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EcAMSat spaceflight measurements of the role of σ^s in antibiotic resistance of stationary phase *Escherichia coli* in microgravity

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ABSTRACT

We report the results of the *EcAMSat* (*Escherichia coli* Antimicrobial Satellite) autonomous space flight experiment, investigating the role of σ^s in the development of antibiotic resistance in uropathogenic *E. coli* (UPEC) in microgravity (μ-**g**). The presence of σ^s, encoded by the *rpoS* gene, has been shown to increase antibiotic resistance in Earth gravity, but it was unknown if this effect occurs in μ-**g**. Two strains, wildtype (WT) UPEC and its isogenic Δ*rpoS* mutant, were grown to stationary phase aboard *EcAMSat*, an 11-kg small satellite, and in a parallel ground-based control experiment; cell growth rates for the two strains were found to be unaltered by μ-**g**. After starvation for over 24 h, stationary-phase cells were incubated with three doses of gentamicin (Gm), a common treatment for urinary tract infections (which have been reported in astronauts). Cellular metabolic activity was measured optically using the redox-based indicator alamarBlue (aB): both strains exhibited slower metabolism in μ-**g**, consistent with results from previous smallsat missions. The results also showed that μ-**g** did ***not*** enhance UPEC resistance to Gm; in fact, both strains were more susceptible to Gm in μ-**g**. It was also found, via a second ground-control experiment, that multi-week storage in the payload hardware stressed the cells, potentially obscuring small differential effects of the antibiotic between WT and mutant and/or between μ-**g** and ground. Overall, results showed that the Δ*rpoS* mutant was 34–37% less metabolically active than the WT for four different sets of conditions: ground without Gm, ground with Gm; μ-**g** without Gm, μ-**g** with Gm. We conclude therefore that the *rpoS* gene and its downstream products are important therapeutic targets for treating bacterial infections in space, much as they are on the ground.

1. Introduction

With NASA setting the course to return to the Moon and venture out to Mars, maintaining the health of astronauts during long-term spaceflight will be paramount. One area of particular concern is the reported increase in virulence and antibiotic resistance of microorganisms in previous space experiments (Aunins et al., 2018; Juergensmeyer et al., 1999; Nickerson et al., 2004; Taylor, 2015; Urbaniak et al., 2018; Wilson et al., 2007; Zea et al., 2017; Zhang et al., 2012). Combined with a depressed immune response in astronauts (Sonnenfeld and Shearer, 2002), there is an increased risk of opportunistic bacterial infection (Taylor, 2015). An important microbe in this regard is uropathogenic *E. coli* (UPEC), a causative agent of the urinary tract infections (UTIs) (Matin et al., 2017) frequently reported in astronauts (Barratt and Pool, 2008). Given that antibiotic resistance remains an urgent issue on Earth (Ventola, 2015; Lushniak, 2014), including in UPEC (Rijavec et al., 2006), mechanistic understanding of this resistance and development of new therapeutic targets will be necessary to treat bacterial infections both here and in space (Singh and Matin, 2016; Taylor and Sommer, 2005).

The sigma factor, σ^s (product of the *rpoS* gene), has been found to regulate the general stress response (GSR) in UPEC, making the bacteria comprehensively resistant to diverse antimicrobial agents (Hengge-Aronis, 2002a; Matin, 1991; Matin et al., 1989; Wang et al., 2014), including gentamicin (Gm), a common treatment for UTIs. We therefore hypothesized that the stress of spaceflight might activate UPEC's GSR via σ^s, rendering this pathogen more resistant to antibiotic treatment.

Indeed, previous work using high-aspect-ratio vessels to produce low-shear modeled microgravity (LSMMG) demonstrated an increase in UPEC's resistance to Gm compared to a control, an increase dependent

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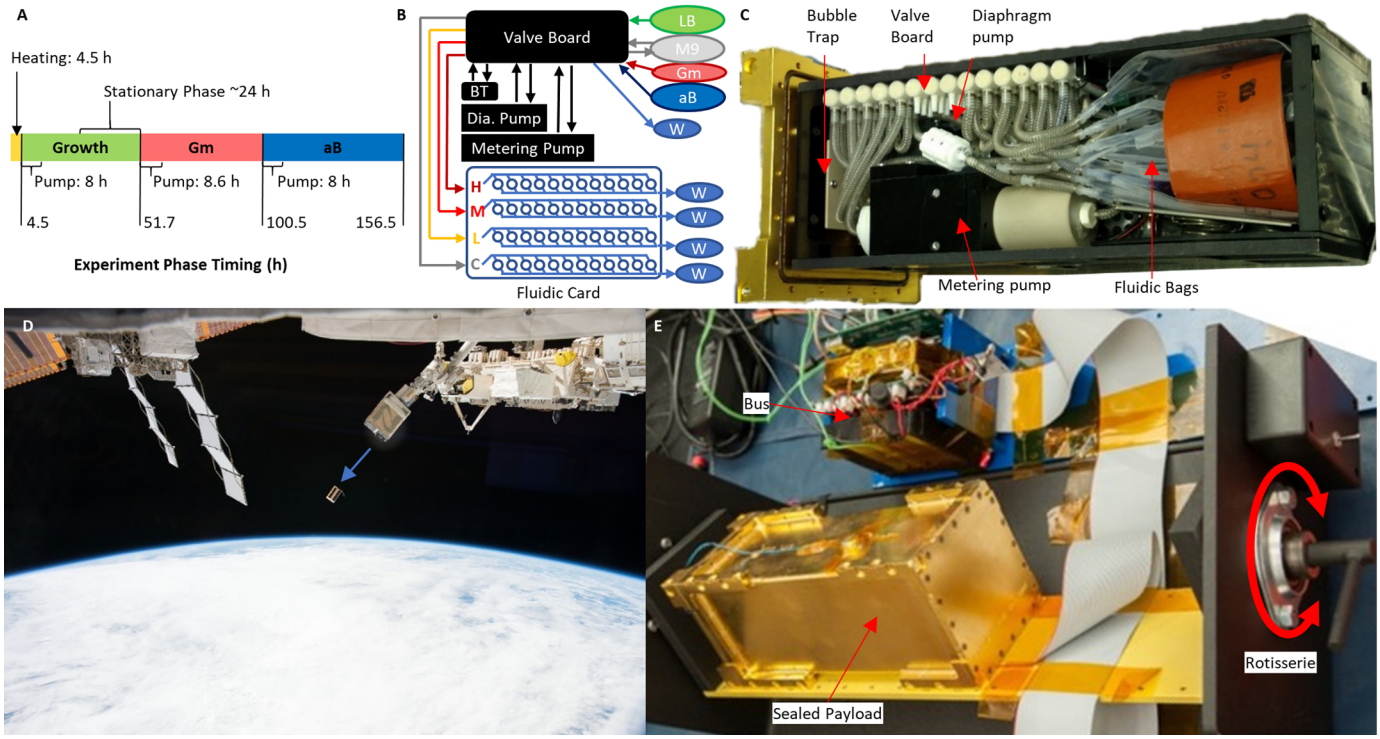

Fig. 1. (A) Experiment timeline showing the heating phase (yellow, to raise the temperature to 37 °C), pumping of LB, growth and stationary phase (green), antibiotic gentamicin (Gm) pumping and challenge (red), and alamarBlue (aB) pumping and reduction phases (blue). (B) Schematic of fluidic system showing flow directions through valve board. Each bank of 12 wells is supplied by a common inlet and common outlet manifold: fluid exchange occurs in parallel. A more detailed schematic has been published previously (Matin et al., 2017). BT = bubble trap; Dia. Pump = diaphragm pump; W = waste bag, H = high dose bank, M = medium dose bank; L = low dose bank; C = control bank. (C) Assembled bio-module of the flight payload prior to installing in hermetic payload can, with labels for components from B. (D) Deployment of *EcAMSat* (arrow) from the ISS on November 20, 2017. (E) The DMTG control payload on a rotisserie, commanded by a flight-like bus. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

on σ^s (Matin et al., 2017). LSMMG, however, is only an approximation of space microgravity (μ-**g**): findings under such modeled conditions are not invariably replicated in spaceflight (Hammond et al., 2013; Wilson et al., 2002). Reported instances of increased resistance have been contradicted by other findings of either no change (Morrison et al., 2017) or enhanced sensitivity to antimicrobials (Leys et al., 2004).

One spaceflight effect that could influence the phenotype of microbes grown in liquid culture and hence their GSR is the absence (in authentic μ-**g** but not in LSMMG) of thermal-gradient-driven convection, resulting in reduced bulk fluid volume exchange in the immediate environment of the cell: this can inhibit both the removal of waste and the nutrient flux to (nonmotile) cells (Klaus et al., 1997). Several studies have found that the stress of the μ-**g** environment can alter the behavior of microorganisms, although the differences between spaceflight and ground experiments have been confounded by species, culture conditions, and other experimental parameters (Taylor, 2015). For example, a number of studies concluded that growth yield of *E. coli* increased and the duration of the lag phase decreased under μ-g (Klaus et al., 1997; Brown et al., 2002; Kacena et al., 1999a,1999b, 1999c), while other reports indicated the opposite (Bouloc and D'Ari, 1991; Gasset et al., 1994; Vukanti et al., 2012).

GeneSat-1 (Kitts et al., 2006; Parra et al., 2008; Ricco et al., 2007), *PharmaSat* (Ricco et al., 2011), *O/OREOS* (Mattioda et al., 2012; Nicholson et al., 2011) and other nanosatellite systems, developed by NASA Ames Research Center, have augmented the space study possibilities offered by the International Space Station (Ricco et al., 2011; Nicholson et al., 2011; Woellert et al., 2011). Multiple aspects of microbial biology, including drug dose dependence (Parra et al., 2008; Ricco et al., 2011), were successfully tested in situ using these nanosatellites. To test the hypothesis that increased antibiotic resistance could be caused by spaceflight, we flew the *E. coli* Antimicrobial Satellite (*EcAMSat*) in μ-**g** (Matin et al., 2017). The AMG1 UPEC strain used here and in previous work was obtained from a patient at Stanford School of Medicine (Stone et al., 2002).

Here we report parallel low-Earth-orbit μ-**g** and ground-based experiments in which the wild type (WT) and the Δ*rpoS* knockout mutant strains (Stone et al., 2002) were inoculated in microwells of a microfluidic card, grown to stationary phase, starved for at least 24 h, then exposed to various doses of the antibiotic. We chose stationary-phase antibiotic exposure because: (1) this late-growth phase is often experienced by bacteria in the human host due, for example, to lack of nutrients or the presence of oxidative or other stresses (Hengge-Aronis, 2002b; Kolter et al., 1993; Matin, 2009); (2) it is in this phase that bacteria express many of the virulence traits required for disease causation (Dalebroux et al., 2010; Mangan et al., 2006; Mouslim and Hughes, 2014; Navarro Llorens et al., 2010; Roop et al., 2003; Sonenshein, 2005); and (3) antimicrobial agents tend to be ineffective in killing bacteria in this phase (Matin, 1991, 2009). Following antibiotic incubation, the metabolic activity of stationary phase cells was measured by the reduction of the redox indicator alamarBlue (aB).

The spaceflight and duration-matched terrestrial-gravity (DMTG) control experiments were designed to address the hypothesis that μ-**g** impacts antibiotic resistance in UPEC by answering several specific questions relevant to the mechanistic details of such an effect: (1) Does microgravity affect growth and, if so, does it do so differentially for the WT and the Δ*rpoS* mutant? (2) Does microgravity affect metabolic activity in the stationary phase without antibiotic addition; if so, is the effect different for the two strains? (3) Does microgravity affect metabolic activity when the strains are treated with Gm? (4) Does microgravity cause UPEC to become more resistant to Gm and, if so, is this impacted by the lack of *rpoS*? Lastly, a second control experiment was designed to address an implementation-related question: (5) How does

the payload hardware, in particular the physicochemical environment within the payload, affect growth and metabolic activity of the biological specimens?

2. Materials and methods

2.1. Spaceflight experimental protocol

The development of the *EcAMSat* hardware platform, shown schematically in Fig. 1(B) and in the photograph of Fig. 1(C), established the functionality of its payload hardware for precise mixing and exchange of fluids in the fluidic cards (Matin et al., 2017). Key characteristics of the bio payload and satellite, as well as a summary of its launch and deployment into low Earth orbit (Fig. 1(D)), are given in Supplemental information (SI). The flight experiments were programmed to start autonomously after four days to allow spacecraft motional and thermal stabilization in orbit. Daily communications with the spacecraft were conducted at Santa Clara University.

A detailed timeline of experimental steps is shown in Fig. 1(A). The heating phase permitted all subsequent steps to be carried out at 37 °C. 1/6th strength LB (Luria broth) was added to all microwells in the cards to initiate the experiments. Cells remained in 1/6 LB for ~48 h in order to reach and remain in stationary phase for at least 24 h to activate GSR (see Introduction); their growth was monitored by turbidimetry using the payload optical system (Matin et al., 2017). The cells were then challenged (for at least 46 h) with three different Gm doses; zero Gm served as control. aB was then added and its reduction was followed to measure the effect of the antibiotic on cell metabolic activity. Optical measurements were recorded every 15 min at red, green, and blue wavelengths (Matin et al., 2017) for each well over the course of the 156.5-h experiment.

2.2. Parallel ground controls

Two separate ground-control experiments were conducted. The aim of the DMTG control was to facilitate the assessment of how μ-**g** affects aspects of UPEC biology, hence its experimental protocol and timeline matched the space experiments as closely as possible. A "rotisserie" apparatus was used to rotate the payload (1.5 rpm) alternately clockwise and counterclockwise (**Fig. 1**(E)) to prevent cell sedimentation that would have interfered with the optical measurements. This slow rotation also ensured cell distribution throughout the fluid volume of the microwells instead of sedimentation against microwell walls or bottoms.

The second ground control, the payload-enclosure-stress test (PEST) control, was identical in operation to the first, but the cells were handled differently in one important regard. In both the spaceflight and the gravitational control experiments, the fluidic cards housing the *E. coli* were sealed inside a payload enclosure for ~8 weeks. This vessel also contained the payload electronics, meaning that materials such as the conformal coating on the printed-circuit boards (PCBs), along with pumps, valves, wiring, and other components were all in vapor-phase contact with the microwells via the permeable layer covering their tops and bottoms. Despite the fact that the PCBs, fluidic cards, and other components were extensively outgassed prior to the integration of microbes (see SI), concern over the possibility of remnant (semi)volatile compounds prompted the PEST control experiment, in which the cell-containing fluidic card was stored in a separate container without electronics, PC boards, or other components; integration with the fluidics-and-measurement system occurred just hours prior to initiation of cell growth.

2.3. Data analysis

The method of converting the raw optics data to absorbance has been previously described (Matin et al., 2017). Detectors for each well provided frequency readings that were proportional to the intensity of the light reaching the detector after transmission through the microwell (pathlength: 7.7 mm). Because LED and detector sets can vary, each was calibrated using two points with known absorbance: that corresponding to M9 in the well (zero absorbance at all three wavelengths) and to that of 1× aB. The absorbance of the reduced (pink) and oxidized (blue) forms of aB was measured with a spectrophotometer using the same wavelengths as the LEDs (470, 525, and 615 nm) to calculate the relative concentrations of these aB forms. Since the absorbance of either form of aB at 470 nm was negligible, that wavelength was used to track changes in OD. Using a measured absorbance spectrum for this strain of *E. coli*, the OD470 was converted to OD615. For both spaceflight and ground experiments, $\ln[aB_{ox}]$ vs. time plots were generated. For the first 3–5 h, the plots were linear, corresponding to first-order kinetics. The slopes of these linear regions were calculated, and the statistical comparisons reported below were performed with Welch's unequal variance *t*-tests.

3. Results and discussion

3.1. Answering question 1: μ-g does not affect UPEC growth

To determine the μ-**g** effect, parallel experiments were performed in space and under Earth gravity conditions, the latter as controls, using the same protocol. WT and Δ*rpoS* strains were tested in all cases. The cells in the flight experiment were in stasis for over 8 weeks, as were those used in the gravitational control experiment. Following LB addition, both the WT and the Δ*rpoS* strains showed similar growth patterns in μ-**g** and on the ground, with growth continuing for some 20 h (Fig. 2) until reaching stationary phase. As reported previously for terrestrial laboratory measurements (Matin et al., 2017), unlike the WT, the mutant exhibited a 2-h lag phase under both gravity conditions. Nonetheless, it attained the same maximum OD at 615 nm as the WT at roughly the same time. The lag seen in the mutant may have been a response to the stress of the microfluidic environment, a smaller starting population of viable cells, or the mutant taking more time to recover from stasis, but whatever the reason, μ-**g** did not affect this phenomenon.

3.2. Answering questions 2 and 3: μ-g does affect metabolic activity in the stationary phase, in both absence and presence of antibiotic

Fig. 3 shows the time-dependent decrease of the concentration of alamarBlue following its addition to stationary-phase WT and Δ*rpoS* UPEC, under conditions of terrestrial gravity and μ-g, in both absence (Fig 3(A)) and presence (Fig 3(B)) of Gm. Plots of $\ln[aB_{ox}]$ vs. time

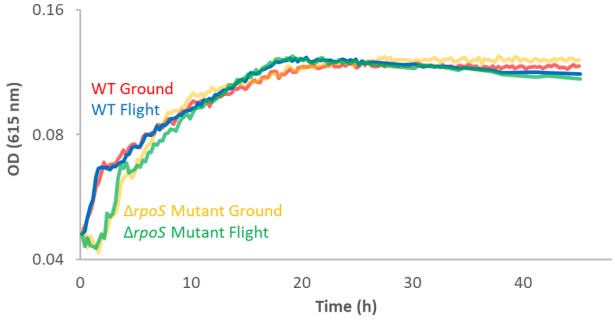

Fig. 2. OD of *E. coli* at 615 nm during growth and stationary phases of experiment. Average growth is shown for both strains in one bank of 12 microwells on ground and flight ($n = 6$ per strain). The OD axis is plotted on a log scale so that regions of exponential growth appear linear. While there was a few-hour initial lag in the growth of the mutant compared to the WT in both environments, the growth phase showed no major difference between flight and ground for either strain.

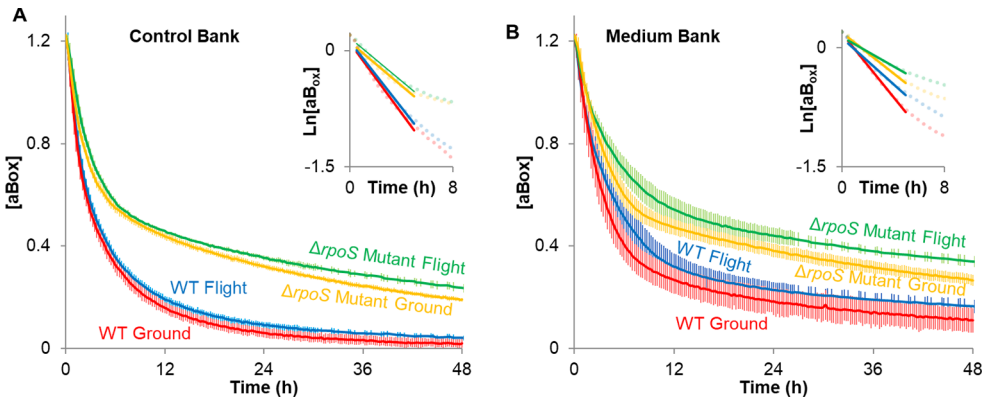

Fig. 3. aB reduction by WT and Δ*rpoS* in flight and on the ground at two Gm dose conditions. (A) Average reduction of alamarBlue over time for WT and Δ*rpoS* mutant in the Gm = 0 Control bank for flight and ground (n = 6; error bars: ± 1 SD (standard deviation)). (B) Average reduction of alamarBlue over time for WT and Δ*rpoS* mutant in the Gm = 15 μg/mL (Medium) bank for flight and ground (n = 6; error bars: ± 1 SD). In both panels, insets show the first 8 h of the response (dotted lines) plotted on a semilog scale ($\ln[aB_{ox}]$ vs. time), showing the trendlines (solid lines) from which the initial slopes were calculated.

(Fig. 3 insets) displayed an initial linear region (lasting 3–5 h), indicative of first-order kinetics. This region of the plot was selected because it started with a consistent initial concentration of aB across all wells and provided a measurement of the *E. coli* before their reducing metabolites (might) become depleted by the reaction with aB. Calculating the slope provided an easy and consistent metric for comparison of the two strains under various conditions, using several hours' worth of metabolic activity (as opposed to using a single-point conversion percentage, for example).

For both strains, differences in the initial slopes between flight and ground, summarized in Fig. 4, were statistically significant: $p < 0.05$ for Gm = 0 (Control); $p < 0.01$ for Gm = 15 μg/mL (Medium). In addition, as noted during previous laboratory testing of the *EcAMSat* platform (Matin et al., 2017), the Δ*rpoS* mutant reduced aB more slowly than did the WT under terrestrial gravity, with results from space showing this to be true in μ-**g** as well; possible reasons are considered below.

3.3. Answering question 4: μ-g does not enhance UPEC resistance to Gm

If WT UPEC had become more resistant to Gm due to the stress of spaceflight, more rapid aB reduction kinetics would have been expected. Instead, as the difference between red and blue bars in the Medium Gm condition of Fig. 4 shows, Gm in spaceflight caused a 25% decrease ($p < 0.01$) in the rate of aB reduction (from initial slopes) by the WT relative to the same strain on the ground. The same comparison for the Δ*rpoS* mutant also showed a 25% decrease ($p < 0.01$); both strains were more susceptible to Gm in μ-**g**. In the presence of the medium dose of Gm, the WT demonstrated a 12% decrease in slope relative to the control on the ground and a 30% decrease in spaceflight. Similarly, the Δ*rpoS* mutant showed 11% and 30% decreases, respectively. The effect of the medium dose of Gm on the metabolic activity of the cells relative to the zero-dose control was the same for both strains on the ground. This was also true in μ-**g**, although as stated above the effect was greater. In the low dose case (3.5 μg/mL), the minimal effect on either strain (see Fig. S1) was unsurprising. The high dose (52 μg/mL) is discussed in the next section.

3.4. Results for high Gm concentrations support a role for differential mass-transport rates

As shown by Fig. 3 and summarized in Fig. 4, cells in the flight payload reduced aB more slowly than those on the ground. This was in concert with flight and ground comparisons from *PharmaSat* and *O/OREOS* (representing aB-measured metabolic activity of *S. cerevisiae* and *B. subtilis* during growth, respectively) (Ricco et al., 2011; Nicholson et al., 2011). Furthermore, in the *GeneSat*-1 experiment, while aB was not used, light scattering by cells analogously showed that the rate of increase of *E. coli* populations in all nine of its microfluidic wells was slower in space than in the ground controls during the growth phase (Ricco et al., 2007). An explanation for this result, common to four different spaceflight missions involving three different microorganisms, is that, on Earth, even small thermal gradients result in movement of liquids: less dense, warmer liquid rises as colder liquid sinks. In μ-**g**, in contrast, thermally induced density differences do not cause liquid motion; mass transport of nutrients and cellular waste products, in absence of active mechanical convection or cell motility, is by diffusion alone.

In the case of the highest Gm dose, no statistically significant

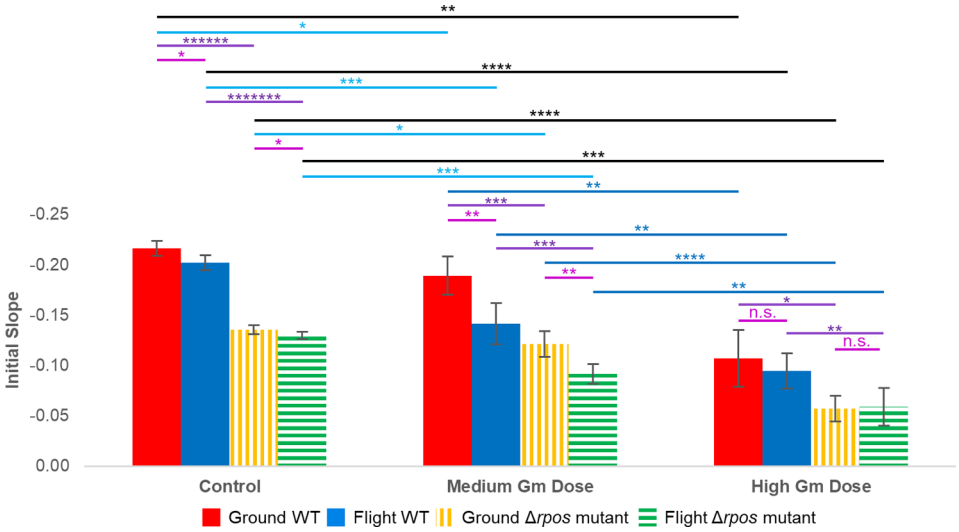

Fig. 4. Natural log of initial slope of the reduction of aB for Ground and Flight experiments for WT and mutant UPEC for Control, Medium and High Gm dose. The slopes were calculated from the aB reduction curves on a semi-log plot (see Fig. 3, insets). In all cases the WT exhibited higher slopes (faster metabolism) than the Δ*rpoS* mutant. Key to comparisons: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, **** $p < 0.0001$; ******$p < 10^{-6}$; ******* $p < 10^{-7}$; n.s:. not significant. Light purple horizontal bars compare Ground and Flight for a given strain and set of conditions; dark purple bars compare WT and Δ*rpoS* mutant; light blue bars compare Control and Medium Gm doses for a given strain and set of conditions; dark blue bars compare Medium and High Gm doses for a given strain and set of conditions; bold black bars compare Control and High Gm doses for a given strain and set of conditions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

differences were found in the initial slopes between flight and ground ($p > 0.05$) for either strain. This is consistent with, though not proof of, mass transport being less important for high Gm than in the other cases: if the high dose slowed the aB conversion process enough, diffusion might cease to be the rate-limiting step. A lack of dependency on microgravity here supports a model in which Gm impacts most or all of the *E. coli* cells similarly: they still metabolized aB, but they did so much more slowly than without the high Gm.

3.5. Answering question 5: materials inside the flight payload enclosure DO adversely impact cell viability

In attempting to discern the impact(s) of μ-**g** from these experiments, we identified four different stressors that could affect comparative metabolic activity (or viability) measurements of the WT and Δ*rpoS* strains for this experimental configuration: (i) growth of cells inside the microwells of a fluidic card (higher surface-area-to-volume ratio than conventional culture vessels; hindered O_2 exchange); (ii) extended exposure of the static cultures to the environment inside the sealed spacecraft payload enclosure (in particular, volatile chemicals associated with the presence of PCBs, despite their extensive outgassing, Matin et al., 2017); (iii) microgravity, which can result in slower local mass transport of nutrients and cellular waste products (Klaus et al., 1997); and (iv) the antibiotic, which in fact includes three different stress levels corresponding to Low, Medium, and High Gm doses.

To help elucidate the relative contributions of the four stress factors enumerated in the previous paragraph, a second ground control experiment, the payload-enclosure-stress test, was conducted to allow comparison of the impact of keeping cells in fluidic cards within the payload enclosure for two very different durations: several hours vs. 8 weeks. The cells in both the spaceflight payload and the DMTG control were in their fluidic cards for more than 8 weeks, the UPEC-inoculated cards being stored inside the enclosure for over 80% of that time. In contrast, the PEST control utilized UPEC cells that were in stasis within the same type of fluidic card for some 11 weeks, but this card was sealed inside a payload enclosure with PCBs and other components for only a few hours before starting the cell-growth experiment.

Fig. 5(A) shows the optical density due to growth of both UPEC strains in the PEST control experiment. As in the spaceflight and DMTG control (Fig. 2), time to stationary phase was ~20 h, and the Δ*rpoS* mutant had a multi-hour lag phase not exhibited by the WT. In contrast to this similarity of growth, there was a major difference in the rate of metabolism of aB by the WT as measured by the initial slopes of the ln [aB_{ox}] curve, summarized in Fig. 5(B). In the PEST control, the initial slope was ~2.3× as large as that of the terrestrial gravity control (black vs. red bars, $p < 0.0001$). Despite outgassing prior to integration, the electronic components inside the payload still slowly released volatile compounds. The accumulation of these compounds over time in the hermetically sealed enclosure caused additional stress on the *E. coli*, with much greater impact on metabolic activity than weeks-long stasis in a fluidic card.

Two other comparisons in Fig. 5(B) provide potential insight as to the role of *rpoS* as the bacteria cope with two different types of stress. Comparing the black and brown bars of the PEST control shows that deleting *rpoS* left the bacteria much less able to cope with the stress of storage and growth in a microfluidic card: the initial slope for the mutant was less than half that of the WT. In contrast, 8 weeks' storage inside the payload container stressed the WT UPEC in such a manner that its initial metabolic activity (slope) was already diminished to just over half that of the PEST WT; compounding this by deleting *rpoS* attenuated metabolic slope further still (yellow vs. red bar), but the change was not nearly as dramatic as the gene-deletion consequence of the PEST control, i.e. when there was no payload-storage stress. One logical conclusion from these comparisons is that *rpoS* is more helpful to *E. coli* for dealing with the environmental stresses of storage and life in a fluidic card than it is for dealing with chemical stress imparted by substances within the payload container. These results also raise concerns that payload-container-related stresses could (partially) obscure some of the stress effects of the μ-**g** environment.

To confirm that the adverse effect of long-term storage was not related to the fluidic card itself, we conducted separate single-material biocompatibility tests (see SI). These, in combination with the PEST control results (see above), indicate minimal stress due to storage, growth, and metabolism in the fluidic card.

3.6. Deleting rpoS matters to drug treatment of UPEC in μ-g

In our experiments, the mutant had already been sufficiently stressed by the payload environment, whether on the ground or in space, that its percentage decrease in metabolic activity due to the added stress of Gm was about the same as for the WT. Our experiments indicate that the stresses of the flight payload container environment and the stresses of Gm and the stresses of spaceflight were not additive: the slopes of the aB reduction curves showed that the Δ*rpoS* mutant was 34–37% less metabolically active than the WT for four different sets of conditions: ground without Gm, ground with Gm; μ-**g** without Gm, μ-**g** with Gm.

4. Summary and conclusions

The *EcAMSat* mission achieved full success in completion of the experimental protocol and receipt of the required set of data (~100 kB of payload data) from space. The entire satellite functioned nominally throughout the experiment and during the 45-day mission, and the flight and two ground-control units successfully performed the same experimental protocol.

The scientific aim of the *EcAMSat* spaceflight experiment and its ground controls was to elucidate the degree to which microgravity may stress UPEC in a manner similar to other stresses (e.g., the presence of an antibiotic) in order to better understand the implications of such stress for future treatment of infections in space flight by hampering

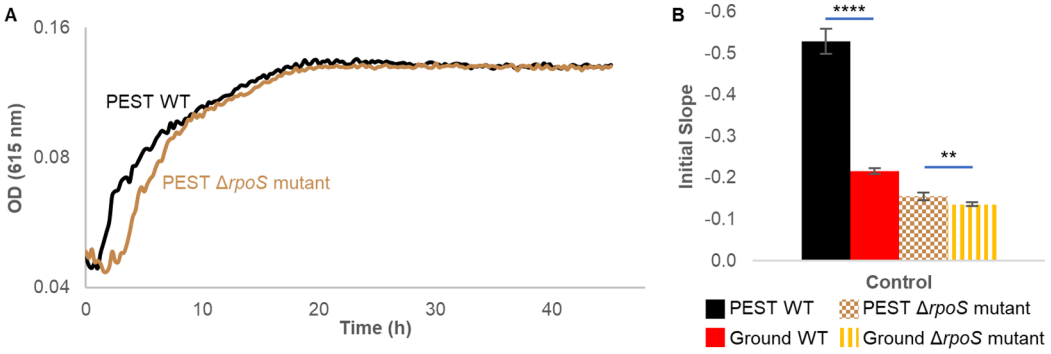

Fig. 5. Growth and metabolic activity of the cells during PEST control. (A) Average OD at 615 nm for the WT and Δ*rpoS* mutant ($n = 6$). The OD axis is plotted on a log scale so that regions of exponential growth appear linear. (B) Comparison of the initial slopes for the PEST and ground experiments ($n = 6$; $^{**}p < 0.01$; $^{****}p < 0.0001$). (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

RpoS. An increase in resistance to Gm in the WT UPEC attributable to microgravity was not found, however, in contrast to earlier experiments conducted on ISS (Aunins et al., 2018; Zea et al., 2017) and consistent with two other prior spaceflight experiments (Juergensmeyer et al., 1999; Morrison et al., 2017). The conflicting results may be attributed to differences in species or experimental protocol and hardware. Additionally, the ability of the two strains to form biofilms, which previous studies have shown to be affected by μ-**g** (Zea et al., 2017; Lynch et al., 2006; Tirumalai et al., 2017), cannot be ruled out as a contributing factor, but this would require additional experiments as the approach described in this article was not inherently sensitive to biofilm formation.

Weeks of storage in the payload hardware provided an exogenous stress on the *E. coli* that complicated interpretation of spaceflight results. Nonetheless, shutting down RpoS was shown still to be an effective approach to help fight bacterial activity: in the stressful payload environment, upon administration of Gm, UPEC with blocked RpoS metabolized aB significantly more slowly than normal UPEC with active RpoS, and that held true both on the ground and in μ-**g**.

The overall results of *EcAMSat* are consistent with the fact that the loss of σ^s renders UPEC more susceptible to several stresses, including killing by gentamicin, consistent with the fact that antagonizing the action of the RpoS protein (as well as the products of several other genes that it regulates) enhances the efficacy of antibiotics in combating bacterial growth (gentamicin was tested, but results are likely to apply also to others). Whether the same phenomenon would be found in other bacteria remains to be determined; given the generality of the RpoS functionality (and its analogous proteins), the effect is likely to be general. Thus, measures to counter the activity of proteins like RpoS that control the bacterial stress response promise to lead to better treatment for bacterial infections both on Earth and in long-duration spaceflight.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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