The Influence of Spaceflight and Simulated Microgravity on Bacterial Motility and Chemotaxis

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The influence of spaceflight and simulated microgravity on bacterial motility and chemotaxis

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As interest in space exploration rises, there is a growing need to quantify the impact of microgravity on the growth, survival, and adaptation of microorganisms, including those responsible for astronaut illness. Motility is a key microbial behavior that plays important roles in nutrient assimilation, tissue localization and invasion, pathogenicity, biofilm formation, and ultimately survival. Very few studies have specifically looked at the effects of microgravity on the phenotypes of microbial motility. However, genomic and transcriptomic studies give a broad general picture of overall gene expression that can be used to predict motility phenotypes based upon selected genes, such as those responsible for flagellar synthesis and function and/or taxis. In this review, we focus on specific strains of Gram-negative bacteria that have been the most studied in this context. We begin with a discussion of Earth-based microgravity simulation systems and how they may affect the genes and phenotypes of interest. We then summarize results from both Earth- and space-based systems showing effects of microgravity on motility-related genes and phenotypes.

BACKGROUND

Earth-based organisms, including microorganisms, have developed under the influence of gravity. As interest in spaceflight grows, it is important to understand the effects of microgravity on single-celled organisms including Bacteria, Archaea, and eukaryotes such as yeast and microalgae. In the microgravity environment particles experience weightlessness, such as in the case of constant free-fall in orbit aboard the International Space Station (ISS). The effects of microgravity exposure on microorganisms are of intense interest for medical and bioengineering applications, and many studies have been conducted over the past few decades, of which there are several comprehensive reviews1–6. However, a systematic summary of the effects of microgravity on microbial motility has not yet been done. Microbial motility is important for normal function of the human microbiome (e.g., in the gut and oral cavity)7,8 as well as for pathogenesis of some bacteria involved in common food- and water-borne infections9–12. Alterations in motility affect the distribution of microorganisms in tissues, encourage or inhibit bacterial invasiveness, and affect biofilm formation11,13. These changes may have important implications for astronaut health, especially combined with host factors; studies suggest that astronauts aboard the ISS suffer from compromised immune systems14,15 and altered microbiota16, potentially making them susceptible to opportunistic bacterial infections.

Microorganisms rapidly adapt to their environment by altering their gene expression to increase survivability. While earlier studies of microgravity effects on microorganisms were largely phenotypic, more recently “omics” techniques have become practical: genomics, transcriptomics, and proteomics, quantifying DNA, mRNA, and proteins, respectively17–19. Genomic studies are the most prevalent and in general the easiest to perform for bacteria, since many organisms have been fully sequenced and DNA-sequencing technology is widely available and relatively inexpensive20. RNA sequencing remains costly for transcriptomic studies, although less expensive microarray technologies may also be used19. Proteomics is a rapidly emerging field, but fractionation of bacteria for proteomic studies, particularly Gram-negative strains, is challenging21,22. Beginning with microarray studies of simulated microgravity responses in 200223, gene expression in microbes has been studied in both ground-based24–26 and spaceflight studies27,28. Transcriptomic and proteomic studies have also begun to appear29,30. Development of technologies for “omics” studies in space has been progressing but is not yet routinely used31. Sorting through the plethora of information available is rapidly becoming increasingly difficult, with numerous databases and software tools devoted to the task32–35.

An important consideration is the relationship between the presence of motility genes (and their transcripts) and phenotype. The genes of the flagellar regulon are expressed as a cascade, and transcriptional activators influence expression of motility genes based upon environmental conditions36,37. This illustrates the importance of performing linked transcriptomic/proteomic and phenotypic studies when working with a test strain to study changes over short time periods (minutes to hours). Despite this complexity, the overall presence of flagellar and chemoreceptor genes has been used to predict community-wide motility behaviors38,39, combined “omics” studies (genomic/transcriptomic) can capture diel and seasonal variations in motility40.

Spaceflight analog devices used on Earth, such as those shown in Fig. 1, mimic specific hallmarks of the microgravity environment including low fluid shear, lack of sedimentation, and low turbulence41. Thus, we will refer to the culture environment in spaceflight analog devices as ‘simulated microgravity.’ The impact of simulated microgravity studies goes beyond prediction of microbial changes during spaceflight. In fact, low fluid shear conditions also exist in vivo under special circumstances, such as in the microvilli of the intestines42. In these environments, organisms colonize and infect their hosts. Therefore, simulated microgravity devices can also be an invaluable tool to understand the mechanisms of the infection process43–45.

This review begins by summarizing the technologies available for simulating microgravity, with some recent analysis of the fluid mechanics of spaceflight analog devices. The possible effects of
the different types of vessels on microorganisms apart from their simulation of weightlessness are discussed, as well as other complications that may arise from attempting to compare ground-based with ISS-based microbial gene expression studies. We then detail recent studies in gene expression regarding motility and chemotaxis in selected Gram-negative bacterial strains: Escherichia coli, Salmonella enterica serovar Typhimurium, Pseudomonas aeruginosa, and Vibrio fischeri.

SIMULATED MICROGRAVITY AND FLUID MECHANICS

Due to the difficulty of conducting experiments in space, ground-based bioreactors were developed by the NASA Johnson Space Center (Houston, TX) to study simulated microgravity on Earth. These devices are generally called rotating wall vessels (RWV), though this terminology can be confusing as it includes different configurations such as: high aspect ratio vessels (HARVs), slow turning lateral vessels (STLVs), and rotating wall perfused vessels (RWPVs). Other devices used to simulate microgravity and of slightly different design are clinostats and random positioning machines (RPMs). Though they have been used to study simulated microgravity on plants, and human cells and tissues, this review’s focus is limited to effects on microbes.

Fluid behavior under simulated microgravity is characterized by low fluid shear, often referred to as low shear modeled microgravity (LSMMG). Low fluid shear values associated with the simulated microgravity environment are of the order ~10⁻² dyne/cm². The low fluid shear condition is dependent on many factors including vessel geometry, particle size, vessel rotation speed, and fluid properties. Though all vessels create simulated microgravity, analysis differs among types. Flow characterization can be approximated with the Reynolds number (Re), which is a ratio of the inertial to viscous forces. For example, for flow in a cylindrical vessel, the Reynolds number is given by Eq. (1):

\[ Re = \frac{\rho V D}{\mu} \]  

where \( \rho \) is the fluid density, \( V \) is the fluid velocity, \( D \) is the particle diameter, and \( \mu \) is the kinematic viscosity.

Common principles and the development of RWVs have been outlined in many sources. Ground-based systems replicate weightlessness by rotating cells such that gravity vectors are nullified, and organisms do not have the opportunity to adapt to a specific gravity orientation. In other words, gravity is not altered; rather, the summation of gravity effects cancels out. When the vessel is filled completely (zero headspace), the chamber contents resemble a rigid body. Fluid within the vessel is assumed both incompressible and Newtonian with approximately uniform density and viscosity. Vessel orientation determines whether microbes are under normal gravity (vertical axis) or simulated microgravity (horizontal axis) as seen in Fig. 2. A common method of control is to inoculate two chambers and grow cells in both configurations. However, caution should be exercised when comparing differences between these two configurations. It is possible that there are fluid dynamic effects under simulated microgravity that do not appear in the normal gravity configuration. Different studies have employed both analytical and numerical techniques such as computational fluid dynamics (CFD) to elucidate the fluid mechanical effects and when they must be considered. This review aims to provide a brief overview of these analyses specific to device type when possible, focusing on those devices primarily used in microbial-simulated microgravity studies.

We begin with HARVs, as these devices were used for most simulated microgravity studies presented in this review. These devices are cylindrical in shape with a membrane on the back to facilitate gas exchange. Ayyaswamy and Mukundakrishnan outlined experimental conditions necessary for simulated microgravity in HARVs and STLVs including low fluid shear at the cell surface, adequate mass transport, and if microcarriers are used, such as when studying biofilm development, to exercise careful thought about size and shape so as to minimize collisions with the vessel walls and adverse fluid effects.

The STLV consists of an inner and an outer cylinder, both of which may rotate along a horizontal axis, in either the same or opposite directions. Oxygenation is provided through a membrane along the inner column. The fluid behavior was initially reviewed in Hammond and Hammond in 2001, and further studies have been done since then. Gao et al. showed that particles tended to migrate radially when the particle density and fluid density differed. Liu et al. later replicated and expanded on this work, providing a comprehensive analysis of the forces on the particle. They also proposed rotational speeds consistent with simulated microgravity on inert particles and reaffirmed difficulties present with larger particle sizes, reporting fluid shear stress values several orders of magnitude higher than simulated microgravity.

The RPM was developed from the clinostat, and consists of two frames that can be independently driven. Therefore, an RPM can be operated in different modes: clinostat mode (only one axis rotates), 3D clinostat mode (two axes rotated at constant speed), and 3D Random mode (two axes rotated at different speeds). Wuest et al. studied the fluid motion using a numerical
approach, comparing the classic clinostat rotation with the 3D clinostat mode. They found that the fluid motion in the cell flask varied as a function of position and never seemed to reach a steady state, as seen in Fig. 3. Fluid shear stress values were found to be highest at the walls and could reach up to a 100 mPa. They urge that experimental design on microbes or tissues must consider fluid dynamic effects.

Although each device simulates microgravity, they do not all do so in the exact same manner. Consequently, organisms grown in one device may not react the same as another device.47

Fig. 2 3D orientation of a rotating wall vessel (RWV) with the direction of the gravity vector included. Left: Simulated microgravity showing a typical microbe path with gravity vectors canceling in the completely vertical orientation. Right: Normal gravity control with a horizontal orientation.

Fig. 3 Simulated shear stress values along the walls in an RPM. Shear stress values vary throughout with no clear steady state. Reprinted from Wuest et al.58 with no alterations under the CC BY 4.0 License (https://creativecommons.org/licenses/by/4.0/).
When conducting a study of *P. aeruginosa*, Crabbe et al. found differing levels of gene expression when culturing in a HARV vs. in an RPM. They probed this difference by injecting dye into one HARV port as seen in Fig. 4. In their supplemental videos, they show how the dye spread to the center of chamber more slowly in the HARV than in the RPM indicating a subtle difference in fluid behavior. This could be a possible reason for the difference in gene expression.

**Simulated microgravity and imaging**

Experimental calculation of fluid shear stress requires estimation of particle velocities, but imaging under simulated microgravity conditions on Earth is challenging. Although any of the traditional methods described above can be used to study simulated microgravity, changes in gene expression can occur in minutes. This is particularly relevant for observing dynamic phenotypic changes in motility and chemotaxis. To address this, one approach is to attach a microscope to a simulated microgravity device, as was done with the clinostat microscope developed by the German Aerospace Research Establishment in 1996. The microscope is positioned to rotate horizontally and was used to study the behavior of *Paramecium biaurelia* in both simulated microgravity and flight. Another example of this was when Pache et al. attached a digital holographic microscope to an RPM, upgraded by Toy et al. to include a widefield epifluorescence microscopy module. Yew et al. developed a lab-on-a-chip clinorotation system as a more cost-effective alternative to the systems previously described. It uses 2D clinostat rotation and requires pauses for imaging.

**SIMULATED MICROGRAVITY, SPACEFLIGHT AND GENE EXPRESSION**

Gene regulation is a process in which gene expression is upregulated or downregulated and can be in microgravity and spaceflight. It uses 2D clinostat rotation and requires pauses for imaging.

**Overview of hfq, motility and chemotaxis**

Hfq has emerged as an important post-transcriptional factor that facilitates the pairing of small RNAs with their target mRNAs; its role in bacteria has been recently reviewed. To highlight its importance, in some organisms it can impact expression of up to 20% of all genes. In a landmark study in 2007, Wilson et al. identified hfq as a global regulator in response to the spaceflight and simulated microgravity environment based on their global microarray and proteomic analyses. Additionally, changes in hfq gene expression can influence virulence of bacterial pathogens including *S. Typhimurium* thus making this gene a recent focus in simulated microgravity studies.

**Motility and chemotaxis**

Changes in microbial motility can result from alterations in gene expression of motility machinery including flagella, fimbriae, and pili. Motor assembly and chemosensory machinery requires about 50 genes in *E. coli* and *S. Typhimurium*. Studies have been conducted to identify genes and proteins involved in bacterial motility, with the *E. coli* flagellar assembly shown in Fig. 6. Flagellar genes are organized into regulatory hierarchies of classes depending on the organism, with each hierarchy affecting the next. Flagella are not only important for motility; they have also been shown to play a role in pathogenicity and biofilm formation. Motility and cell shape have been shown to influence cell growth. Interestingly, transmission electron microscope (TEM) images have shown phenotypic changes in *E. coli* after spaceflight, with decreased size and increased presence of outer membrane vesicles as seen in Fig. 7. Benoit and Klaus reviewed microbial motility as it related to final cell populations, comparing motile and non-motile cultures during spaceflight, simulated microgravity and normal gravity controls. They revealed that spaceflight and simulated microgravity compared to normal gravity controls resulted in higher final cell counts in non-motile bacteria grown in liquid. Microbial motility is governed by an electromotive gradient of ions across the cell membrane, so concentration

![Image](https://example.com/image.png)
Fig. 5 Motility and chemotaxis gene expression categorized by study and organism. Color indicators: Green: upregulation, Yellow: no change reported, Red: downregulation. Dashes indicate no data available. Normal Gravity Rotation control is represented by NG.

E. coli: hfq, motility, and chemotaxis

E. coli is an organism with motile and non-motile strains and is the most well studied and most thoroughly characterized organism on Earth101. E. coli K12 MG 1655 is motile with peritrichous flagella and fully sequenced102. E. coli Nissle (ECN) 1917 is another fully sequenced103, probiotic, non-pathogenic strain of E. coli lacking pathogenic adhesion factors; it carries genes that help limit the proliferation of other bacteria104.

E. coli and hfq. Tsui et al.105 showed that an hfq insertion mutation caused pleiotropic phenotypes, thus highlighting the importance of hfq expression in E. coli. Soni et al.10 describe the role of hfq in both motility and chemotaxis for various enterobacteria. Cultures of E. coli MG1655, DH5α, and AS11 were grown in Lennox broth using HARVs and compared with normal gravity rotation. They found hfq expression to be downregulated.

E. coli: motility and chemotaxis. In E. coli, motility is critical for biofilm formation106. Tucker et al.107 cultured E. coli MG1655 in both minimal MOPS medium and Luria broth, comparing cultures in HARVs vs. normal gravity rotation. In the MOPS media, flagellar genes fiqBDEK and flcCDZ were upregulated, whereas no changes were observed with the Luria broth compared to normal gravity controls, respectively. Regarding chemotaxis, they found an upregulation of cheZ in the MOPS media vs. normal gravity rotation. Motility and chemotaxis are used by bacteria to identify and obtain nutrients; thus, expression levels vary with the level of nutrients in the environment. This study underlines the importance of this factor in designing studies of motility changes under simulated microgravity.

Tirumalai et al. conducted two studies77,79 analyzing gene mutation following different HARV cleaning protocols. In one study77, the HARVs were cleaned using steam sterilization; in a later study79, they used chloroamphenicol to prevent contamination. In both studies, they cultured 1000 generations of E. coli K12 MG 1655, chosen because they could be visually distinguished. Following the chloramphenicol treatment, the lac+ cultures showed mutations in the predicted fimbiae-like adhesion proteins yadL. Flagellar and motility proteins also had loss of function mutations in the predicted fimbiae-like adhesion proteins. These changes were not present in the steam sterilization study, they were thought to aid in antibiotic resistance under simulated microgravity conditions. Additionally, they found loss of function mutations in the chemotaxis-related genes cheABRWYZ. Although they did not look at gene expression changes in motility specifically, Lynch et al.61 found that biofilm coverage of E. coli on microcarrier particles cultured in Luria Bertani broth in 10 mL HARVs was more pronounced than under normal gravity. Vukan et al.56 cultured E. coli in Luria broth in an STLV and found a downregulation of fimDFG. Yim et al.108 grew EcN in M9 minimal media in 10 mL HARVs. Sample collection followed exponential and stationary growth. They found upregulation of flIC, fimACDGI after exponential growth, but changes did not persist in samples taken from stationary growth. Regarding chemotaxis, they also found a downregulation of cheZ following the same conditions.
**S. Typhimurium: motility and chemotaxis**

*S. Typhimurium* is motile with peritrichous flagella, a fully sequenced pathogenic organism shown to have increased virulence after simulated microgravity exposure and spaceflight.

S. Typhimurium and *hfq*. In non-space-related studies, Sittka et al. showed that in *S. Typhimurium* Hfq influences 87% of genes in the flagellar system and 84% of genes in the chemotaxis system. Monteiro et al. showed that Hfq controls biofilm formation through regulation of CsgD in *S. Typhimurium*. Since changes in *hfq* expression have been shown to impact expression of genes related to motility, chemotaxis, and biofilm formation in *S. Typhimurium* in Earth-based studies, studies were conducted to ascertain whether these changes persisted under simulated microgravity and spaceflight. In a study conducted by Wilson et al. of bacterial gene expression after spaceflight, *hfq* expression was down-regulated. Noting that simulated microgravity induced acid resistance in a previous study at late log phase, they cultured both the wild type and an *hfq*-deficient mutant in Lennox broth in HARVs, comparing survivability. The wild type showed greater survivability in normal gravity rotation vs. simulated microgravity. However, this difference in survivability was not observed between simulated microgravity and normal gravity rotation for the *hfq*-deficient strain. Pacello et al. cultured *S. Typhimurium* in Luria Bertani broth in 10 mL HARVs using normal gravity as a control. They found that simulated microgravity increased acid resistance even in the absence of *hfq* as compared to normal gravity. Soni et al. cultured *S. Typhimurium* in Lennox broth in HARVs using normal gravity rotation as a control. They observed a downregulation of *hfq*.

**S. Typhimurium: motility and chemotaxis**

*S. Typhimurium* motility has been shown to increase invasiveness, therefore making changes in motility important from a health perspective.

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**Fig. 6** Bacterial flagellar motor (*E. coli*) with associated protein components. Eco02040 reprinted with modification under the CC BY 4.0 License (https://creativecommons.org/licenses/by/4.0/) based on KEGG, Kanehisa et al. 88.
P. aeruginosa: hfq, motility and chemotaxis

**P. aeruginosa** is motile with a single polar flagellum, fully sequenced, and occasionally a part of human flora. In spaceflight and simulated microgravity, it has shown increased biofilm formation.

P. aeruginosa and hfq. Crabbe et al. investigated global changes in gene expression including the *hfq*-dependent response under simulated microgravity using both a HARV and an RPM in *P. aeruginosa* PAO1. *Hfq* was upregulated using the HARV as compared to normal gravity rotation, but not differentially expressed when comparing the RPM to normal gravity rotation. In *P. aeruginosa*, the loss of *hfq* can result in reduction in growth. Interestingly, comparison of total bacterial counts showed no significant differences between HARV, RPM, and normal gravity. Crabbe et al. also investigated the *hfq*-dependent response after spaceflight, comparing their results with their earlier simulated microgravity study. In contrast to the HARV results, they found a downregulation of *hfq* after spaceflight. This finding was significant because following *S. Typhimurium*, it showed Hfq as a regulator acting across bacterial species.

P. aeruginosa: motility and chemotaxis. *P. aeruginosa* is associated with infections in immunocompromised hosts. Motility, particularly the presence of *flIC*, plays a key role in its pathogenesis. Crabbe et al. cultured *P. aeruginosa* in Lennox broth in HARVs and RPMs and cataloged changes in motility, finding upregulation of motility genes *flACFG, flELNP, flGM* but no apparent changes under the RPM. No significant changes in motility gene expression were reported after spaceflight. In contrast to what is seen in *E. coli* and *S. Typhimurium*, the chemotactic response pathway in *P. aeruginosa* involves more than 20 *che* genes and 26 MCP-like genes. Crabbe et al. showed upregulation of chemotaxis genes *cheWYZ* in the HARV and to a lesser extent in the RPM compared to normal gravity. Spaceflight showed an upregulation of *PA2573*, an MCP homolog compared to ground controls. Kim et al. compared biofilm formation in *P. aeruginosa* PA14 during spaceflight with ground controls finding that spaceflight not only increased biofilm formation but also revealed a different biofilm architecture than normally appears on Earth that they termed “column-and-canopy”. Additionally, they investigated biofilm formation in the wild type, mutants deficient in flagella-driven motility, methyl-accepting chemotaxis, and type IV pili-driven motility. The wild type and Δ* pilB* made the column-and-canopy architecture, while the Δ*motABCD* did not, thus underscoring the importance of motility in the formation of this architecture.

V. fischeri: hfq, motility and chemotaxis

**V. fischeri** is a motile marine bacterium with a single polar flagellum, completely sequenced. It forms a symbiotic relationship with the bobtail squid *Euprymna scolopes* and as such is a good model for understanding how such relationships can change in simulated microgravity.

V. fischeri and hfq. Hfq expression was also studied in *V. fischeri* and its resulting effect on *E. scolopes*. Grant et al. placed both symbiotic partners in HARVs and examined changes in *V. fischeri’s* colonization of *E. scolopes* at different stages of development, using both the wild type and a mutant with a non-functioning hfq gene. Under simulated microgravity, both the wild type and mutant reached higher cell counts than under normal gravity. Hfq was also downregulated under simulated microgravity. No differences in the ability of *V. fischeri* to colonize *E. scolopes* were observed between the wild type and *hfq* mutant, though colonization occurred more quickly under simulated microgravity with both strains. *V. fischeri* is also critical for morphogenesis of the light organ in *E. scolopes* by triggering developmental events. The wild type, during simulated microgravity, negatively impacted these developmental events, while the *hfq* mutant did not. This coupled with the fact that the *hfq* mutant under normal gravity also negatively impacted these developmental events showed that *hfq* while not necessary for colonization, was still necessary for light organ morphogenesis.

V. fischeri: motility and chemotaxis. Motility is necessary for *V. fischeri* to successfully colonize *E. scolopes*. Duscher et al. investigated *V. fischeri* exposure to simulated microgravity using HARVs. The 12- and 24-h cultures of *V. fischeri* (wild type) and associated global regulator *hfq* protein knockout strains (Δ*hfq*) were grown in seawater tryptone. They used a heat map to qualitatively depict changes in gene expression as shown in Fig. 8. When comparing the wild type grown in simulated microgravity vs. normal gravity after 12 and 24 h, no genes showed significant differences. However, comparison of the wild type after 12- and 24-h exposure to simulated microgravity showed an upregulation in *flAG* similar to an upregulation of *flagEM* when viewing changes under normal gravity. The Δ*hfq* strain after 12 h under simulated microgravity showed upregulation of *flaDEF*, *flaACEK* as compared to normal gravity. After 24 h under simulated microgravity, these changes did not seem to persist. The ability to chemotax is an advantage for *V. fischeri* when colonizing *E. scolopes*, though not strictly necessary. Duscher et al. did not report many changes to chemotaxis gene expression. Under simulated microgravity, only the Δ*hfq* showed a downregulation of *V. fischeri* chemotaxis genes.

**CONCLUSIONS**

There is still much work needed to understand microbial response to microgravity. Gene expression can vary based on perspective. Wilson et al. compared cultures of *S. Typhimurium* grown in Lennox broth in HARVs and normal gravity, finding 163 genes differentially expressed under simulated microgravity, including downregulation of *flmA, flIB*. They reported an upregulation of *mcpB*, a gene identified in chemotaxis in *S. Typhimurium*. In a later study, they compared spaceflight *S. Typhimurium* samples with controls on the growth kept under similar temperature and nutrient conditions. They showed downregulation in *flEST, flgM, flhD*; however, *flIC* was upregulated. Regarding chemotaxis, they found a downregulation of *cheY*. In another study, they cultured *S. Typhimurium* in M9 media during spaceflight and showed eight genes in *flgACFG, flICMT, flIB* downregulated compared to ground-based controls. Additionally, they found a downregulation of *cheF*.

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environmental factors including temperature, nutrients, and fluid shear stress ranges. A recent study argued that there is no identifiable common bacteria "spaceflight response," although another suggested Hfq as a general spaceflight regulon.

The two gravity-dependent processes believed to most influence bacteria are indirect, namely (a) settling (of both cells and nutrients) and (b) buoyant convection. Both affect non-motile microorganisms more than motile cells, since flagellar motility stirs the liquid surrounding the cell as well as permitting the cell to avoid settling. Thus, increased growth seen under microgravity conditions was initially hypothesized to be restricted to non-motile cells; however, later studies showed that this was not universally the case. Instead, phosphate and/or oxygen availability are decreased in microgravity due to lack of convection, leading to altered microbial behavior, such as in P. aeginosa. Reduced convection leading to substrate concentration gradients has been proposed as a general mechanism underlying all microbial alterations seen in microgravity. These models are supported by studies using diamagnetic levitation to simulate weightlessness. Levitation prevents settling but increases convection because of the diamagnetic properties of oxygen. What is known about low-oxygen and low-phosphate environments can help inform microgravity studies, but careful attention to experimental and strain differences is essential. Low-nutrient environments have different effects on motility depending upon the motility features (e.g., run-and-tumble vs. flagellar assembly/chemotaxis).

Fig. 8  Heat map depicting the clustering patterns of the eight treatments by KEGG pathways associated with the proposed function of V. fischeri genes at 12 and 24 h. Gene changes governing flagellar assembly and bacterial chemotaxis labeled at the top. Colors represent the differential abundance of individual genes listed by V. fischeri identification number (VF-ID) for both wild type (WT) and Δhfq mutants under simulated microgravity (M) and normal gravity (G) conditions. Figure and modified caption reprinted from Duscher et al. under the conditions of the CC BY 4.0 License.
forward-reverse\textsuperscript{128} and stress responses of the particular strain. A significant number of studies have been done with human pathogens, since limiting environments are frequently encountered during the infection process, for example in intestinal villi, and can trigger the transition from planktonic to biofilm in many Gram-negatives\textsuperscript{129}. Reduced phosphate leads to increased virulence and swarming motility in \textit{P. aeruginosa}\textsuperscript{30}. Oxygen availability influences microbial pathogenicity at all stages of the infection process\textsuperscript{131}; S. Typhimurium grown in low oxygen environments shows greater adhesion to and invasion of epithelial cells\textsuperscript{132}. In the mutualistic \textit{V. fischeri}, which transitions from motile to non-motile as it enters stationary phase, the low-nutrient microenvironments in microgravity may simulate the transition to stationary phase\textsuperscript{30}.

Downregulation of \textit{hfq} expression was the most consistent finding in the studies we focused on here and is a common theme in stress-response studies as well\textsuperscript{133–136}. As mentioned previously, Hfq is a global transcriptional regulator that has been found in approximately half of all known bacterial genomes. It is an RNA chaperone which can serve as both a positive and a negative regulator. Hfq stabilizes small RNAs (sRNAs) and acts as a platform for sRNA–mRNA interaction; regulation by sRNAs requires Hfq. Hfq-dependent sRNAs play a key role in regulation of flagellar genes by acting on the master regulator, FlhD, as well as other factors (refer again to Fig. 6)\textsuperscript{137}. Almost 90% of flagellar genes are Hfq-regulated in common Gram-negative pathogens\textsuperscript{138}. The general downregulation of \textit{hfq} expression seen in spaceflight and simulated microgravity studies may be related to oxygen and micronutrient availability.

Understanding changes in gene expression is an important step in understanding phenotypic changes. The results shown here suggest that swimming speeds and patterns of microbes could be altered under simulated microgravity conditions. Motility and chemotaxis have evolved to provide some microbes an evolutionary advantage. It remains to be seen whether prolonged exposure to simulated microgravity could fundamentally alter both motility gene expression and swimming phenotypes. Experiments involving imaging during and after exposure to simulated microgravity and spaceflight to quantify motility and chemotaxis behaviors remain to be performed.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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