RpoS-dependent stress tolerance in Pseudomonas aeruginosa

Frieda Jorgensen,† Marc Bally, Virginie Chapon-Herve, Gerard Michel, André Lazdunski, Paul Williams and G. S. A. B. Stewart

Pseudomonas aeruginosa is able to persist during feast and famine in many different environments including soil, water, plants, animals and humans. The alternative sigma factor encoded by the rpoS gene is known to be important for survival under stressful conditions in several other bacterial species. To determine if the P. aeruginosa RpoS protein plays a similar role in stationary-phase-mediated resistance, an rpoS mutant was constructed and survival during exposure to hydrogen peroxide, high temperature, hyperosmolarity, low pH and ethanol was investigated. Disruption of the rpoS gene resulted in a two- to threefold increase in the rate of kill of stationary-phase cells. The rpoS mutant also survived less well than the parental strain during the initial phase of carbon or phosphate–carbon starvation. However, after 25 d starvation the remaining population of culturable cells was not significantly different. Stationary-phase cells of the RpoS-negative strain were much more stress resistant than exponentially growing RpoS-positive cells, suggesting that factors other than the RpoS protein must be associated with stationary-phase stress tolerance in P. aeruginosa. Comparison of two-dimensional PAGE of the rpoS mutant and the parental strain showed four major modifications of protein patterns associated with the rpoS mutation.

Keywords: RpoS, stress tolerance, stationary phase, Pseudomonas aeruginosa

INTRODUCTION

Pseudomonas aeruginosa is an adaptable opportunistic human pathogen which can be found in many different environments including soil, water, plants, animals and humans. To successfully persist in a changing environment a micro-organism must sense such change and react appropriately. The adaptation to stressful and growth-limiting conditions encountered in host tissue, as well as in natural environments, may rely upon mechanisms common to the response observed when bacteria in culture are depleted of nutrients or exposed to other stresses (Kolter et al., 1993). Analysis of the response of different bacterial species to such unfavourable environmental conditions often coincides with the induction of multiple stress tolerance and production of virulence factors (Huisman et al., 1996; Hengge-Aronis, 1996; Mahan et al., 1996). It is because the ability to mount such a response will help the successful pathogen to persist in the environment and evade host defence systems that much recent work has focused on elucidating the molecular mechanisms governing global stress tolerance.

Studies in Escherichia coli have demonstrated that the stress response is very complex, consisting of changes in global gene expression including general as well as stress-specific components, and is accompanied by physiological and morphological changes. It is now clear that the rpoS-encoded σ5 subunit of RNA polymerase is an important regulator of the general stress response in E. coli (Muffler et al., 1997). Initially, the transcription of the rpoS gene was shown to increase during entry into stationary phase (Lange & Hengge-Aronis, 1991) but the cellular level of the protein has now been demonstrated to also increase upon exposure to hyperosmotic stress (Muffler et al., 1996), acid stress (Lee et al., 1995), exposure to low temperature (Sledjeski et al., 1996) as well as heat shock (Muffler et al., 1997). Consistent with a role for the RpoS protein in con-
tributing to multiple stress tolerance, stationary-phase rpoS mutants of E. coli show increased sensitivity to starvation, high osmolarity, heat stress, hydrogen peroxide and acid stress (McCann et al., 1991; Small et al., 1994). rpoS gene homologues have been identified in other bacteria including Salmonella spp., Klebsiella pneumoniae, Shigella flexneri, Yersinia enterocolitica and Vibrio cholerae (Fang et al., 1992; Loewen & Henge-Aronis, 1994; Small et al., 1994; Badger & Miller, 1995; Yildiz & Schoolnik, 1998). In these bacteria the RpoS homologues play a similar physiological role to that in E. coli. Moreover, a direct requirement for rpoS for virulence in orally infected mice has been demonstrated in Salmonella typhimurium mainly due to activation of the plasmid-encoded spv genes but rpoS regulated chromosomally encoded genes also play a contributory role (Fang et al., 1992). rpoS mutants of Y. enterocolitica and V. cholerae were, however, not attenuated for virulence in mice but a functional rpoS gene was required for production of haemagglutinin/protease in V. cholerae and full expression of the Yst enterotoxin in Y. enterocolitica (Yildiz & Schoolnik, 1998; Badger & Miller, 1995; Iriarte et al., 1995). The existence of RpoS homologues in bacteria such as Sinorhizobium (Rhizobium) melloti and Acetobacter métan hooplasticus has been inferred from the experiments carried out by Miksch & Dobrowolski (1995), suggesting that RpoS-mediated regulatory mechanisms are conserved in more distantly related Gram-negative species.

P. aeruginosa possesses an RpoS homologue (Tanaka & Takahashi, 1994). RpoS levels in P. aeruginosa increase upon entry into stationary phase as observed in other Gram-negative bacteria (Fujita et al., 1994). Compared with E. coli relatively little is known about the mechanisms of the regulation of the RpoS protein in P. aeruginosa. However, the cell-density-dependent signalling mechanism termed quorum sensing was recently linked to the expression of the rpoS gene in this bacterium (Latifi et al., 1996). Two quorum sensing regulons have been identified in P. aeruginosa in which the LuxR homologues LasR and RhlR are activated by N-[(3-oxododecanoyl)-d-homoserine lactone (OdDHL) and N-butanoyl-d-homoserine lactone (BHL), respectively (Latifi et al., 1996). These two circuits regulate a wide array of exproducts (proteases, toxin and secondary metabolites) which are essential for virulence (Latifi et al., 1995). Expression of the rpoS gene was abolished in a P. aeruginosa lasR mutant and in the BHL-negative mutant PANO67 (Latifi et al., 1996). In E. coli an rpoS-lacZ fusion was regulated directly by RhlR/BHL (Latifi et al., 1996). It is also plausible that additional factors participate in the regulation of RpoS synthesis. Recently, Kim et al. (1998) identified a link between regulation of exopoly saccharide alginate synthesis, cellular levels of ppGpp and survival of P. aeruginosa in stationary phase. It was suggested that this latter effect might be related to the expression of the RpoS protein in response to high levels of ppGpp, a modulator of the synthesis of the RpoS protein in E. coli (Gentry et al., 1993). Pseudomonas spp. are known to develop stress tolerance and increase their production of antibiotics upon entry into stationary phase (Givskov et al., 1994; Klotz & Hutcheson, 1992; Sarniguet et al., 1995). Growth-phase-dependent expression of azurin, a protein required for the cellular response of P. aeruginosa to redox stress, involves expression of the rpoS gene (Vijgenboom et al., 1997). In a Pseudomonas fluorescens rpoS mutant antibiotic production was affected and survival during oxidative and osmotic stress and on plant surfaces was decreased (Sarniguet et al., 1995).

In the present study we report on the role of the RpoS protein in conferring stress tolerance in P. aeruginosa towards a variety of environmental stresses.

**METHODS**

**Bacterial strains and media.** P. aeruginosa wild-type strain PAO1 (Holloway collection) and its rpoS-null derivative PAOS (this study) were grown at 37 °C with shaking, at 200 r.p.m. in LB broth, Lennox (Difco) or in a chemically defined medium (CDM) containing 24 mM glucose, 48 mM (NH₄)₂SO₄, 3-84 mM K₂HPO₄, 3H₂O, 0-74 mM KCl, 0-66 mM NaCl, 0-48 mM MgSO₄.7H₂O, 60 mM MOPS and 0-01 mM FeSO₄.7H₂O (Kadurugamuwa et al., 1987). The omission of either glucose (CDM-C) or glucose and K₂HPO₄.3H₂O (CDM-PC) from CDM provided carbon and carbon-phosphate starvation media, respectively. Unless otherwise stated all chemicals were purchased from BDH. PAOS was grown in the presence of 300 μg carbenicillin ml⁻¹. Growth of the cultures was measured as OD₆₃₀ with a Gallenkamp Visi-Spec spectrophotometer. A starter culture (20 ml inoculated with a single colony and incubated for 16 h) was used to initiate the exponential and stationary-phase cultures used in the stress tolerance assays. Exponential-phase cultures were obtained by diluting the starter culture 1:50 in fresh media. Cells were then grown to an OD₆₃₀ of 0-7, diluted again to an OD₆₃₀ of 0-1 and finally grown to mid-exponential phase (corresponding to 4-6 x 10⁹ cells or OD₆₃₀ = 0-7). Stationary-phase cultures were obtained by diluting the starter culture to an OD₆₃₀ of 0-05 and then incubating for 24 h reaching a final cell density of 8-9 x 10⁸.

**Plasmids and DNA procedures.** The suicide plasmid pUR was constructed by cloning of a 0-55 kb Hincl–PstI DNA fragment internal to the rpoS gene (Tanaka & Takahashi, 1994) into pUC18. The suicide plasmid pFLR5 was obtained by insertion of a 18 kb KpnI–HindIII fragment from pDB18R containing a functional rpoS gene (Tanaka & Takahashi, 1994) into the broad-host-range vector pLAFR3 (Friedman et al., 1982). Plasmid DNA was prepared and digested with restriction enzymes according to techniques described by Sambrook et al. (1989). Recombinant plasmids were transferred to P. aeruginosa by electroporation as previously described by Smith & Iglewski (1989). Chromosomal DNA preparations were carried out according to the protocol established by Chen & Kuo (1993). Hybridization probes were prepared with DNA fragments extracted from agarose gels (Sambrook et al., 1989), labelled with digoxigenin and detected by using chemiluminescence (DIG Luminescent Detection kit; Boehringer Mannheim).

**Construction of rpoS mutant strain PAOS.** The suicide plasmid pUR was introduced into PAO1 by electroporation and
transformed colonies were screened on plates containing 300 μg carbenicillin ml⁻¹. Correct integration was confirmed by Southern blot analysis (Sambrook et al., 1989). Immunodetection of the RpoS protein by Western blot analysis was performed as described elsewhere (Latifi et al., 1995).

**Two-dimensional gel electrophoresis.** Cells were grown at 37 °C in MNP medium at 180 r.p.m. (Holloway, 1955). Late-exponential-phase cells were labelled with 18 MBq [³⁵S]methionine ml⁻¹ for 1 h. Labelling was stopped by the addition of 1 mM non-radioactive methionine on ice, then the cells were centrifuged (10000 g, 5 min, 4 °C) and washed with cold 10 mM Tris/HCl (pH 7.5) containing 10% (w/v) sucrose. Proteins were solubilized in the solubilization buffer of O'Farrell (1975) after several freeze-thawing treatments and separated by two-dimensional gel electrophoresis according to O'Farrell (1975). The pH gradient in the focusing gel was established using 2% (w/v) Ampholines (LKB) containing 1.6% (w/v) Ampholines (pH 5–7) and 0.4% (w/v) Ampholines (pH 3–5). Isoelectric focusing gels were loaded with approximately 100 μg protein and electrophoresis was carried out at 400 V for 18 h (7200 V h). In the second dimension, proteins were separated by SDS-PAGE (12%, w/v, acrylamide gels) according to Laemmli (1970).

**Measurement of resistance to hydrogen peroxide, high temperature, low pH, ethanol and high salt.** *P. aeruginosa* cultures were assayed for the ability to survive oxidative stress (50 mM or 10 mM H₂O₂), heat stress (53 °C), osmotic stress (3 M NaCl), pH stress (media acidified to pH 3 using HCl) and ethanol stress (10%, v/v, ethanol) in LB and CDM. The stress media were pre-warmed to 37 °C or 53 °C (heat stress assay only) and were all prepared immediately prior to the measurement of stress tolerance. Stationary-phase or exponential-phase cultures were diluted in the respective growth medium so that by adding 10 μl of this diluted culture to the pre-warmed stress medium, a final cell density of 1 x 10⁷ c.f.u. ml⁻¹ was obtained. Less than 5 min elapsed from the initial dilution of the cultures until exposure to the stress. Samples were removed immediately and, at timed intervals, diluted in their respective growth medium and plated onto Tryptic Soy Agar (TSA; Difco). Colonies were counted after 2 d at 37 °C. When assaying PAOS cultures, carbenicillin (300 μg ml⁻¹) was added to the stress medium and to the TSA plates. Each stress...
tolerance assay was performed in duplicate or triplicate and performed at least twice unless otherwise stated.

**Measurement of starvation survival rates.** Exponential-phase cells grown in CDM prepared as described above were harvested by centrifugation (8000 g, 10 min), washed twice in CDM-C or CDM-PC and then suspended in the respective starvation medium to give a final cell density of 5–7 x 10^7 c.f.u. ml^-1. These cultures were held at 37 °C at 200 r.p.m. for up to 33 d. Samples were taken in duplicate at intervals, diluted in LB, plated onto TSA and counted after 2 d at 37 °C.

**RESULTS**

**Construction of an rpoS-null mutant**

The rpoS gene in PAO1 was mutated by insertional inactivation as described in Methods and illustrated in Fig. 1(a, b). After electrotransformation of PAO1 by the suicide plasmid pUR', a relatively large number of carbenicillin-resistant colonies were obtained. One of these clones, designated PAO5, was further examined. The possible disruption of the rpoS gene, resulting from the homologous recombination between pUR' and the PAO1 chromosome, was verified by Southern blot analysis. The chromosomal DNA extracted from PAO5 and its parental strain PAO1 was digested with BanHI and its parental strain PAO1 was digested with BanHI and HindIII, respectively, and then hybridized with the Kpd+R probe. The 5.7 kb BamHI-EcoRI fragment, encompassing the 5' and 3' extremities of the rpoS gene, respectively, were unchanged between the parental and the mutant strain. The absence of RpoS protein in the mutant was confirmed by Western blot analysis (Fig. 1d). Introduction of pLFR5, a pLAFR3 derivative carrying the rpoS gene, into the PAO5 mutant strain re-established the synthesis of the RpoS protein (Fig. 1d). These analyses permitted us to conclude that the insertional mutation occurred within the rpoS gene in the PAO5 strain.

**Stress resistance of stationary-phase cells grown in complex and chemically defined media is affected by an altered rpoS allele**

Survival of stationary-phase PAO5 and wild-type PAO1 strains of P. aeruginosa after exposure to hydrogen peroxide, heat stress, high osmolarity, low pH and ethanol was examined. The PAO5 strain was significantly more sensitive than the wild-type strain to all of the stresses examined both when grown in a complex medium (LB) and in a chemically defined medium (CDM) (Table 1). When survival was compared by single time point analysis, the number of wild-type PAO1 strain survivors was 5- to 80-fold greater than the number of PAO5 strain survivors depending on the stress factor and medium (Table 1). Assessing the sensitivity of stationary-phase PAO1 and PAOS cells grown in LB to the same stresses by comparing the survival rate (Figs 2 and 3) also showed a clear difference (two- to threefold).

The medium the cultures were grown and challenged in affected the sensitivity of the cells to stress. Cells in CDM were more sensitive to stress than those grown and challenged in LB. As shown in Table 1, 5 min at

<table>
<thead>
<tr>
<th>Stress*</th>
<th>log₁₀ reduction in cell numbers after exposure to the indicated stress</th>
<th>LB</th>
<th>CDM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAO1</td>
<td>PAO5</td>
<td>P value</td>
</tr>
<tr>
<td>H₂O₂ (50 mM)</td>
<td>1.50 (0.120)</td>
<td>2.8 (0.22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heat (53 °C)</td>
<td>0.44 (0.040)</td>
<td>2.2 (0.36)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NaCl (3 M)</td>
<td>0.85 (0.559)</td>
<td>2.3 (0.17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH 3 (HCl)</td>
<td>2.60 (0.180)</td>
<td>4.0 (0.19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ethanol (10 %, v/v)</td>
<td>0.89 (0.160)</td>
<td>1.6 (0.29)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* Exposure to H₂O₂ was maintained for 25 min in LB and 10 min in CDM; exposure to 53 °C was maintained for 25 min in LB and 5 min in CDM; exposure to NaCl was maintained for 12 h in LB and 4 h in CDM; exposure to pH 3 was maintained for 20 min in LB and 10 min in CDM; exposure to ethanol was maintained for 2.5 h in LB and 1.75 h in CDM.
Stress tolerance in *Pseudomonas aeruginosa*

53 °C resulted in a decrease of 1.4 log₁₀ (c.f.u. ml⁻¹) for cells grown and challenged in CDM whereas in LB, 25 min at 53 °C only resulted in a decrease of 0.44 log₁₀ (c.f.u. ml⁻¹). It is well known that measured resistance can be affected by the growth and challenge media used (Hansen & Riemann, 1963).

The RpoS-negative strain survived less well than the parental PAO1 strain during the initial phase of carbon starvation (Fig. 4a) and phosphate–carbon starvation (Fig. 4b). However, after 25 d starvation the remaining population of culturable cells was not dramatically different for strain PAOS (mean percentage survival: 1.4% and 2.3% in CDM-C and CDM-CP, respectively) compared with strain PAO1 (mean percentage survival: 1.8% and 2.1% in CDM-C and CDM-CP, respectively) (Fig. 4).

**Fig. 2.** Survival of stationary-phase (filled symbols) and exponential-phase (open symbols) cells of *P. aeruginosa* PAO1 (squares) and PAOS (circles) in LB with 50 mM H₂O₂ (a) or LB with 10 mM H₂O₂ (exponential-phase cells only) (b). Values for stationary-phase cells represent means of at least two experiments each performed in duplicate. Error bars represent standard errors of the means. Values for exponential-phase cells are from one experiment performed in duplicate. All cultures were grown in LB.

**Fig. 3.** Survival of stationary-phase (filled symbols) and exponential-phase (open symbols) cells of *P. aeruginosa* PAO1 (squares) and PAOS (circles) in LB, at 53 °C (a), with 3 M NaCl (b), at pH 3.0 (c) and in 10% (v/v) ethanol (d). Values for stationary-phase cells represent means of at least two experiments each performed in duplicate. Error bars represent standard errors of the means. Values for exponential-phase cells are from one experiment performed in duplicate. All cultures were grown in LB.
Fig. 4. Long-term survival of *P. aeruginosa* PAO1 (■) and PAOS (○) cells during carbon starvation (a) and phosphate-carbon starvation (b). Values represent duplicate samples from a typical experiment and each experiment was done twice. All cultures were grown in CDM.

Relative contribution of the RpoS protein to survival in exponential-phase cultures

Comparison of the survival rates of exponential-phase PAO1 and PAOS cells did not reveal significant differences (Figs 2 and 3). However, stationary-phase RpoS-negative *P. aeruginosa* was markedly more stress resistant than exponential-phase RpoS-positive cultures (Figs 2 and 3).

Effects of inactivation of the rpoS gene on global gene expression in *P. aeruginosa*

The *rpoS* mutant PAOS and its isogenic parent PAO1, grown in minimal medium, were pulse-labelled with [35S]methionine in the exponential- to stationary-phase transition and the proteins were separated by two-dimensional gel electrophoresis (Fig. 5). Comparison of the two-dimensional PAGE autoradiograms showed that the synthesis of four proteins was reproducibly modified between the two strains. Protein C18 (18 kDa) was absent in the PAOS strain while C32 (32 kDa) was not detected in the parental strain. Furthermore, the synthesis of two other proteins, B19 (19 kDa) and D16 (16 kDa), was decreased in PAOS compared to PAO1. Other proteins were also shown to be differentially expressed in the two strains. However, these modifications in the two-dimensional patterns were found to fluctuate between several experiments, suggesting that expression of the corresponding proteins could be determined by additional factors, possibly independent of rpoS gene functionality.

DISCUSSION

Stationary-phase bacterial cells possess complex mechanisms which can increase their potential to survive following exposure to diverse stress conditions. A regulator of central importance for the development of the resistant state during entry into stationary phase in *E. coli* is the alternative sigma factor RpoS (Hengge-Aronis, 1996). In this work the significance of the RpoS protein for stress resistance and protein expression in stationary-phase cells of *P. aeruginosa* was assessed.

Disruption of the *P. aeruginosa* PAO1 rpoS gene resulted in a two- to threefold increase in the rate of kill of stationary-phase cells following exposure to heat, low pH, high osmolarity, hydrogen peroxide and ethanol. The extent, however, of the increased sensitivity in the RpoS-negative stationary-phase cells was not as pronounced as in studies with *E. coli*. This was particularly the case for survival during oxidative, osmotic and heat stress, where, depending on the strain, growth medium and challenge conditions, 6- to 30-fold differences in the survival rate between RpoS-positive and RpoS-negative stationary-phase *E. coli* cells have been reported (Lange & Hengge-Aronis, 1991; Hengge-Aronis et al., 1991; McCann et al., 1991; Cheville et al., 1996). However, it is important to note that various environmental factors have been demonstrated to have a profound influence on the extent of the requirement for RpoS protein for stationary-phase resistance in other bacterial species. The ability of a *Y. enterocolitica* rpoS mutant to survive a variety of stresses was affected at 37 °C but not at 26 °C (Badger & Miller, 1995). The degree of requirement for RpoS protein in *Sh. flexneri* for stress resistance was also shown to depend on environmental factors such as pH or anaerobiosis (Small et al., 1994). The survival assays performed with carbon- and carbon-phosphate-starved *P. aeruginosa* cells indicate that the effect of the RpoS protein is only transient in such conditions. One possible explanation could be that other resistance mechanisms may prevail on RpoS-dependent stress tolerance systems in aged *P. aeruginosa* cultures. A similar transient effect was not observed in starvation studies in *E. coli* but in those experiments starvation survival was not studied for as long a period of time as in this work (McCann et al., 1991; Lange & Hengge-Aronis, 1991).

Stationary-phase cells of *E. coli* and *Sh. flexneri* rpoS mutants have been shown to be more sensitive to acid
stress than their exponential-phase parent strains (Small et al., 1994). Similar results have been observed with respect to the sensitivity to oxidative, osmotic and heat stress, e.g. starved cultures of an *E. coli* rpoS mutant were more sensitive than the exponential-phase parental strain (McCann et al., 1991). Stationary-phase RpoS-negative cells of *P. aeruginosa*, however, were much more resistant than exponentially growing RpoS-positive cells, suggesting that factors other than the RpoS protein which are associated with entry into stationary phase contribute to stationary-phase stress tolerance in *P. aeruginosa*. In *E. coli* and *Sal. typhimurium* *pcm*, *surE*, *mcbA*, *osmC* and *slyA* are expressed in an rpoS-independent manner during entry into stationary phase (Li et al., 1997; Bohannon et al., 1991; Gordia & Gutierrez, 1996; Buchmeier et al., 1997). Some of these genes have also been linked to protection against oxidative stress (*slyA*) and osmotic stress (*osmC*) in

---

**Fig. 5.** Two-dimensional protein maps of *P. aeruginosa* PA05 (a) and PA01 (b) strains. Cells were grown in minimal medium and labelled at late-exponential phase with \[^{35}S\]methionine (1.8 MBq ml\(^{-1}\)) for 1 h. Proteins were separated by two-dimensional PAGE and detected by autoradiography. Modifications of the protein pattern are indicated by arrows. Proteins were designated by an alphanumeric nomenclature. The letter corresponds to a pH zone and the number to an approximate molecular mass. Positions of the molecular mass standards are indicated on the right (a).
stationary-phase cells (Gordia & Gutierrez, 1996; Buchmeier et al., 1997).

Much of our understanding of the underlying molecular changes resulting in the dramatic increase in general stress resistance when exponentially growing cells enter stationary phase has been formed on the basis of studies in E. coli. Several genes known to be RpoS-dependent in E. coli encode proteins which have been shown to be important for the resistance of stationary-phase cultures to environmental stress, e.g. dps (protecting DNA), katE (catalase), otsA/otsB (general stress protectant) and xthA (DNA repair) (Hengge-Aronis, 1996). Some of the protective mechanisms employed by Pseudomonas to survive stress are similar to the mechanisms found in E. coli, e.g. the synthesis of antioxidant enzymes such as superoxide dismutases and catalases to combat oxidative stress and the accumulation of compatible solutes to survive high osmolarity environments (Brown et al., 1995; Dsouzaault et al., 1993). The sodA gene-encoded superoxide dismutase in P. aeruginosa is mainly expressed during the stationary phase of growth as in E. coli and has been shown to be important for oxidative stress resistance (Hassett et al., 1993; Polack et al., 1996; Nyström et al., 1996). The P. aeruginosa katB and katA genes which encode catalases have been shown to be required for optimal resistance when exposed to hydrogen peroxide (Brown et al., 1995). KatB is inducible by hydrogen peroxide in a manner similar to HPII (KatG) in E. coli, whereas katA is transcribed throughout growth in contrast to the RpoS-mediated growth-phase-dependent expression of HPII (KatE) in E. coli (Loewen et al., 1985; Storz et al., 1990; Mulvey et al., 1988). Differences in the regulation of genes involved in stationary-phase resistance may help explain some of the variation observed with respect to the RpoS-dependency of stationary-phase resistance among different species. In this respect, it is worth noting that although a number of proteins were affected by a disruption of the rpoS gene in P. aeruginosa only four proteins (one absent, one elevated, two decreased) showed a consistent difference, which appears to be a less dramatic change when compared to similar studies performed in E. coli (32 absent or reduced; McCann et al., 1991) or Sh. flexneri (27 absent or reduced, two elevated; Waterman & Small, 1996).

Apart from its importance in stress and starvation survival, it is possible that the RpoS protein may have a role in P. aeruginosa virulence. There are several examples of specific roles for alternative sigma factors in microbial pathogenesis. In P. aeruginosa biogenesis of flagella is under the control of the specific alternative sigma factor FlIA, and flagellar motility is required for virulence (Starnbach & Lory, 1992; Drake & Montie, 1988). Another sigma factor, RpoN, is involved in the expression of pili which are also required for full virulence of the organisms (Ishimoto & Lory, 1989). AlgU (also known as AlgT; Goldberg et al., 1993), a homologue of the σE factor in E. coli, controls conversion to a mucoid phenotype in P. aeruginosa but is also important for heat resistance (Boucher et al., 1996). Given that the rpoS gene is regulated in P. aeruginosa via quorum sensing (Latifi et al., 1996), it is possible that the RpoS protein plays a role in co-ordinating the cell-density-dependent expression of virulence determinants. In this respect, it is worth noting that the P. aeruginosa cytotoxic lectins PA-I and PA-II (Avichazar et al., 1992) which are regulated via the RpoS protein are not produced in mutants defective in the production of N-acylhomoserine lactones (unpublished). Whether other P. aeruginosa virulence determinants are co-ordinately regulated via quorum sensing and RpoS protein remains to be established.

ACKNOWLEDGEMENTS

We would like to thank K. Tanaka for providing the RpoS antibody and pDB18R plasmid, G. Ball for technical assistance and Z. Hindle for critical comments on the manuscript. F. Jørgensen was supported by the European Commission (EU) (contract no. FAIRCT96-5066) and V. Chapon-Herve was supported by the Ministry of Research and Technology, France. The work was also supported by the Biotechnology and Biological Sciences Research Council (to P. Williams and G. S. A. B. Stewart) and from the EU contract no. B102-CT950119.

REFERENCES


Received 25 June 1998; revised 16 November 1998; accepted 9 December 1998.