_I sgaC strain (ZK2563) (□) (D). Asterisks indicate that viable counts fell below detectable levels ($<10^3$ CFU/ml). The patterns of GASP takeovers were identical in six replicate mixtures, including ones where the selectable markers were switched between competing strains.

as a minority and take over the population. Like the G_{II} mutant (Fig. 2A), the G_I sgaA mutant grew on the first day of the competition (Fig. 2B), while the G_I sgaB and G_I sgaC mutants experienced a 1-day lag period before growth (Fig. 2C and D).
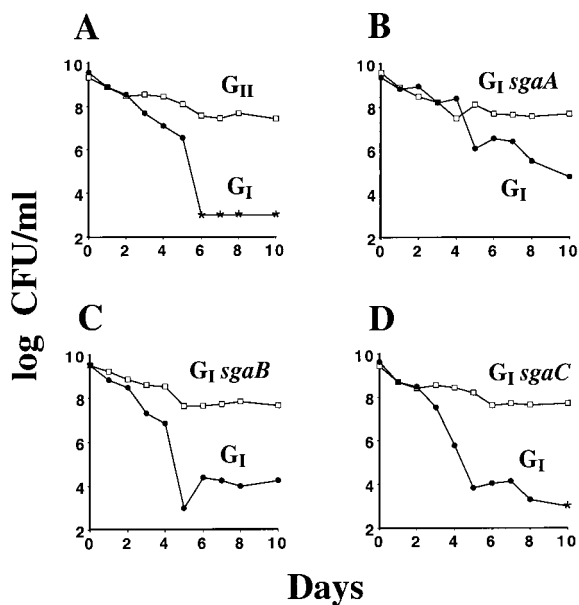

FIG. 3. The G_{II} alleles confer a competitive advantage to G_I cells. A 1-day-old culture of the G_I mutant (ZK2552) (●) was mixed 1:1 with a 1-day-old culture of the G_{II} strain (ZK2555) (A), the G_I sgaA strain (ZK2561) (B), the G_I sgaB strain (ZK2559) (C), or the G_I sgaC strain (ZK2563) (□) (D). Asterisks indicate that viable counts fell below detectable levels ($<10^3$ CFU/ml). The patterns of GASP takeovers were identical in six replicate mixtures, including ones where the selectable markers were switched between the competing strains.
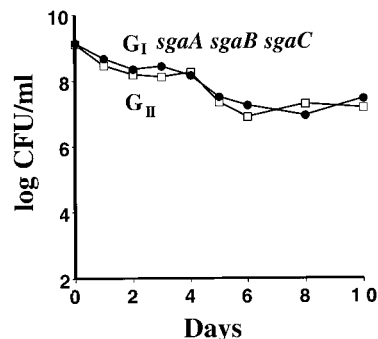

FIG. 4. The sgaA, sgaB, and sgaC alleles are sufficient for the G_{II} GASP phenotype of ZK1141. A 1-day-old culture of the reconstructed G_{II} strain G_I sgaA sgaB sgaC (ZK2564) (●) was mixed 1:1 with a 1-day-old culture of the G_{II} strain (ZK2554) (□). Neither strain was outcompeted in eight competitions.

However, none of the G_I mutants with a single G_{II} GASP allele was able to eliminate the majority population as rapidly or as completely as the G_{II} mutant, suggesting that their fitness advantages are additive, a possibility addressed below.

Another component of the GASP phenotype is the ability of the GASP mutant to directly outcompete the parent, resulting in the death of the parent. This ability is assayed by mixing the two cultures in a 1:1 ratio and looking for the decline of one of the two populations during the starvation period. Figure 3 demonstrates that all three of the G_I mutants with a G_{II} GASP allele are capable of outcompeting the G_I parent. Interestingly, during the first 2 days of the 1:1,000 and 1:1 mixes for both G_{II} and G_I sgaA mutants (Fig. 2A and B and 3A and B), the GASP mutant could grow as a minority population but did not yet outcompete the G_I parent when mixed in equal numbers. This finding implies that while there may be utilizable carbon for the strains available during the first 2 days, the competition for those nutrients does not become lethal for the parental strain until the environmental conditions change as a result of continued starvation.

The three G_{II} GASP mutations are necessary and sufficient for the G_{II} GASP phenotype. Our selection method identified three new distinct GASP loci on the chromosome of G_{II}: sgaA, sgaB, and sgaC. To determine whether there are additional GASP mutations in G_{II}, we examined whether the three GASP mutations identified so far were both necessary and sufficient for the G_{II} GASP phenotype. Our first approach was to compete the G_{II} mutant with a constructed G_I sgaA sgaB sgaC mutant. Neither strain had a competitive advantage when competed in a 1:1 mix (Fig. 4). Furthermore, the G_{II} mutant was unable to grow when inoculated as a 1:1,000 minority into a culture of the G_I sgaA sgaB sgaC (data not shown). These results demonstrate that the G_{II} strain lacks any additional GASP mutations that would confer a competitive advantage over the constructed strain. Additionally, the G_I sgaA sgaB sgaC strain grew immediately and completely displaced the G_I strain as the majority when inoculated as a 1:1,000 minority into the G_I culture (data not shown); this GASP phenotype was indistinguishable from that of the G_{II} strain (Fig. 2A). The results from these three different competition experiments thus demonstrate that the sgaA, sgaB, and sgaC mutations are sufficient for the G_{II} GASP phenotype.

We next asked if all three G_{II} GASP alleles were necessary for the G_{II} GASP phenotype. We competed 1:1 the G_{II} strain versus one of three constructed strains harboring two of the three G_{II} GASP mutations: G_I sgaA, sgaB, G_I sgaA sgaC, or G_I sgaB sgaC. In every case, the G_{II} strain outcompeted the con-
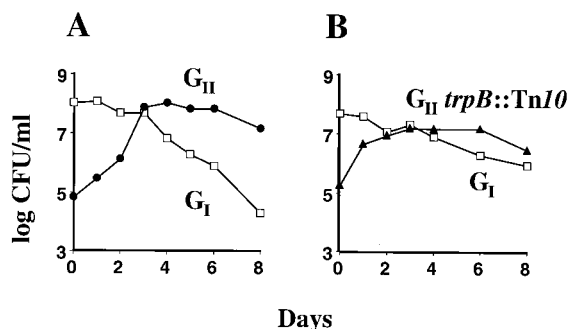
A

B

Days

FIG. 5. GASP in a chemically defined medium. Stationary-phase cultures grown in M63-serine (0.5%)-isoleucine (0.03%)-valine (0.03%)-leucine (0.03%)-NaCl (0.5%) (plus tryptophan [0.004%] for the G_{II} trpB::Tn10 strain [ZK2618]) were mixed 1:10,000 to assay the GASP phenotype. Both the G_{II} strain (ZK1141; ●) (A) and the tryptophan auxotrophic G_{II} trpB::Tn10 strain (ZK2618; ▲) (B) express the GASP phenotype as a minority versus the G_I strain (ZK820; □). The patterns of GASP takeovers were identical in four competitions.

structed strain (data not shown). Hence, all three reconstructed strains, each lacking one of the three G_{II} alleles, were less fit than G_{II}, indicating that each of the three G_{II} alleles is necessary for the full fitness gain of the G_{II} strain. That each is necessary for the G_{II} GASP phenotype indicates that they act additively to confer higher and higher fitnesses in stationary phase.

GASP mutants obtain nutrients from dying cells in a chemically defined medium. Because growth in LB ceases due to carbon limitation (37), it has been assumed that the GASP mutants obtain the nutrients required for growth from the dying majority population. To demonstrate directly that the dying cells release nutrients which the GASP mutants can utilize during growth, we identified a chemically defined medium in which the G_{II} GASP strain could grow and outcompete the G_I strain. Figure 5A shows the GASP phenotype of the G_{II} strain versus G_I after both cultures were grown to stationary phase in M63 salts medium supplemented with serine (0.5%), isoleucine, valine, and leucine (0.03% each), and NaCl (0.5%). As in LB, the G_{II} strain grew rapidly as a 1:10,000 minority to take over the population. Like the prototroph, a tryptophan auxotrophic derivative of the G_{II} strain (trpB::Tn10) was able to grow and take over the G_I minority (Fig. 5B). This finding indicates that the G_{II} strain can scavenge enough tryptophan to meet its growth requirement during the population takeover. As no exogenous tryptophan was supplied by the medium, the only remaining source of tryptophan is the cells of the dying majority population. This is the first evidence that the GASP mutants can scavenge nutrients released by the dying cells during carbon starvation.

The three G_{II} GASP alleles and the rpoS819 allele confer faster growth on amino acids. We reasoned that the primary selective force acting on carbon-starved cells is the ability to utilize the carbon released by the dying majority population.

Therefore, we asked whether the GASP mutations confer faster growth on carbon sources that may resemble the nutrients released by dying cells. The three G_{II} GASP mutations were tested in the G_I background, as it was from this background that G_{II} was selected. We also tested the growth phenotypes of the G_I GASP mutation, rpoS819, by comparing its growth rate with that of the G_I strain carrying the rpoS+ allele (this is essentially a G_0 strain, as the rpoS819 mutation is sufficient to confer the G_I GASP phenotype of ZK819 [38]). We first assayed growth on LB, a rich medium containing many of the building blocks, vitamins, and energy sources necessary for growth. The growth rates on LB were indistinguishable between the different mutant strains (data not shown).

The composition of the medium during prolonged carbon starvation has not been characterized. However, we speculated that amino acids are the most abundant nutrients released by the dying cells, given that amino acids account for most of the dry weight of *E. coli* (26). Hence, we determined the relative growth rates of the GASP mutants on mixtures of amino acids, either as a combination of monomers and short peptides (tryptone) or only as monomers (Casamino Acids). While none of the individual GASP alleles had much of an effect, the G_{II} strain harboring all three G_{II} GASP mutations grew significantly faster than G_I on the monomer and peptide mixture (Table 3). In contrast, all four GASP mutations conferred significantly faster growth on the mixture of the amino acid monomers. Interestingly, in every case, the strains with more GASP mutations grew faster, indicating that the GASP mutations act additively to confer faster growth on mixtures of amino acids.

Since all four GASP mutations confer faster growth on mixtures of amino acids, we attempted to identify individual amino acids that the GASP mutants could catabolize more rapidly. We assayed growth in liquid media containing M63 salts and the amino acid in question at 0.5% (wt/vol). Because of the potential problem of amino acids such as serine and cysteine inhibiting isoleucine biosynthesis and thus preventing growth on single amino acids (9, 10), we supplemented all of the growth media with isoleucine (0.03%). We assayed for the ability of the GASP mutants to grow on each of the 20 amino acids singly as the sole source of carbon and energy. At least one of the six strains tested could grow on alanine, asparagine, aspartate, glutamate, glutamine, proline, serine, or threonine; none grew on the other 11 amino acids. While *E. coli* K-12 can grow on tryptophan (32), our strains were not expected to grow, as they lack tryptophanase activity due to the tna2 mutation.

All four GASP alleles conferred growth advantages on several amino acids, manifested as a higher growth rate or, in some cases, a new ability to grow on the particular amino acid (Table 4). The rpoS819 GASP allele conferred upon the cell the new ability to utilize asparagine and glutamine as sole sources of carbon and energy. However, the rpoS819 mutants grew to an OD at 600 nm of only about 0.3 on glutamine. It is therefore uncertain whether the cells grew incompletely on

TABLE 3. Relative growth rates on amino acid mixtures

Carbon and energy source (1.0%)	Relative growth rate ($\pm$SD, $n = 3$)[a]					
	G_0 (ZK2557)	G_I (ZK2553)	G_I sgaA (ZK2561)	G_I sgaB (ZK2559)	G_I sgaC (ZK2563)	G_{II} (ZK2555)
Tryptone	1.00 ($\pm$0.01)	0.98 ($\pm$0.01)	1.00 ($\pm$0.03)	1.03 ($\pm$0.01)	1.03 ($\pm$0.02)	1.14 ($\pm$0.03)
Casamino Acids	1.00 ($\pm$0.02)	1.16 ($\pm$0.01)	1.34 ($\pm$0.01)	1.31 ($\pm$0.05)	1.21 ($\pm$0.02)	1.43 ($\pm$0.02)

[a] A relative growth rate of 1.00 indicates a generation time (in hours) of 0.59 on tryptone or 1.28 on Casamino Acids.

TABLE 4. Relative growth rates on single amino acids as carbon sources

Amino acid	Relative growth rate ($\pm$SD, $n = 2$)[a]					
	G_0 (ZK2557)	G_I (ZK2553)	G_I sgaA (ZK2561)	G_I sgaB (ZK2559)	G_I sgaC (ZK2563)	G_{II} (ZK2555)
Alanine	1.00 ($\pm$0.01)	1.04 ($\pm$0.02)	0.88 ($\pm$0.00)	1.25 ($\pm$0.01)	1.11 ($\pm$0.02)	1.21 ($\pm$0.01)
Asparagine	0	1.00 ($\pm$0.03)	1.90 ($\pm$0.04)	0.69 ($\pm$0.03)	0.72 ($\pm$0.04)	1.60 ($\pm$0.03)
Aspartate	0	0	1.00 ($\pm$0.01)	0	0	0.53 ($\pm$0.00)
Glutamate	1.00 ($\pm$0.03)	2.93 ($\pm$0.01)	9.14 ($\pm$0.00)	1.92 ($\pm$0.07)	2.17 ($\pm$0.03)	9.29 ($\pm$0.00)
Glutamine[b]	0	1.00 ($\pm$0.02)	0.87 ($\pm$0.04)	0.75 ($\pm$0.09)	0.76 ($\pm$0.03)	0.82 ($\pm$0.00)
Serine	1.00 ($\pm$0.05)	1.63 ($\pm$0.36)	0.86 ($\pm$0.06)	9.81 ($\pm$0.66)	2.30 ($\pm$0.14)	9.19 ($\pm$0.28)
Threonine	1.00 ($\pm$0.02)	2.17 ($\pm$0.11)	1.16 ($\pm$0.02)	3.66 ($\pm$0.00)	3.92 ($\pm$0.11)	5.01 ($\pm$0.04)
Proline[c]	1.00 ($\pm$0.1)	0.72 ($\pm$0.11)	2.1 ($\pm$0.1)	0.45 ($\pm$0.05)	1.6 ($\pm$0.1)	10.5 ($\pm$0.7)

[a] For all amino acids except proline (see footnote c), growth rates in liquid M63 plus 0.5% amino acid (plus 0.03% isoleucine) medium were determined. A relative growth rate of 1.00 indicates a generation time (in hours) of 4.50 on alanine, 14.0 on asparagine, 11.2 on aspartate, 58.5 on glutamate, 12.0 on glutamine, 61.2 on serine, or 304 on threonine; a relative growth rate of zero indicates no growth.

[b] Cultures stopped growing at an OD of 600 nm of 0.2 to 0.3. This was not due to alkalinization of the medium, as the pH remained below 7.5, nor was it due to end product inhibition, as the cultures grew readily when spiked with 0.2% glucose.

[c] Overnight LB cultures were diluted into M63 medium and plated on M63 proline (0.5%) plus isoleucine (0.03%). Values indicate relative colony areas ($\pm$SD, $n = 20$) after 4 days of incubation.

glutamine or grew on an impurity in the glutamine supply instead. The rpoS819 allele alone conferred faster growth on glutamate, serine, threonine, and alanine. The sgaA allele conferred the new ability to grow on aspartate and conferred faster growth on asparagine and glutamate. The sgaB and sgaC alleles both conferred faster growth on alanine, threonine, and serine. Comparison of the growth rates for the G_0, G_I, and G_{II} strains indicates that both the repertoire of amino acids and the growth rates on the amino acids increase as more GASP alleles are added and demonstrates that these GASP alleles act additively to increase the overall capacity to catabolize amino acids.

We were not able to obtain relative growth rates on proline in liquid cultures, because the cultures were consistently taken over by faster-growing mutants. However, we were able to obtain estimates of relative growth rates by comparing the sizes (surface area) of colonies grown on M63-proline (plus isoleucine) plates (Table 4). The sgaA and sgaC alleles conferred faster growth on proline, and their effects on growth were strikingly additive, as the G_{II} colonies were significantly larger than those of the G_I mutants with either single allele alone.

Interestingly, the four GASP alleles conferred slower growth on several of the amino acids (Table 4). The rpoS819 mutation conferred slower growth on proline; the sgaA mutation conferred slower growth on alanine, glutamine, and serine; the sgaB mutation conferred slower growth on asparagine, glutamate, glutamine, and proline; and the sgaC mutation conferred slower growth on asparagine, glutamate, and glutamine. This indicates that while the individual GASP alleles confer fitness gains on some amino acids, each also confers a fitness loss on other amino acids. We discuss the implications of these observations below.

DISCUSSION

Previous work identified an allele of rpoS, rpoS819, as a mutation that can confer the GASP phenotype on E. coli (40). Genetic analysis of an isolate (G_{II}) from a starved culture of the rpoS819 GASP strain (G_I) has revealed three new GASP mutations: sgaA, sgaB, and sgaC. All four GASP mutations of this isolate map to different regions of the chromosome, suggesting that there are many loci that when mutated can confer fitness advantages in stationary phase.

As each of the three new GASP mutations acquired by the G_{II} strain can confer a GASP phenotype on the G_I parental

strain, it is most likely that they were acquired as a result of three successive GASP takeover events. G_{II} was isolated from a culture starved for a total of about 2.5 weeks, which suggests that in our experimental system population takeovers during starvation can happen faster than once per week. The fact that the three G_{II} GASP mutations are necessary (and sufficient) for the G_{II} GASP phenotype demonstrates directly that the acquisition of multiple GASP mutations can provide successively higher fitnesses for starved bacteria. The continual accumulation of GASP mutations by cells within the surviving population can account for the multiple rounds of population takeovers observed in starved cultures (7, 40). Our results thus support the growing body of evidence that starved populations are highly dynamic and undergo frequent population takeovers as a result of fitness differences among the competing subpopulations (7, 8, 40).

A major purpose of our investigation was to understand how GASP mutations alter cell physiology to provide fitness gains in stationary phase. Previous studies have demonstrated that fitness gains during conditions of limited substrate availability in chemostats or selections for growth on novel substrates were manifested as increases in catabolic potential for those substrates (11, 17, 20, 24, 33, 36). In our system, we have observed that dying cells in stationary-phase cultures release nutrients (e.g., tryptophan) that can be utilized by the growing GASP mutants, and we hypothesized that the GASP mutants selected are those that outcompete their parents for these limited substrates. We observed a direct correlation between relative GASP fitness and relative ability to catabolize amino acids, which are likely to be the most abundant nutrients released by dying cells (26). The G_I GASP mutation rpoS819 and the three G_{II} GASP mutations sgaA, sgaB, and sgaC all confer higher growth rates on an amino acid mixture (Casamino Acids). To our knowledge, this is the first report that mutations in rpoS can alter amino acid catabolism during exponential growth, extending previous observations that wild-type σ^S affects the physiology of both arrested and growing cells (12, 27).

All four of the GASP mutations examined in this study also increase the ability of the cell to utilize certain amino acids singly as the sole source of carbon and energy. These changes were manifested as either a higher growth rate or a new ability to grow on the particular amino acid. The GASP mutations are pleiotropic in this respect, as each affects growth on several amino acids. The subsets of amino acids on which they confer a growth advantage are overlapping but distinct. In general,
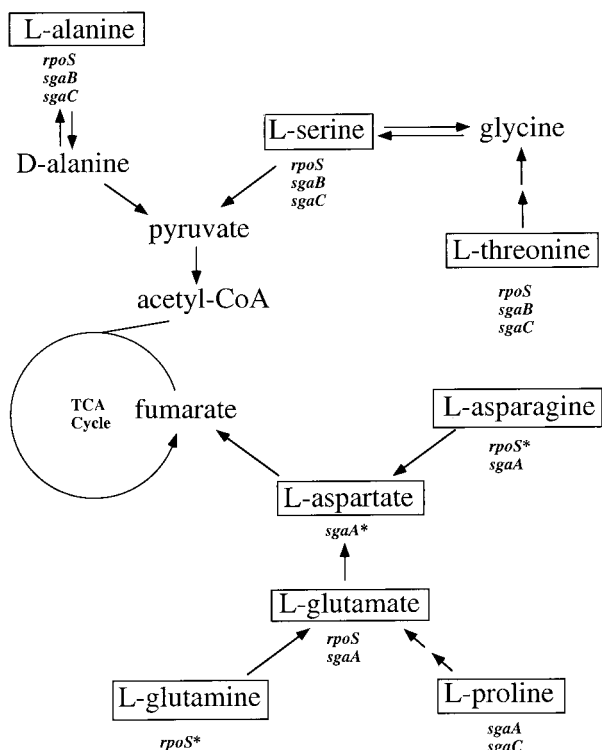
FIG. 6. The primary catabolic pathways for the amino acids on which the GASP mutants have a growth advantage (reviewed by McFall and Newman [18]). The arrows indicate enzymatic steps between metabolites. Boxed amino acids denote amino acids that can serve as sole sources of carbon and energy for at least one of the six strains tested. Listed under the boxed amino acids are the loci whose GASP alleles confer a growth advantage on the amino acid. An asterisk indicates the GASP allele that confers the novel ability to grow on the amino acid.

these subsets contain amino acids that enter catabolism along the same major degradative pathways (reviewed in reference 18) (Fig. 6). The *sgaA* allele confers a growth advantage on amino acids that enter the central metabolic pathway through aspartate and fumarate, while the *sgaB* and *sgaC* alleles confer growth advantages on amino acids that enter this pathway
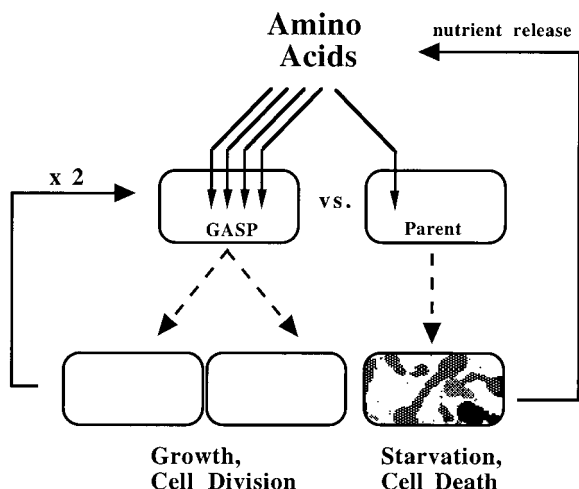

FIG. 7. A model for the physiological basis of the GASP phenotype.

through pyruvate (the effect of *sgaC* on proline metabolism is the one exception). The *rpoS819* mutation, on the other hand, affected both of these degradative pathways. It is tempting to speculate that the GASP mutations, especially *rpoS819*, alter the regulation of the enzymes of these degradative pathways. We are currently investigating the mechanistic bases of these physiological changes.

Based on our findings, we propose a model for a physiological basis of GASP (Fig. 7). We hypothesize that the primary selective force acting on carbon-starved cells is the ability to scavenge carbon sources released by the dying cells for the purposes of cell maintenance and growth. Cells unable to obtain a sufficient supply of carbon and energy can no longer maintain activities essential for viability, and they die. The fact that in every case studied the GASP fitness correlates directly with the capacity to catabolize amino acids leads us to propose that the most significant nutrients released are catabolizable amino acids. At the onset of starvation, the population is composed almost entirely of cells of the parental genotype that compete with equal fitness for carbon sources. Hence, the death during the first few days of starvation is stochastic, as all parental cells have an equal chance of scavenging the nutrients and surviving. However, the rare mutants within the population expressing the GASP phenotype outcompete their parents for the carbon resources because of their enhanced catabolic capabilities. These advantages provide the GASP cells with enough resources not only to survive but to grow and divide during starvation conditions. Once the GASP mutants grow to a significant cell density, they effectively decrease the amount of carbon available to the parental cells. These parental cells die as a result and release their nutrients into the medium. This model involves two positive feedback loops which can account for both the rapid growth of the GASP mutant and the rapid death of the parent. Supporting our model is the finding that if the G_{II} strain ZK1141 lacks the respiratory enzyme NADH dehydrogenase I, essential for the utilization of several amino acids catabolized by G_{II} (29), it loses the GASP phenotype versus the G_I parental strain, ZK819 (38).

Interestingly, we observed that while G_I mutants with a single G_{II} allele grow faster than G_I with certain amino acids as sole carbon sources, they grow slower with others. This result may seem inconsistent with our model for the physiological basis of GASP. However, growth on mixed amino acids may more accurately reflect the growth of the GASP mutants during starvation, since all amino acids are likely to be released at similar rates from the dying cells. We have demonstrated that all four GASP alleles confer an overall advantage when growing on mixed amino acids (Casamino Acids). Hence, our results suggest that when the GASP mutants are competing with their parents for the complex mixtures of nutrients released by the dying cells, their faster growth on some amino acids outweighs their slower growth on others.

REFERENCES

1. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. J. Mol. Biol. **215:**403–410.
2. Berlyn, M. K., K. B. Low, and K. E. Rudd. 1996. Linkage map of *Escherichia coli* K-12, edition 9, p. 1715–1902. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley,

M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. ASM Press, Washington, D.C.

3. **Blackburn, N., T. Fenchel, and J. Mitchell.** 1998. Microscale nutrient patches in planktonic habitats shown by chemotactic bacteria. Science **282:**2254–2256.

4. **Bohannon, D. E., N. Connell, J. Keener, A. Tormo, M. Espinosa-Urgel, M. M. Zambrano, and R. Kolter.** 1991. Stationary-phase-inducible "gearbox" promoters: differential effects of *katF* mutations and the role of σ^{70}. J. Bacteriol. **173:**4482–4492.

5. **Caetano-Annoles, G.** 1993. Amplifying DNA with arbitrary oligonucleotide primers. PCR Methods Appl. **3:**85–92.

6. **Connell, N., Z. Han, F. Moreno, and R. Kolter.** 1987. An *E. coli* promoter induced by the cessation of growth. Mol. Microbiol. **1:**195–201.

7. **Finkel, S. E., and R. Kolter.** 1999. Evolution of microbial diversity during prolonged starvation. Proc. Natl. Acad. Sci. USA **96:**4023–4027.

8. **Finkel, S. E., E. Zinser, S. Gupta, and R. Kolter.** 1997. Life and death in stationary phase. Mol. Microbiol. **103:**3–16.

9. **Hama, H., T. Kayahara, M. Tsuda, and T. Tsuchiya.** 1991. Inhibition of homoserine dehydrogenase I by L-serine in *Escherichia coli*. J. Biochem. **109:**604–608.

10. **Hama, H., Y. Sumita, Y. Kakutani, M. Tsuda, and T. Tsuchiya.** 1990. Target of serine inhibition in *Escherichia coli*. Biochem. Biophys. Res. Commun. **168:**1211–1216.

11. **Helling, R. B., C. N. Vargas, and J. Adams.** 1987. Evolution of *Escherichia coli* during growth in a constant environment. Genetics **116:**349–358.

12. **Hengge-Aronis, R.** 1996. Regulation of gene expression during entry into stationary phase, p. 1497–1512. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.

13. **Kleckner, N., J. Bender, and S. Gottesman.** 1991. Uses of transposons with emphasis on Tn*10*. Methods Enzymol. **204:**139–180.

14. **Lam, H. M., and M. E. Winkler.** 1990. Metabolic relationships between pyridoxine (vitamin B$_6$) and serine biosynthesis in *Escherichia coli*. J. Bacteriol. **172:**6518–6528.

15. **Lange, R., and R. Hengge-Aronis.** 1991. Growth phase-regulated expression of *bolA* and morphology of *Escherichia coli* cells is controlled by the novel sigma factor, σ^S (*rpoS*). J. Bacteriol. **173:**4474–4481.

16. **Lawther, R. P., D. H. Calhoun, C. W. Adams, C. A. Hauser, J. Gray, and G. W. Hatfield.** 1981. Molecular basis of valine resistance in *Escherichia coli* K-12. Proc. Natl. Acad. Sci. USA **78:**922–925.

17. **Lenski, R. E., J. A. Mongold, P. D. Sniegowski, M. Travisano, F. Vasi, P. J. Gerrish, and T. M. Schmidt.** 1998. Evolution of competitive fitness in experimental populations of *E. coli*: what makes one genotype a better competitor than another? Antonie Leeuwenhoek **73:**35–47.

18. **McFall, E., and E. B. Newman.** 1996. Amino acids as carbon sources, p. 358–379. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.

19. **Miller, J. H.** 1992. A short course in bacterial genetics. Cold Spring Harbor Press, Cold Spring Harbor, N.Y.

20. **Miller, R. D., D. E. Dykhuizen, L. Green, and D. L. Hartl.** 1984. Specific deletion occurring in the directed evolution of 6-phosphogluconate dehydrogenase in *Escherichia coli*. Genetics **108:**765–772.

21. **Moriarty, D. J. W., and R. T. Bell.** 1993. Bacterial growth and starvation in aquatic environments, p. 25–53. *In* S. J. Kjelleberg (ed.), Starvation in bacteria. Plenum Press, New York, N.Y.

22. **Morita, R. Y.** 1988. Bioavailability of energy and its relationship to growth and starvation survival in nature. Can. J. Microbiol. **34:**436–441.

23. **Morita, R. Y.** 1993. Bioavailability of energy and the starvation state, p. 1–23. *In* S. Kjelleberg (ed.), Starvation in bacteria. Plenum Press, New York, N.Y.

24. **Mortlock, R. P.** 1982. Metabolic acquisitions through laboratory selection. Annu. Rev. Microbiol. **36:**259–284.

25. **Mulvey, M. R., P. A. Sorby, B. L. Triggs-Raine, and P. C. Loewen.** 1988. Cloning and physical characterization of *katE* and *katF* required for catalase HPII expression in *Escherichia coli*. Gene **73:**337–345.

26. **Neidhardt, F. C., and H. E. Umbarger.** 1996. Chemical composition of *Escherichia coli*, p. 13–28. *In* F. C. Neidhardt, R. Curtiss III, J. L. Ingraham, E. C. C. Lin, K. B. Low, B. Magasanik, W. S. Reznikoff, M. Riley, M. Schaechter, and H. E. Umbarger (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, D.C.

27. **Notley, L., and T. Ferenci.** 1996. Induction of RpoS-dependent functions in glucose-limited continuous culture: what level of nutrient limitation induces the stationary phase of *Escherichia coli*? J. Bacteriol. **178:**1465–1468.

28. **Pratt, L. A., and R. Kolter.** 1998. Genetic analysis of *Escherichia coli* biofilm formation: roles of flagella, motility, chemotaxis and type I pili. Mol. Microbiol. **30:**285–293.

29. **Prüß, B. M., J. M. Nelms, C. Park, and A. J. Wolfe.** 1994. Mutations in NADH:ubiquinone oxidoreductase of *Escherichia coli* affect growth on mixed amino acids. J. Bacteriol. **176:**2143–2150.

30. **Reynolds, A. E., J. Felton, and A. Wright.** 1981. Insertion of DNA activates the cryptic *bgl* operon of *E. coli*. Nature **293:**625–629.

31. **Singer, M., T. A. Baker, G. Schnitzler, S. M. Deischel, M. Goel, W. Dove, K. J. Jaacks, A. D. Grossman, J. W. Erickson, and C. A. Gross.** 1989. A collection of strains containing genetically linked alternating antibiotic resistance elements for genetic mapping of *Escherichia coli*. Microbiol. Rev. **53:**1–24.

32. **Snell, E. E.** 1975. Tryptophanase: structure, catalytic activities and mechanisms of action. Adv. Enzymol. **42:**287–333.

33. **Sonti, R. V., and J. R. Roth.** 1989. Role of gene duplications in the adaptation of *Salmonella typhimurium* to growth on limiting carbon sources. Genetics **123:**19–28.

34. **Taylor, B. L.** 1983. How do bacteria find the optimal concentration of oxygen? Trends Biochem. Sci. **8:**438–441.

35. **Vanden Boom, T. J., K. E. Reed, and J. E. Cronan, Jr.** 1991. Lipoic acid metabolism in *Escherichia coli*: isolation of null mutants defective in lipoic acid biosynthesis, molecular cloning and characterization of the *E. coli lip* locus, and identification of the lipoylated protein of the glycine cleavage system. J. Bacteriol. **173:**6411–6420.

36. **Weikert, C., U. Sauer, and J. E. Bailey.** 1997. Use of a glycerol-limited, long-term chemostat for isolation of *Escherichia coli* mutants with improved physiological properties. Microbiology **143:**1567–1574.

37. **Zambrano, M. M.** 1993. Ph.D. thesis. Harvard University, Cambridge, Mass.

38. **Zambrano, M. M., and R. Kolter.** 1993. *Escherichia coli* mutants lacking NADH dehydrogenase-I have a competitive disadvantage in stationary phase. J. Bacteriol. **175:**5642–5647.

39. **Zambrano, M. M., and R. Kolter.** 1996. GASPing for life in stationary phase. Cell **86:**181–184.

40. **Zambrano, M. M., D. A. Siegele, M. Almirón, A. Tormo, and R. Kolter.** 1993. Microbial competition: *Escherichia coli* mutants that take over stationary phase cultures. Science **259:**1757–1760.