INCREASED BACTERIAL RESISTANCE AND VIRULENCE IN SIMULATED MICROGRAVITY AND ITS MOLECULAR BASIS

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ABSTRACT

Experiments conducted during space flights showed that space conditions affect basic aspects of bacterial physiology, such as growth, conjugation efficiency, and secondary metabolite production. Since logistical constraints hamper research during space flights, use of ground based systems has greatly stimulated progress in this field. A commonly used system is the rotating bioreactor which simulates some aspects of microgravity. Studies using this system have shown that bacteria grown under diminished gravity become more virulent and resistant to a variety of antimicrobial treatments [referred to as "comprehensive cellular resistance (CCR)]. In this respect, the diminished gravity effect resembles the well studied bacterial general stress response. The latter is centrally controlled by an alternate sigma factor, called σ^s. However, the diminished gravity-conferred CCR is not controlled by this sigma factor in rapidly growing cells. Moreover, the proteins known to confer CCR under the Earth conditions do not appear to have a role in this resistance under the diminished gravity conditions. Growth in the HARV system altered the of σ^s regulation pattern at transcriptional, translational and posttranslational levels. Since macromolecular folding patterns play a role in this regulation, the findings raise the possibility that diminished gravity may alter the folding pattern of macromolecules. The effect of diminished gravity on bacterial biofilms, which are responsible for serious and recalcitrant diseases, has recently become possible on earth by the designing of a novel HARV adaptation. The results show that diminished gravity stimulates biofilm formation and makes them highly resistant to antimicrobial agents. Given that the human immune response is compromised in space, these results highlight the serious danger that microbes can potentially pose in space.

INTRODUCTION

Space travel, although tantalizing, is fraught with hazards. There are first the purely human factors, such as the psychological stress originating from the fear of the unknown, isolation, and the claustrophobic atmosphere of a confined environment; and upsetting of the normal life rhythm that results in sleep deprivation and malnourishment. Then there are the peculiarities of space: unusually strong radiation and diminished gravity. Life on Earth evolved in the presence of gravity and thus the latter aspect is an unprecedented experience for the earthlings.

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A good deal of research in recent years has concerned with determining the effect of diminished gravity on human physiology as well as on bacterial characteristics. And the answer seems to be that diminished gravity or 'microgravity' as it is termed, weakens humans and makes bacteria stronger, more difficult to kill, and more accomplished at causing disease. In this review, we will discuss the findings underlying these conclusions.

Bone decalcification and loss are well documented in astronauts during space travel and residence. This predisposes them to bone fracture as well as kidney stones from resorbed bone material. In microgravity, muscles atrophy and blood production decreases. The latter results in diminished pumping by the heart and, combined with the concomitant blood shift to the upper torso, can damage heart muscles. In addition, microgravity compromises the human immune response. This appears to result from several factors: the proportion and number of circulating lymphocytes and their cytokine production are detrimentally altered, lymphocytes produce lower levels of human leukocyte antigen, there is increased apoptosis of peripheral blood mononuclear cells, and dendritic cells become defective in phagocytosis (Sonnenfeld and Shearer, 2002). Additionally, pharmacodynamics and pharmacokinetics are expected to be altered during spaceflight (Graebe et al., 2004).

The study of spaceflight effects on bacteria originated during the advent of manned spaceflight programs in the 1960's. Since that time, many bacterial studies have been conducted onboard spacecraft, and several published reviews of these studies exist (Cioletti, 1991; Dickson, 1991; Moore, 1996; Pierson and Mishra, 2000; Klaus, 2002). In general, these studies have shown that spaceflight causes increased final cell populations, shortened lag phase duration, increased conjugation efficiency, and increased production of secondary metabolites. Other findings, which pose a more direct threat to human health, include decreased antibiotic effectiveness during spaceflight experiments (Tixador et al., 1985; Lapchine et al., 1988; Tixador et al., 1994) and increased spacecraft contamination. Observations of spacecraft contamination include the discovery of biofilms in the Space Shuttle water system (Koenig and Pierson, 1997), free-floating condensate containing microbes onboard the International Space Station (Ott et al., 2004), and microbial contamination onboard the Mir Space Station (Novikova, 2004). The combination of potentially less effective antibiotic treatment, increased

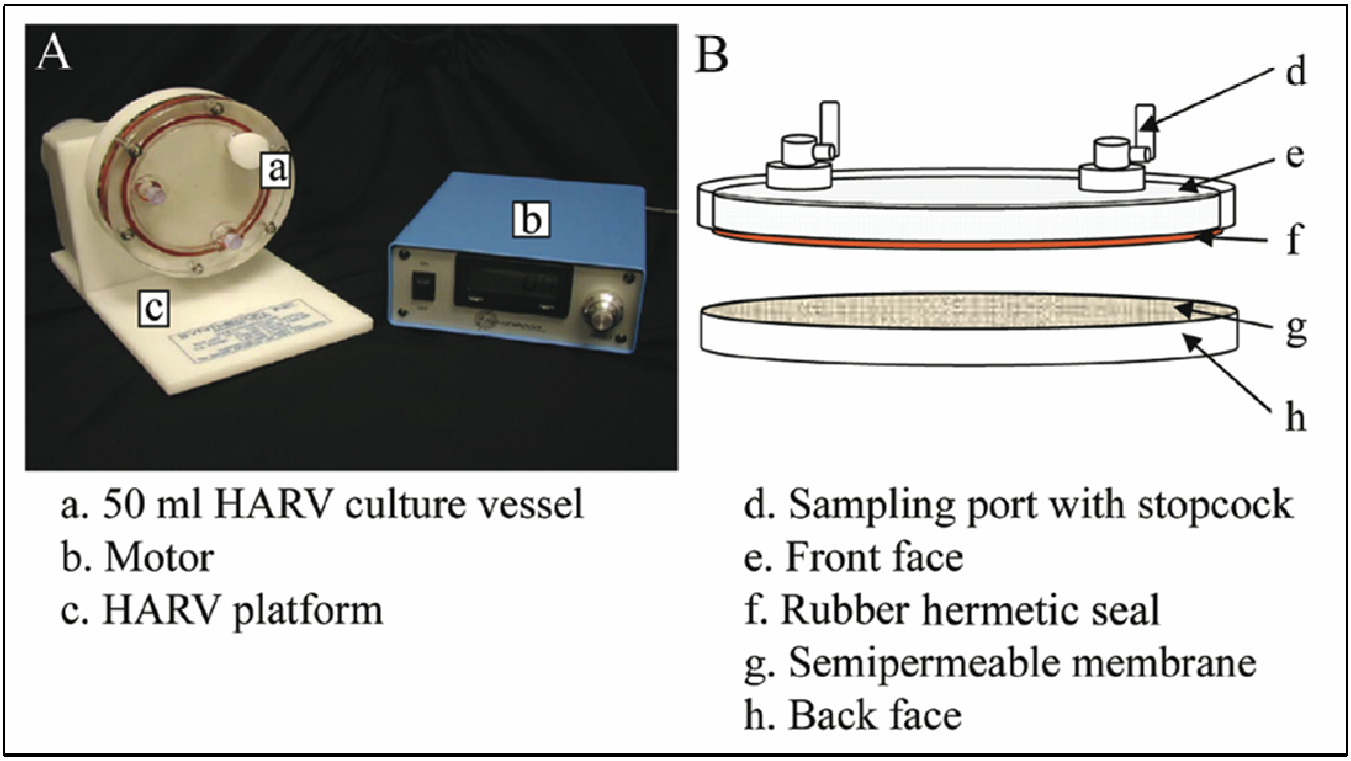

Fig. 1A *HARV system used to simulate conditions of microgravity in Earth-based investigations.* **B.** *HARV vessel components. (Synthecon, Inc.)*

bacterial growth, contaminated spacecraft, and suppressed astronaut immune systems presents a serious threat to crew health. This threat will become exacerbated with increased mission duration (Pierson, 2001), and future missions planned for travel outside of low Earth orbit will not be capable of a swift return home in the case of an untreatable infectious disease.

Studies on the effect of diminished gravity on bacteria as well as mammalian cells have been greatly facilitated by the invention of earth based systems that reproduce some aspects of microgravity. The clinostat, which was developed over a century ago for studying gravitropic plant responses (Dedolph and Dipert, 1971), rotates a container of suspension cultures about an axis that is perpendicular to the gravity vector. Thorough descriptions of this device and its limitations have been published (Albrecht-Buehler, 1992; Klaus, 2001). More recently, rotating systems such as the high aspect ratio vessel (HARV) bioreactor (Fig. 1a), have been designed for studying mammalian tissue culture (Wolf and Schwarz, 1991; Schwarz et al., 1992; Wolf and Schwarz, 1992). The HARV has front and back faces that are separable; the front face contains two sampling ports, and the back face is equipped with a semi-permeable membrane for aeration (Fig. 1b). The vessel is completely filled with the medium and inoculum so that no head space remains. Air bubbles are removed, to prevent fluid mixing. Two such vessels are used per experiment, of which one is rotated about a vertical axis, perpendicular to the gravitational vector, and the other about a horizontal axis, parallel to this vector.

In both the clinostat and the HARV, virtually no relative fluid motion exists and after an initial start-up time, solid body rotation of the fluid ensues. Since mixing occurs by gentle rotation in the absence of stirring vanes, and aeration is affected by dissolved gasses with no headspace or bubbles, there is no shear-field inhomogeneity;vessels rotated around either axis thus possess a low-shear environment. In the vessel rotated about the horizontal axis, particles quickly reach a terminal settling velocity, but cumulative sedimentation is avoided due to hydrodynamic forces, viz., shear, centrifugal and Coriolis (Hammond and Hammond, 2001). The gravity vector is continually re-oriented as the cells and fluid rotate together, which dramatically influences large cells, e.g., those of plants with gravity sensing mechanisms (Dedolph and Dipert, 1971).

Both the clinostat, and more recently the HARV, have been used to study bacteria. Although planned experiments to demonstrate the effectiveness of the HARV as a spaceflight analog have yet to be conducted (Niesel et al., 2005), ground-based clinostat experiments have been correlated with spaceflight experiments yielding similar results, at least in terms of increased final cell populations (Kacena et al., 1999; Brown et al., 2002). Due to the small size of bacteria, rotating culture systems operated at the correct rotation rate can reduce gravitational cell motion to less than Brownian motion (random diffusion) for the duration of the experiment (Todd and Klaus, 1996), thereby making a suitable simulation of the reduced fluid motion provided by weightlessness. This condition has been referred to as Low Shear Modeled MicroGravity (LSMMG) (Nickerson et al., 2003; Nickerson et al., 2004) or simply Simulated

MicroGravity (SMG) (Lynch and Matin, 2005; Matin and Lynch, 2005).

An interesting alternate method of simulating weightlessness was devised by Benoit and Klaus (2005). A genetically engineered, gas vesicle-forming strain of *Escherichia coli* was used. These vesicles are organelles with a protein coat which is permeable to ambient gases, and certain bacteria produce them to stay afloat in the water column in nutritionally favorable regions. The engineered *E. coli* strain remained suspended without the need for rotation and exhibited growth characteristics similar to the isogenic wild type (not producing gas vesicles), when the latter was grown in a rotated clinostat.

EFFECT OF SMG ON BACTERIAL VIRULENCE

Nickerson and co-workers demonstrated that when mice were inoculated with SMG grown *Salmonella enteritica* serovar Typhimurium, they died faster than mice inoculated with an equal number of bacteria ($2x10^6$ CFU) grown under normal gravity conditions (Fig. 2) On the 10^{th} day of inoculation, while only 40% of the mice inoculated with the NG grown cells had died, the number of mice that had died following inoculation with SMG grown bacteria was 100% (Nickerson et al., 2000). Autopsy showed a more extensive colonization by the latter; thus, for instance, 10,000 vs. 300 cells were recovered from the spleens of mice infected with SMG and NG-grown bacteria, respectively.

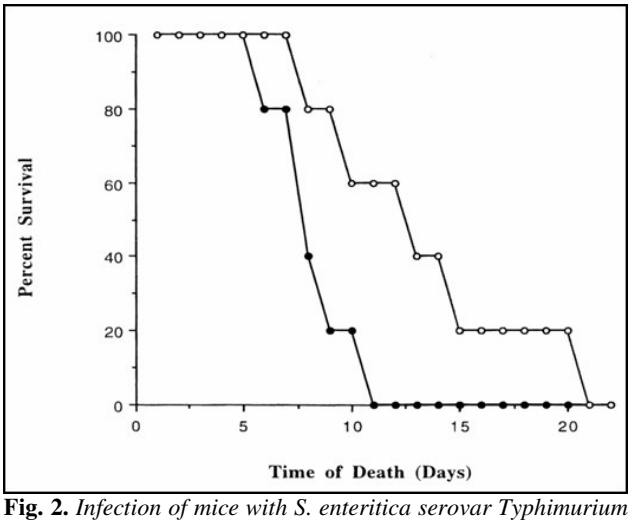

Fig. 2. *Infection of mice with S. enteritica serovar Typhimurium cultured in SMG (solid symbols) results in shortened time to death compared to S. enteritica cultured under normal gravity conditions (open symbols). Reproduced with permission from Nickerson et al. (2000).*

EFFECT OF SMG ON BACTERIAL RESISTANCE TO ANTIMICROBIAL STRESSES

A large body of work conducted with bacteria grown under conventional normal gravity conditions over the last two or so decades has shown that increased bacterial virulence is accompanied with increased resistance to antimicrobial stresses. To test if this was true of the SMG effect, Lynch et al. (2004) determined the effect of SMG growth on the resistance of the bacterium *E. coli* to two treatments that are detrimental to bacteria, exposure to high salt or to high acidity. In these experiments, two growth phases were examined, the exponential phase of rapid growth and the stationary phase, in which little or no net growth is seen and bacteria exist in a resting state. While both phases occur in nature, it is the stationary phase or condition resembling this phase that is most commonly experienced by bacteria in nature (Matin, 2001).

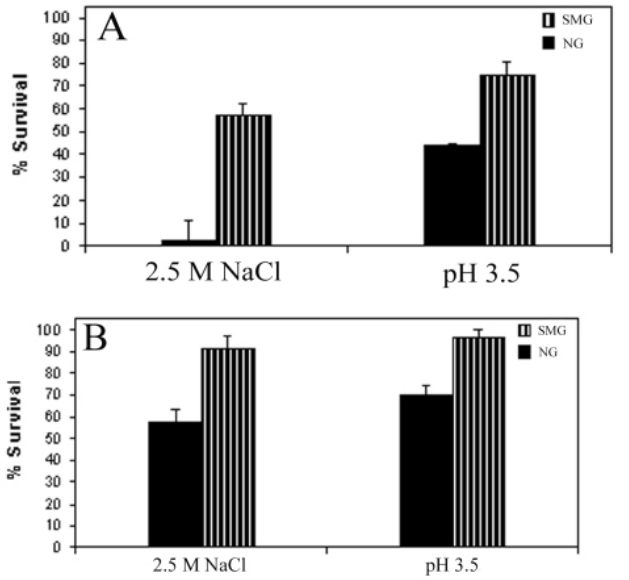

Fig. 3. *SMG-cultured E. coli demonstrates increased resistance to both osmotic and acid stresses during both exponential (A) and stationary (B) phases of growth. Reproduced with permission from Lynch et al. (2004).*

Growth under SMG conditions increased exponential phase *E. coli* resistance to each of the separately tested stresses (Fig. 3A); in the case of the salt treatment, for instance, nearly all the NG-grown cells but only some 40% of the SMG-grown cells, respectively, were killed. It is well established that stationary phase bacteria grown under conventional flask cultures under normal gravity exhibit increased resistance compared to their exponential phase counterparts (Jenkins et al., 1990; Matin, 1991). As shown in Fig. 3B, this is also true of the HARV NG cultures. The striking thing, however, is that growth under SMG caused the already resistant stationary phase cells to become even more resistant, becoming virtually invincible to antimicrobial treatments. That *E. coli* grown under SMG (compared to shake flask) conditions were less susceptible also to ethanol exposure, a common method to disinfect, was shown by Gao et al. (2001).

RESEMBLANCE OF THE SMG EFFECT TO THE GENERAL STRESS RESPONSE

The three antimicrobial treatments named above against which growth under SMG made *E. coli* more resistant harm bacteria in different ways. Exposure to low pH is

detrimental because it acidifies the cytoplasm and leads to decomposition or denaturation of vital cell constituents. High salt dehydrates the cell, and exposure to ethanol damages the cell envelope. That SMG made cells resistant to these three disparate stresses, which kill bacteria by causing different kinds of injuries, indicates that the mechanism of resistance activated by SMG encompasses preventing and/or repairing injury to many different cell constituents.

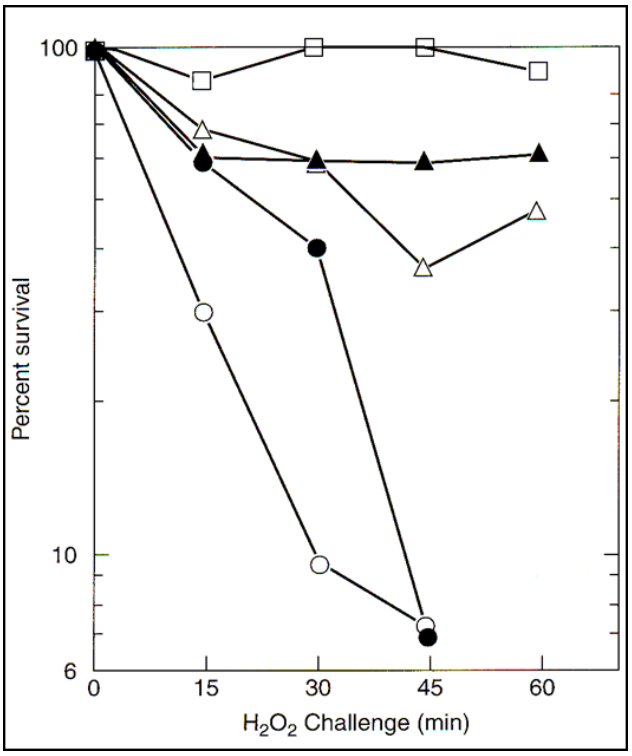

Fig. 4. *Comparison of the H_2O_2 resistance of exponential phase (○), or glucose-starved (□) E. coli cultures to growing cultures stressed by heat (Δ), H_2O_2 (▲), or ethanol (●). Reproduced with permission from Matin (2001).*

In this respect, the SMG effect resembles the general stress response (GSR) that has been extensively studied under conventional culture conditions of normal gravity (Matin, 2001). This phenomenon is illustrated in Fig. 4. When bacteria are exposed to small doses of H_2O_2, they acquire the capacity to resist much higher and normally lethal doses of this toxic agent. But the striking thing is that protection against lethal doses of H_2O_2 results from prior exposure of cells to a large number of separate unrelated antimicrobial treatments, such as starvation, heat, or ethanol. This sort of cross protection operates against almost any stress so that, for instance, H_2O_2-treated cells become more resistant to the other deleterious agents as demonstrated in Fig. 4; and heat or starvation-stressed cells acquire cross protection to unrelated insults such as osmotic shock, or exposure to low pH. Thus, the essence of GSR is that exposure of bacteria to small sub-lethal dose of an antimicrobial treatment makes them resistant not only to subsequent exposure of lethal doses of the same agent but also to others that injure bacteria by different mechanisms.

MOLECULAR AND BIOCHEMICAL BASIS OF GSR AND SMG EFFECTS

What is the molecular basis that renders cells comprehensively resistant after exposure to small doses of a deleterious agent? Bacterial cells exposed to a large variety of harmful agents respond by increasing the cellular concentration of a "stress" sigma factor, σ^s. This sigma factor replaces the "house keeping" sigma factor (σ^{70}) on the RNA polymerase enzyme, thereby changing its regulatory properties. σ^s-RNA polymerase recognizes a different promoter sequence compared to σ^{70}-RNA polymerase; promoter regions consist of sequences present in front of the coding region of genes. The σ^s-RNA polymerase recognized promoters are present in front of genes that encode proteins, which can protect against different types of cell injuries. Examples of proteins thus induced are molecular chaperones, such as DnaK which prevents damage to and can repair denatured proteins; the SOS response proteins, e.g., PexB that can insulate the cell against DNA damage, and proteins such as D-alanine carboxypeptidase and PexAB that are concerned with cell envelope integrity (Matin, 2001). Consequently, bacteria subjected to sub-lethal dose of one harmful treatment become capable of withstanding multiple types of damage to their cell constituents.

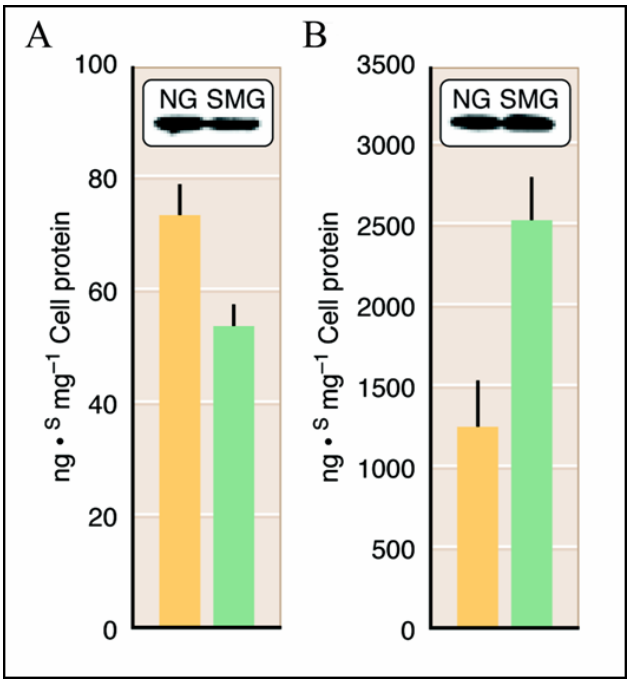

Fig. 5. *Quantification of σ^s protein levels in exponential (A) and stationary (B) phase NG and SMG cultures. Reproduced with permission from Matin and Lynch (2005).*

As we have seen (Fig. 3), growth under SMG also makes cells resistant to mechanistically different killing agents, and since it resembles GSR in this respect, it was logical to suspect that the underlying molecular and biochemical mechanisms may be similar, and that SMG acted in the same way as do other deleterious agents. However, exponential phase cells grown under SMG conditions

possessed lower σ^s levels than their NG-grown counterparts (Fig. 5A; (Lynch et al., 2004)). This is the first instance in which increased cellular resistance to deleterious treatments is found accompanied with lowered levels of σ^s. In contrast, the stationary phase SMG-grown cells that exhibit super resistance (Fig. 3), conformed to the normal paradigm: their increased resistance was accompanied by increased σ^s levels compared to the NG-grown cells (Fig. 5B). The conclusion suggested by these findings, namely, that the SMG-conferred resistance is independent of σ^s in exponential phase, but dependent on it in the stationary phase was confirmed by repeating these experiments with a mutant of *E. coli* missing this sigma factor. The mutant developed resistance normally in the exponential phase (Fig. 6A), when grown under SMG conditions, but was severely weakened under both NG and SMG conditions in stationary phase (Fig. 6B).

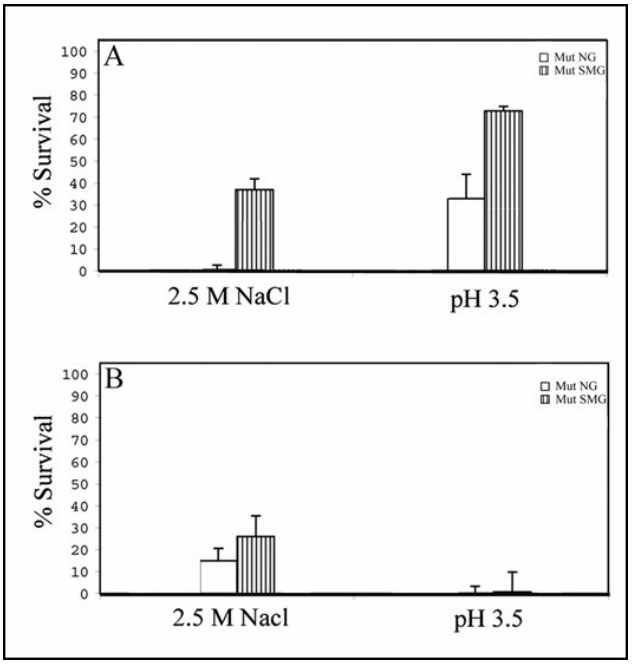

Fig. 6. *Stress resistance of an rpoS mutant strain grown under NG and SMG conditions in exponential (A) and stationary (B) phases of growth. Reproduced with permission from Lynch et al. (2004).*

The Nickerson group has examined the genes that are influenced in exponential phase-cells grown under SMG conditions (Wilson et al., 2002). Expression of over 160 genes was affected by growth under SMG conditions in *S. enterica typhimurium* serovar Typhimurium. Surprisingly, none of the up-regulated genes included those known to encode protective proteins of the type mentioned above. Furthermore, many of the genes involved in determining virulence under the conventional flask culture conditions were in fact down-regulated by SMG growth. The ferric iron uptake protein, Fur, which has been shown to have a role in SMG acid resistance, could have a larger role in regulating gene expression under these conditions, since many of the SMG-induced genes apparently possess Fur-binding sites (Wilson et al., 2002). How gene expression is regulated by SMG growth in the stationary phase has not yet been investigated. It is clear however, that the SMG and conventional normal gravity stress resistance and virulence mechanisms differ in at least two respects: in the exponential phase, it is independent of σ^s and does not involve induction of genes known to be responsible for conferring protection on the bacterial cell; and in that the relationship of this mechanism to σ^s, and possibly also to the known stress genes, reverses during transition from exponential to stationary growth phase.

MOLECULAR REGULATION OF σ^S

These unexpected findings led Lynch et al. (2004) to explore if fundamental cell processes, such as regulation of protein synthesis, were affected by growth under SMG. They addressed this question by looking at σ^s for two reasons, one that under SMG it is up or down regulated depending on the growth phase; and two that its synthesis has been thoroughly studied in conventional gravity conditions, providing guidelines on aspects to study. These prior conventional investigations have established that σ^s synthesis is regulated by a combination of regulatory mechanisms that operate at transcriptional, translational efficiency, and protein stability levels.

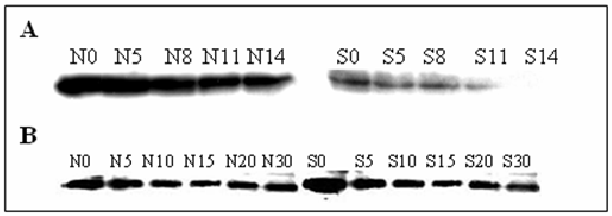

Fig. 7. *Western analysis of σ^s protein degradation over time in exponential (A) and stationary (B) phases of growth under NG and SMG conditions. N, Normal gravity time point; S, Simulated microgravity time point. Reproduced with permission from Lynch et al. (2004).*

σ^s is encoded by the *rpoS* gene. To study the effect of SMG on the transcription rate of this gene, the steady-state levels of the *rpoS* messenger RNA (mRNA) were quantified as well as the half-life of this mRNA in cells grown under NG and SMG conditions, using quantitative PCR. The transcription rate was not altered in exponential phase but was decreased by one-half in the stationary phase in SMG-grown cells (Table 1). A much more pronounced effect of SMG was seen on the stability of σ^s protein (Fig. 7). In the exponential phase, growth under SMG rendered this protein over three-fold less stable. A similar, less pronounced, but reproducible effect occurred also in the stationary phase cells. Translational efficiency of the *rpoS* mRNA, i.e., the amount of σ^s protein synthesized per molecule of the *rpoS* mRNA, was calculated from the rate of σ^s synthesis (calculated in turn from steady state σ^s levels and from the half-life of this protein) and that of *rpoS* mRNA synthesis (Table 2). It increased over two-fold under SMG regardless of the growth phase.

Table 1. *Copies of rpoS mRNA synthesized per minute under NG and SMG conditions in exponential or stationary phases of growth (Lynch et al., 2004).*

	NG	SMG
Exponential phase	1.6×10^7	1.6×10^7
Stationary phase	2.8×10^5	1.2×10^5

Table 2. *Translational efficiency of σ^s protein per copy of rpoS mRNA per minute (Lynch et al., 2004).*

	NG	SMG
Exponential phase	3.6×10^{11}	8.4×10^{11}
Stationary phase	3.3×10^{11}	8.4×10^{11}

It is not known how SMG affects these parameters of σ^s synthesis. It is possible that the levels of molecules that regulate these processes are affected by growth under SMG. Another possibility is suggested by the fact that regulation at all the levels mentioned above involves higher order structures of the concerned molecules. Stability of the σ^s protein depends on whether it is subject to proteolysis by a specific protease, called the ClpXP protease (Schweder et al., 1996; Becker et al., 1999; Hengge-Aronis, 2002). Why it is attacked more readily by the ClpXP protease under certain conditions and not others is not fully understood. Inactivation of another protein, called RssB (or SprE), appears to have a role under carbon starvation conditions. In addition, the folding pattern of σ^s could also be involved. The activity of ClpXP protease is markedly affected by the folding pattern of its substrate (Kenniston et al., 2004). Experiments conducted in space have shown that proteins form crystals more readily under these conditions. Taking these findings together, it has been hypothesized that diminished gravity and low shear may influence protein folding pattern (Fig. 8; (Lynch et al., 2004)). In the present case, this may mean that growth under SMG causes the σ^s protein to acquire a configuration that is more amenable to attack by the ClpXP protease.

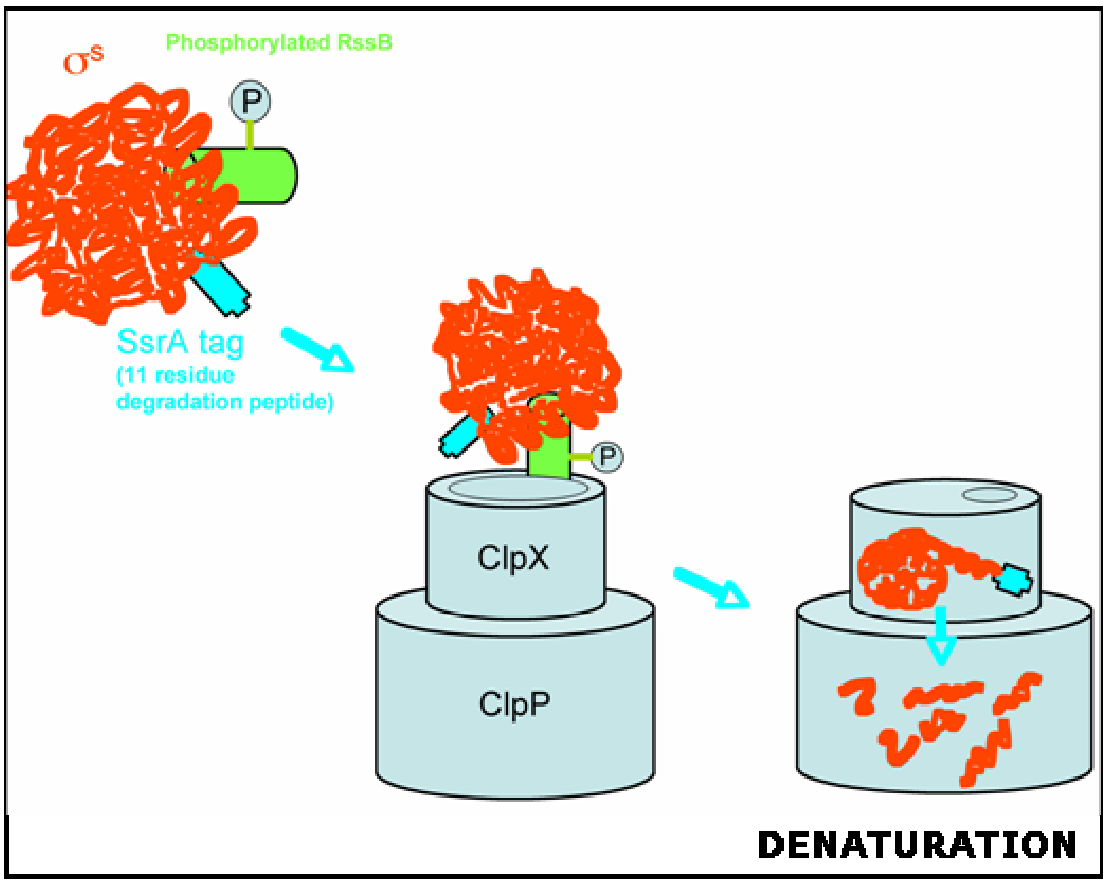

Fig. 8. *Schematic representation of post-translational degradation of σ^s protein involving RssB, the degradation tag (termed SsrA) and the ClpXP protease complex.*

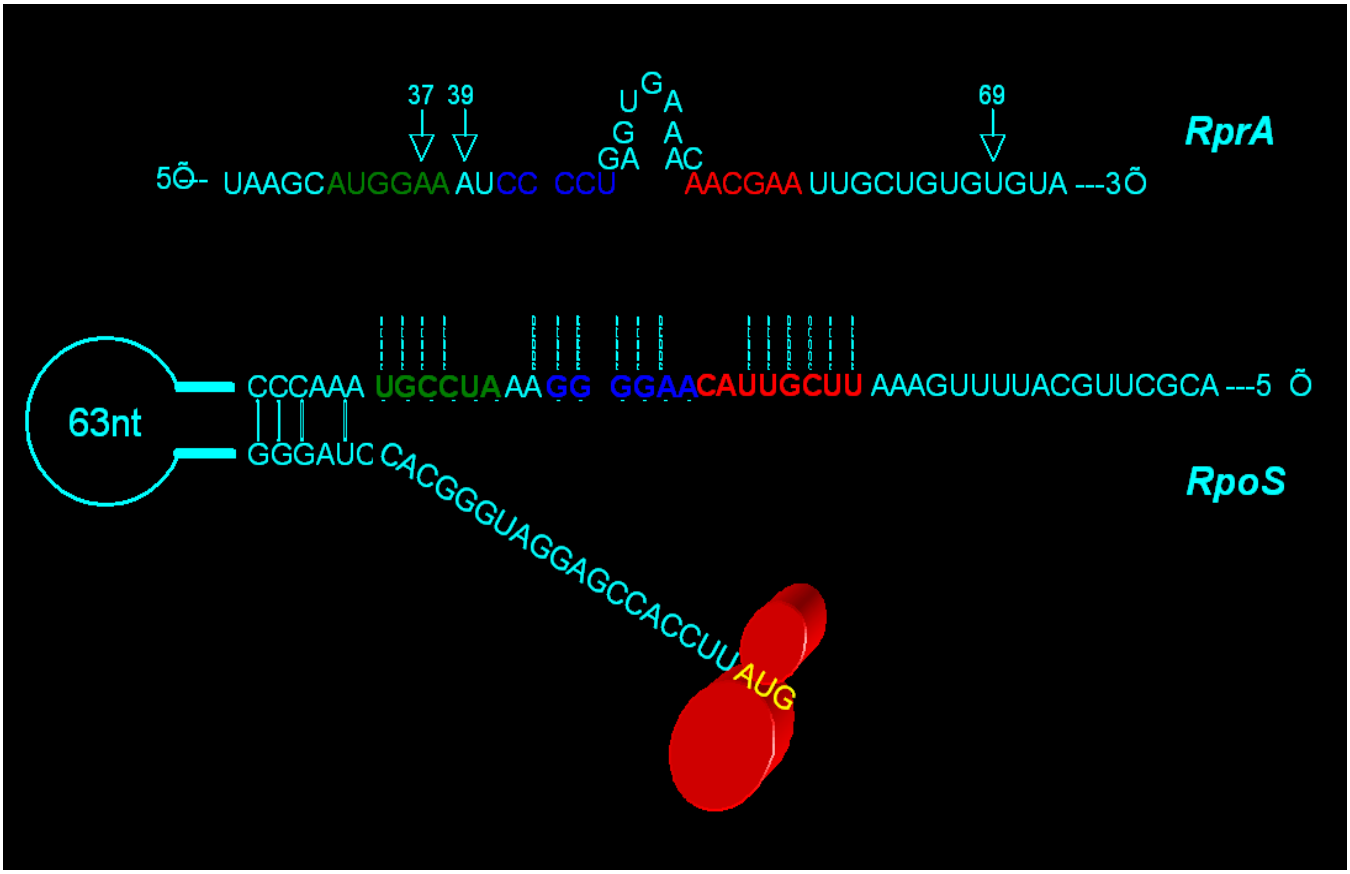

Fig. 9. rpr*A a small non-translated regulatory RNA possesses stretches of sequence homology with the untranslated region of rpoS, permitting binding and relief of translation inhibition of the rpoS mRNA. Reproduced with permission from Matin and Lynch (2005).*

Secondary structure formation also plays a central role in the translational efficiency of *rpoS* mRNA. This messenger molecule possesses internal homologies in its untranslated region that result in the formation of a hairpin structure, which block its translational start site, making it unavailable to the ribosomes. Studies under conventional conditions of normal gravity strongly suggest that this structure is relaxed when cells are exposed to antimicrobial treatments. This is brought about by interaction between the *rpoS* mRNA, a protein called Hfq, and one of several small RNAs, for example, *rprA*. The latter possesses homologies to the interfering sequence responsible for the hairpin structure formation in the *rpoS* mRNA and, in association with Hfq and perhaps other factors, can disrupt this structure. As a result the translational start site becomes untangled and available to the ribosomes, promoting synthesis of σ^s (Fig. 9). SMG could act in stimulating translation by affecting any of the participants in this mechanism, including a direct effect in minimizing secondary structure formation within the *rpoS* mRNA. Only future work will reveal what factors are involved in these phenomena, but if diminished gravity and low shear can influence folding patterns of molecules, implications of space conditions on life processes would be wide ranging indeed.

EFFECT OF SMG ON BACTERIAL BIOFILM RESISTANCE

Biofilms are bacterial communities that are surrounded by a polysaccharide matrix. Different cells within such communities may perform different functions, and biofilms may have a primitive circulatory system to ensure efficient distribution of nutrients and removal of waste products. Bacterial biofilms have a considerable medical importance because they exhibit much greater resistance to antibiotics and other antimicrobial treatments than their constituent individual cells when they exist outside the community. Consequently, diseases in which bacterial biofilms have a major role, such as cystitis, endocarditis, and cystic fibrosis, are chronic and difficult to treat (Fux et al., 2005).

During experiments onboard the Space Shuttle, *Burkholderia cepacia* formed biofilms (Pyle et al., 1999) and *Pseudomonas aeruginosa* biofilms developed on polycarbonate membranes (McLean et al., 2001). Furthermore, biofilms were found in the Space Shuttle water system (Koenig and Pierson, 1997) and on surfaces and equipment onboard the Mir Space Station (Novikova, 2004). These findings point to increased danger to astronaut health. Conversely, however, biofilms are commonly used for nitrification and organic carbon removal of wastewater, making them suitable for

wastewater treatment devices onboard future long-duration space missions (Sharvelle et al., 2002). Because biofilms will be present in the spacecraft environment, understanding the effects of microgravity on biofilms will be important for their control and exploitation.

How SMG growth affects biofilm resistance is therefore of interest. However, this question has not been adequately addressed because of a lack of earth-based system to generate SMG-grown biofilms. Recently, a modification of the HARV apparatus, involving the use of appropriate beads, permitted facile bacterial biofilm cultivation under SMG conditions on Earth (Lynch, Mukundakrishnan, Ayyaswami and Matin, submitted). SMG stimulated more copious biofilm formation and these were much more resistant than their NG grown counterparts to two antibiotics, penicillin and chloramphenicol, as well as to the antimicrobial treatments, such as exposure to high salt and ethanol. The SMG-mediated enhanced resistance to salt and ethanol, but not to the antibiotics, required the presence of σ^s in the cells since an *rpoS* mutant failed to exhibit this phenomenon for the former but not for the latter agents.

What aspect of the biofilms is controlled by σ^s that enhances their resistance to various agents in a differential manner has not as yet been determined. Lynch, Dixon and Matin. (submitted) have, however, recently identified another gene in uropathogenic *E. coli* which controls biofilm resistance and whose mode of action has become partially known. Comparison between biofilms of wild type uropathogenic *E. coli* and a *rapA* mutant shows that the *rapA* gene contributes to the increased resistance of biofilms by promoting matrix exopolysaccharide formation. When penetration of a fluorescent probe of penicillin was visualized, it became clear that the antibiotic penetrates the biofilm of the mutant much more rapidly than that of the wild type. In addition, the *rapA* gene is required for the expression of two genes that appear to encode multidrug resistance pumps. Thus, this gene appears to enable biofilms to utilize a dual strategy for resistance to antimicrobials, retarded penetration and more effective means of effluxing the antimicrobial that does manage to finds its way into the biofilm cells. The studies on the *rapA* gene have so far been done only under the conventional conditions of normal gravity. How SMG may affect these parameters is under investigation.

SMG AND SPACEFLIGHT EFFECTS ON SECONDARY METABOLITE AND SPORE PRODUCTION

In addition to the general stress response and increased virulence, other survival strategies of bacteria include the production of secondary metabolites, such as antibiotics, which kill off competing organisms, and spore production, which allows cells to wait out poor environmental conditions. A few reports exist of increased antibiotic production by bacteria on spacecrafts (Lam et al., 1998; Luo et al., 1998; Lam et al., 2002; Benoit et al., 2005). Following 15 days of *Streptomyces ansochromogenus* growth onboard a Chinese satellite, Luo et al. (1998) found that some mutants produced increased concentrations of Nikomycin compared to ground controls. A 17-day experiment conducted on the Space Shuttle (STS-80) showed increased production of Actinomycin D by *Streptomyces plicatus*. Due to spaceflight hardware constraints, however, the absolute production was low compared to conventional culture methods. In a follow-up study, a newly designed space bioreactor was used allowing antibiotic production at similar concentrations to conventional methods. This study involved a 72-day experiment conducted onboard the International Space Station. Samples taken at days eight and twelve showed increased spaceflight production, but subsequent production was lower than the matched ground controls.

Several ground-based studies of bacterial secondary metabolite production have been conducted with HARV bioreactors. In four of these experiments, secondary metabolite production (of β-lactam antibiotics and rapamycin) was inhibited by SMG compared to normal gravity mode of the HARV (Fang et al., 1997a; Fang et al., 1997c; Fang et al., 2000; Gao et al., 2001). In the fifth experiment, gramicidin synthesis by *Bacillus brevis* was unaffected by SMG (Fang et al., 1997b). However, the site of antibiotic accumulation shifted from inside to the outside of the cell, which is beneficial as it facilitates purification. In addition, secondary metabolite production in the RWV bioreactors was much lower than production in shake flasks for all five studies. Demain and Fang (2001) hypothesized that reduced shear stress in the RWV bioreactors caused the reduced secondary metabolite production. In support of this hypothesis, beads were added to RWV bioreactors to increase shear stress, resulting in increased production of secondary metabolites (Fang et al., 2000; Gao et al., 2001). However, bacterial production of a polymer has been shown to increase for bacteria grown in a HARV compared to shake flask cultures (Thiruvenkatam and Scholz, 2000).

In terms of spore production, Mennigmann and Lange (1986) reported a lower number of spores from *B. subtilis* cultures in spaceflight (5×10^4 spores ml^{-1}) compared to ground controls (8×10^5 spores ml^{-1}). In a follow on experiment, Mennigmann and Heise (1994) reported the opposite trend: the ratio of spores from spaceflight to ground cultures was 12.64 and the ratio of spores per cell was higher for flight cultures (0.64) compared to ground cultures (0.17). A centrifuge was used onboard the Space Shuttle during this experiment to provide 1 g flight control, which showed no significant difference in spore production from 1 g ground control. Launch accelerations and space radiation were therefore ruled out as possible causes of altered spore production. Lam et al. (2002) reported that cultures of *Streptomyces plicatus* flown in space for 17 days sporulated profusely when plated post-flight, but the ground controls lost their ability to sporulate when similarly plated. Finally, Benoit et al. (2005) reported that significantly less spores were recovered from the spaceflight residual viable cultures.

However, the post-flight sporulating ability of the flight cultures was found to be approximately 8 times that of the corresponding ground controls. In addition, scanning electron micrograph images showed that spore morphology was altered by spaceflight.

CONCLUSION

Spaceflight alters bacteria in several ways, but the potential threat to humans is not well understood. Rotating bioreactors such as the clinostat and HARV are effective at simulating some aspects of microgravity for bacterial cell cultures. While much remains to be learned, it is clear already that SMG growth affects bacterial resistance and virulence both in planktonic and biofilm mode of growth in ways as to constitute a serious potential threat to astronaut health. The molecular mechanisms underlying this phenomenon remain largely unexplored but initial indications are that novel, as yet unknown, strategies may be involved. Besides being potentially harmful, bacteria and their biofilms also have beneficial uses, for example, in microbial-based regenerative systems of waste water treatment. These systems, commonly used on Earth, represent a viable alternative to physical and chemical methods in use today on the International Space Station. Long duration missions planned for travel outside of low Earth orbit may demand regenerative systems, which are intended to minimize consumable mass. Thus, continued investigation of molecular physiology of bacteria under SMG is necessary to better control their harmful effects as well as exploit their beneficial roles.

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