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Note

Stress and Survival of Aging *Escherichia coli rpoS* Colonies

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ABSTRACT

In *Escherichia coli*, the expression of the RpoS regulon is known to be crucial for survival in liquid cultures during stationary phase. By measuring cell viability and by transcriptome analysis, here we show that *rpoS* cells as well as wild-type cells survive when they form colonies on solid media.

ESCHERICHIA coli possess seven RNA polymerase σ-factors, which compete for association with the core polymerase subunit, each coordinating the transcription of a set of genes, which allows fine control of adaptation to different physiological conditions (Record *et al.* 1996). In cells that approach stationary phase, the σ-factor σ^{38} (RpoS) competes with the primary σ-factor σ^{70} (RpoD) for association with the core polymerase subunit. To date ~100 genes have been reported to belong to the RpoS regulon whose induction results in physiological and morphological modifications that increase resistance to various stresses such as heat shock, cold shock, acid shock, oxidative stress, osmotic stress, and UV light (Storz and Hengge-Aronis 2000).

Paradoxically, mutations and even inactivation of the *rpoS* gene might be tolerated or advantageous in nature, despite the partial or complete loss of RpoS-dependent resistance to environmental challenges, as suggested by the high degree of allelic variation of the *rpoS* genes from *E. coli* natural isolates (Herbelin *et al.* 2000; Jorgensen *et al.* 2000). Several studies have shown that the fitness of *rpoS* mutants depends on environmental conditions (Ferenci 2001; Farrell and Finkel 2003). However, the influence of the structured environment on fitness of the *rpoS* mutant is unknown. In nature, *E. coli* is often found in structured environments, *e.g.*, microcolonies, aggregates, or biofilms. Hence, in this study we have tested how a structured environment influences the survival of an *E. coli rpoS* mutant to determine whether the selection of *rpoS* alleles found in nature can occur in such environments.

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While the decrease in the number of colony forming units (CFUs) obtained from planktonic cells between 1 day (D1) and 7 days (D7) in culture was 200-fold for *rpoS* mutant and 10-fold for wild-type strain (wt), there was little difference in colonies between D1 and D7 for each strain as well as between the two strains (Table 1a). Furthermore, the measurements of the cell viability in aging colonies, as well as of the colony area, show that the *rpoS* mutant had difficulties relative to wt during the first 24 hr (D1) but there was no difference between the two strains after 7 days (D7; Table 1b), indicating better survival of *rpoS* cells at D7 than at D1, and/or increased cell turnover. To identify the functions that might be responsible for fitness modifications of *rpoS* mutants in structured environments, we established genome-wide transcription profiles of cells in aging colonies of wt and *rpoS* strains using macroarray technology.

The transcriptome analysis supports the observation that the *rpoS* colonies have more difficulties at D1 compared to wt colonies (Tables 1b and 2). For example, at D1, we observed a lower expression of genes coding for translation apparatus [ribosomal proteins (50S and 30S subunits)] in *rpoS* than in wt colonies, which may indicate that the *rpoS* cells have a lower growth rate than wt cells (Gausing 1977). Furthermore, at D1, we observed a higher expression of the *oxyR* and *soxR* genes (coding for transcriptional regulators involved in the response to hydrogen peroxide and superoxide, respectively; Storz and Imlay 1999) in *rpoS* colonies than in wt colonies, indicating that oxidative stress might be the major problem for *rpoS* cells (Table 2). The induction of these two stress responses in *rpoS* mutant may be the consequence of the deficiency in RpoS-mediated protection against oxidants (Dps, KatE; Hengge-Aronis 2002) and/or an increase in production of reactive oxygen species. Moreover, some genes that are not

TABLE 1

Growth and death within wild-type and *rpoS* colonies

a	CFU per milliliter of liquid LB	SD	CFU per colony	SD
wt D1	2.74×10^9	4.1×10^8	2.19×10^9	1.14×10^9
wt D7	2.98×10^8	1.4×10^8	4.05×10^9	2.15×10^9
rpoS D1	3.11×10^9	6.3×10^8	1.9×10^9	0.7×10^9
rpoS D7	2.96×10^7	3.2×10^7	4.32×10^9	1.7×10^9

	Colony area (mm^2)		% of dead cells per colony	
b	D1	D7	D1	D7
wt	1.76	136	2.94	58
rpoS	1.56	147	7.2	59
P-value	0.003	0.36	5.1×10^{-5}	0.56

We used the *E. coli* K12 MG1655 strain (wt) and the derived *rpoS* mutant strain (the *rpoS*359::Tn10 allele was kindly provided by M. E. Winkler, Indiana University, Bloomington, IN). Bacteria were grown in agitated *L*uria-*B*ertani broth (LB) liquid medium at 37° for 1 day or 7 days. For colony analysis, 10^2–10^3 cells from an overnight liquid culture were inoculated onto nitrocellulose filters (NC 45 from Schleicher & Schuell, Keene, NH), laid on fresh LB plates (agar 15 g/liter), and incubated at 37° for 1 day (D1) or 7 days (D7). Afterward, the filter with the growing colony was put in 1 ml of LB and vortexed, which enabled the recovery of all the cells. To calculate the number of CFUs per milliliter of liquid culture or per colony, aliquots of diluted cells were plated on LB and the CFUs were counted 1 day after plating. The average number of CFUs and the standard deviation (SD) from six experiments are indicated in a. The areas of 22 wt colonies and 22 *rpoS* colonies at D1 and D7 were measured using the software Ellix (Microvision, Evry, France). Percentages of dead cells within 7 wt colonies and 7 *rpoS* colonies at D1 and D7 were estimated by flow cytometry analysis (FASCscan, Becton Dickinson) using the two-color fluorescence (syto 9 green/propidium iodide) assay from Molecular Probes (Eugene, OR; live/dead Baclight bacterial viability kits). The null hypothesis of equal mean surface and equal mean percentage of dead cells (H1) between the two strains at each time was tested by calculating the *t*-test and the *P*-value (significant when $P < 0.05$). The area averages, the percentage of dead cell averages, and *P*-value are reported in b.

regulated by OxyR but are induced by H_2O_2 treatment (Zheng *et al.* 2001)—such as the *ydcH* (unknown function) and the *yfiA* gene encoding a protein associated with 70S ribosomes in stationary phase (Maki *et al.* 2000)—were also upregulated in *rpoS* compared to wt at D1. The decrease in viability of *rpoS* cells at D1 could also result from the absence of downregulation of the *ompF* gene (coding for major outer membrane porin; Liu and Ferenci 2001), which is observed in wt. The downregulation of the *ompF* gene reduces membrane permeability and consequently the entry of toxic compounds into cells (Martinez-Martinez *et al.* 2000).

Between D1 and D7, the fraction of dead cells within wt and *rpoS* colonies reached 60%. The difficulties faced by both strains were reflected by the increase in expression of RpoS-independent genes involved in stress resistance and DNA repair: *e.g.*, the σ^{32} (RpoH)/RpoN-regulated heat-shock genes *ibpA*, *ibpB* (Kitagawa *et al.* 2002), the *psp* genes (*p*hage *s*hock *p*roteins; Model *et al.* 1997), some SOS genes (*dinJ*, *dinG*; Courcelle *et al.* 2001), and the *nfo* gene encoding endonuclease IV (Hosfield *et al.* 1999). We also observed, but only in *rpoS*, an increase in transcripts of RpoH-regulated genes (*groES* and *htgA*; Missiakas and Raina 1997), as well as the *sbcB*, *recB* (respectively, the exodeoxyribonuclease I and the exonuclease V subunit; Seigneur *et al.* 1999), *xseB* (exonuclease subunit; Viswanathan *et al.* 2001), *sodA* (manganese superoxide dismutase; Compan and Touati 1993), and *ung* (uracil-N-glycosylase; D'souza and Harrison 2003) genes. Therefore, *rpoS* cells in aging colonies appear to face various stresses that damage DNA and proteins. However, the fact that the *rpoS* strain does not show an increase in mortality relative to wt at D7 suggests that it succeeds in coping with the stresses encountered in aging colonies.

Remarkably, the expression of genes coding for ribosomal proteins globally decreases between D1 and D7 in the wt strain, while it increases in *rpoS* colonies (Figure 1). A similar tendency was observed for genes coding for RNA (*rpoB*, *rpoC*, and *rpoH*) and DNA (*holC*) polymerase subunits (Record *et al.* 1996). Finally, the expression of *fis* (Appleman *et al.* 1998), a gene coding for a protein required for adaptation of cells to rapid growth conditions, increases in *rpoS* D7 colonies relative to *rpoS* D1, as well as compared to wt D7. The resulting increase in transcription, protein, and DNA synthesis in aging *rpoS* colonies may simply be a side effect due to the absence of RpoS regulation. However, this global change may be the result of *rpoS* cell turnover, as some cells grow and divide while others die.

In conclusion, our study shows that RpoS-independent stress responses may be sufficient to ensure the

TABLE 2

Genes with altered expression in *rpoS* compared to wt colonies

Functional categories and gene products	Genes	Fold wt D7 *vs.* wt D1	Fold *rpoS* D1 *vs.* wt D1	Fold *rpoS* D7 *vs.* wt D7	Fold *rpoS* D7 *vs.* *rpoS* D1
Adaptations: atypical conditions					
Heat-shock protein	*htgA*	−1.06	−2.55	1.27	3.04
Heat-shock protein	*htpX*	1.25	−1.05	1.43	1.88
Heat-shock protein	*ibpB*	1.26	−4.69	−1.09	5.41
Heat-shock protein	*ibpA*	1.31	−1.96	−1.19	2.16
Heat-shock protein	*groES*	−1.83	−1.37	2.06	1.54
Heat-shock protein	*hslV*	1.13	−1.95	1.33	2.93
H_2O_2-inducible genes activator	*oxyR*	1.14	2.38	−1.01	−2.11
Manganese superoxide dismutase	*sodA*	−1.15	−2.64	1.35	3.11
Regulatory protein of SoxRS regulon	*soxR*	−1.12	2.00	1.08	−2.09
Ribosome binding protein, H_2O_2 inducible	*yfiA*	1.54	4.74	−1.37	−4.23
RNA polymerase σ-32 subunit	*rpoH*	−1.01	−1.64	1.23	1.99
Unknown, H_2O_2 inducible	*ydcH*	−1.01	2.7	1.64	−1.66
DNA repair					
Damage-inducible protein J	*dinJ*	1.43	−2.14	−1.01	3.02
DNA-damage-inducible protein G	*dinG*	1.68	−1.14	1.05	2.00
Endonuclease IV	*nfo*	1.52	−2.01	1.13	2.50
Exodeoxyribonuclease I	*sbcB*	−1.04	−1.90	1.15	2.11
Exodeoxyribonuclease small subunit	*xseB*	1.01	−1.78	1.63	2.92
Exonuclease V subunit	*recB*	−1.15	−1.55	1.42	1.92
Uracil-DNA glycosylase	*ung*	−1.03	−1.26	1.56	1.90
Replication-transcription					
DNA polymerase III, χ-subunit	*holC*	−1.60	−2.78	1.10	1.90
Factor for inversion stimulation	*fis*	−1.89	−1.55	1.74	1.43
RNA polymerase, α-subunit	*rpoA*	−1.89	−1.58	1.82	1.52
RNA polymerase, β-subunit	*rpoB*	−2.01	−2.74	1.63	2.23
RNA polymerase, β′-subunit	*rpoC*	−1.51	−3.13	1.25	2.59
TCA cycle					
2-Oxoglutarate dehydrogenase	*sucA*	1.19	1.03	2.31	2.65
Aconitate hydratase 2	*acnB*	−1.19	−2.61	−1.11	1.97
Citrate hydro-lyase 1	*acnA*	1.01	−2.04	1.04	2.15
Citrate synthase	*gltA*	−1.15	1.06	2.08	1.71
Dihydrolipoamide succinyltransferase	*sucB*	1.02	1.02	1.81	1.82
Fumarate hydratase class II	*fumC*	2.45	−1.09	1.43	3.85
Malate dehydrogenase	*mdh*	−1.07	−2.08	1.20	2.32
Succinate dehydrogenase	*sdhC*	1.67	−1.08	1.18	2.13
Succinate dehydrogenase	*sdhD*	1.23	−1.02	2.78	3.51
Succinate dehydrogenase flavoprotein	*sdhA*	1.03	−1.05	1.69	1.83
Succinate dehydrogenase iron-sulfur protein	*sdhB*	−1.09	1.02	1.93	1.73
Succinyl-coA synthetase α-chain	*sucD*	−1.02	1.96	3.40	1.70
Succinyl-coA synthetase β-chain	*sucC*	1.09	−1.19	1.90	2.47
Outer membrane constituents					
Outer membrane porin F	*ompF*	−1.11	3.21	−9.32	−33.19
Phage shock proteins					
Phage shock protein A	*pspA*	3.18	2.06	1.18	1.82
Phage shock protein B	*pspB*	1.62	−1.16	2.26	4.25
Phage shock protein C	*pspC*	−1.13	1.07	2.56	2.11
Phage shock protein D	*pspD*	2.17	−1.06	1.71	3.94
Ribosomal proteins					
30S subunit protein S3	*rpsC*	−1.81	−2.02	1.64	1.83
30S subunit protein S5	*rpsE*	−1.70	−1.75	2.16	2.21

(*continued*)

TABLE 2

(Continued)

Functional categories and gene products	Genes	Fold wt D7 *vs.* wt D1	Fold *rpoS* D1 *vs.* wt D1	Fold *rpoS* D7 *vs.* wt D7	Fold *rpoS* D7 *vs.* *rpoS* D1
30S subunit protein S7	*rpsG*	−2.57	−3.26	1.51	1.92
30S subunit protein S8	*rpsH*	−1.92	−2.50	1.43	1.86
30S subunit protein S14	*rpsN*	−1.45	−1.97	1.92	2.61
30S subunit protein S16	*rpsP*	−1.26	−1.30	1.66	1.71
30S subunit protein S19	*rpsS*	−1.31	−1.98	1.37	2.07
50S subunit protein L1	*rplA*	−2.07	−3.15	1.44	2.19
50S subunit protein L2	*rplB*	−1.49	−2.48	1.43	2.38
50S subunit protein L3	*rplC*	−1.83	−2.53	1.19	1.64
50S subunit protein L6	*rplF*	−1.76	−1.81	2.00	2.06
50S subunit protein L10	*rplJ*	−1.79	−2.55	1.24	1.77
50S subunit protein L11	*rplK*	−1.51	−2.98	1.13	2.23
50S subunit protein L7/L12	*rplL*	−1.42	−2.36	1.22	2.03
50S subunit protein L15	*rplO*	−1.44	−2.24	1.81	2.81
50S subunit protein L16	*rplP*	−1.55	−2.01	1.56	2.01
50S subunit protein L19	*rplS*	−1.61	−1.33	1.81	1.50
50S subunit protein L23	*rplW*	−1.53	−1.80	1.37	1.61
50S subunit protein L24	*rplX*	−1.18	−1.58	1.51	2.02
50s subunit protein L25	*rplY*	−1.63	−2.21	1.28	1.74
50S subunit protein L29	*rpmC*	−1.54	−1.76	1.78	2.03
Ribosomal protein L11	*prmA*	−1.60	−1.88	1.78	2.08

After 1 day (D1) and 7 days (D7), total RNA was extracted from several colonies ($\sim 10^9$ cells) and 2 μg was used for production of [α-^{33}P]dCTP-labeled cDNA probe. Panorama arrays (Sigma-Genosis) containing the PCR-amplified 4290 ORFs of *E. coli* genes printed in duplicate were hybridized with the entire labeled cDNA probe following the recommendation of the furnisher. The software XdotsReader (Cose, Dugny, France) was used to calculate the pixel density for each spot. Each hybridization experiment was repeated four times with independent RNA preparation. The eight values for each gene have been used to test the null hypothesis of equal mean expression between the two strains and two different times by calculating the *t*-test and the Wilcoxon test. To analyze the fold change in transcript abundance, the (wt D7/wt D1), (*rpoS* D1/wt D1), (*rpoS* D7/wt D7), and (*rpoS* D7/*rpoS* D1) mRNA ratios were calculated. When the ratio was <1, the inverse ratio was reported with a negative value. This allows a symmetric view of the data (hence the direct value shift from +1 to −1). Genes with the (*rpoS* D1/wt D1) or (*rpoS* D7/*rposS* D1) ratios >1.5 and with a *P*-value ≤0.01 for both the *t*-test and the Wilcoxon test were considered to have significant difference of expression and are reported in this table. Genes upregulated in the *rpoS* strain compared to the wt strain at D1 are underlined. For the other genes listed, an increase in mRNA level was observed in the *rpoS* strain between D1 and D7. (For gene annotation, see http://www.genome.wisc.edu.)

survival of an *rpoS* population in aging colonies, which may not be the case in planktonic cells where the level of stress may be higher, particularly in oxidative stress (Dukan and Nystrom 1998). Furthermore, besides providing a collective defense against antagonists (Ma and Eaton 1992), the cellular aggregation may facilitate access to resources that cannot be efficiently utilized by isolated cells in suspension. This may be the case for reutilization of nutrients from the dead cells concentrated inside the colony (Shapiro 1992). Under such conditions, *rpoS* cells might use the available nutrients more efficiently than wt as suggested by the significant differences in transcriptional modifications of energy-producing pathways between the two strains. A compensatory transcription in *rpoS* colonies is suggested by the expression of genes involved in energy salvage: transcription of the *psp* operon and genes belonging to the tricarboxilic acid and the glyoxylate cycles (Cronan and LaPorte 1996), which are, respectively, RpoN and RpoD dependent, was higher in *rpoS* than in wt D7 colonies (Table 2). Therefore, it may be that the structured environments are favoring the observed enrichment for modified or null *rpoS* alleles in nature by providing an environmental niche in which the handicap resulting from the lack of various RpoS-dependent functions is attenuated through compensation.

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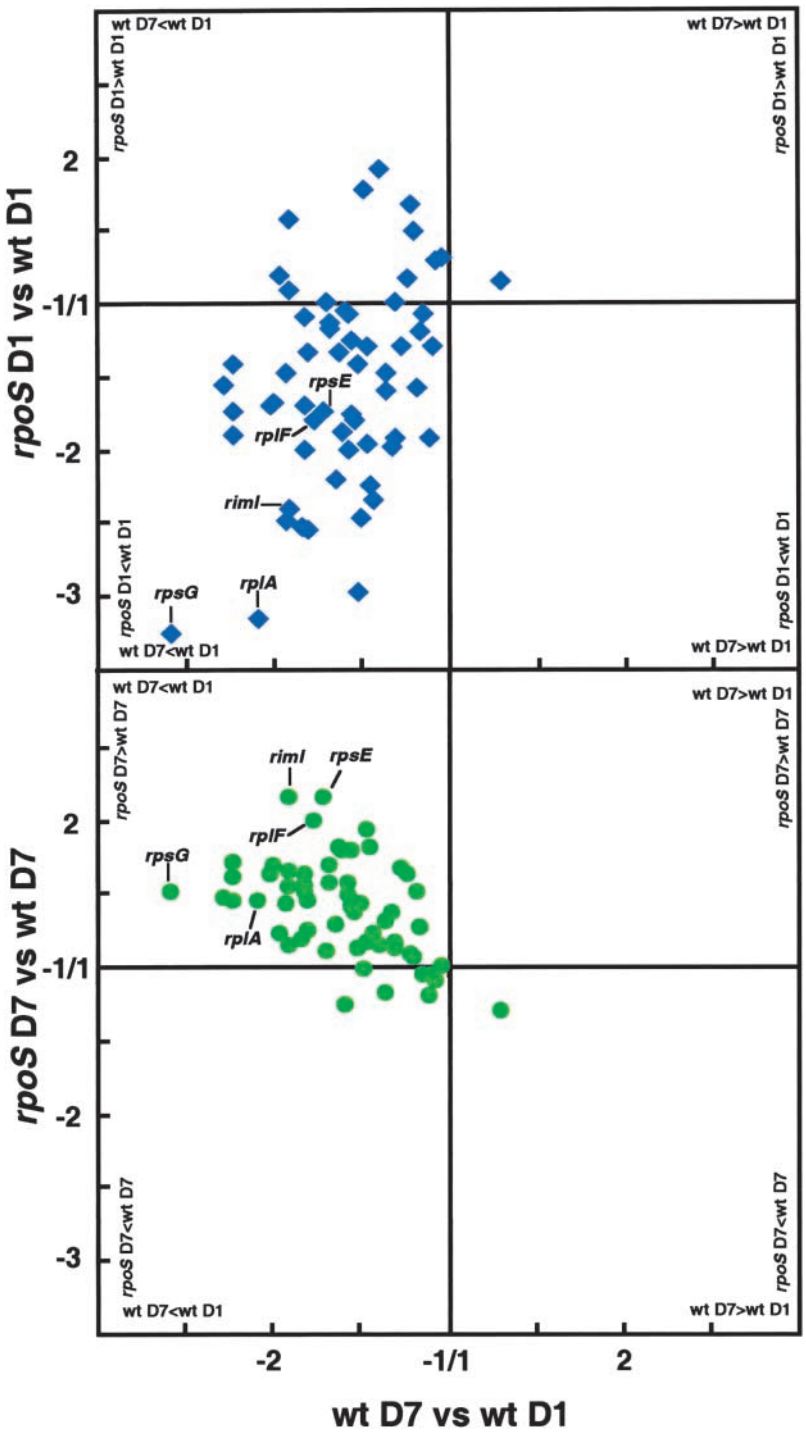

FIGURE 1.—Fold change in transcript abundance of genes coding for ribosomal proteins. The two graphs compare expression of the 60 genes encoding ribosomal proteins in the rpoS strain to wt strain: top after 1 day (D1; blue) and bottom after 7 days (D7; green). The mRNA ratios for each gene between D1 and D7 in the wt strain [(wtD7/wtD1) or if wtD1 > wtD7 (−wtD1/wtD7)] are reported on the *x*-axis. mRNA ratios of the same genes for D1 and D7 between the wt strain and the rpoS strain [(rpoS/wt) or if wt > rpoS (−wt/rpoS)] are reported on the *y*-axis. At D1, most of the values are in the bottom left quadrant, corresponding to wt D7 < wt D1 and rpoS < wt. At D7, most of the values are in the top left quadrant, corresponding to wt D7 < wtD1 and rpoS > wt.

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