Microbial Stress: Spaceflight-Induced Alterations in Microbial Virulence and Infectious Disease Risks for the Crew

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Abbreviations

C4-HSL	*N*-Butanoyl-L-homoserine lactone
CF	Cystic fibrosis
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
Fur	Ferric uptake regulator
HARV	High aspect ratio (or rotating) vessel
ISS	International Space Station
LD	Lethal dose
LPS	Lipopolysaccharide
LSMMG	Low-shear modeled microgravity
MEED	Microbial ecology evaluation device
MMG	Modeled microgravity
NASA	National Aeronautics and Space Administration

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OES	Orbital Environmental Simulator
RT-PCR	Reverse transcriptase-polymerase chain reaction
RWV	Rotating wall vessel
SMG	Simulated microgravity
STLV	Slow turning lateral vessel

Although preventative measures to mitigate infectious disease risks to the crew are stringently enforced prior to the launch of spacecraft, pathogenic organisms are still carried by crewmembers, the spacecraft, and its cargo (Taylor 1974; Castro et al. 2004; Gueguinou et al. 2009) (see also Chap. 25). Of additional concern is spaceflight food, which is randomly monitored for microbial content prior to flight, yet remains a potential route of infection for food-borne pathogens, such as *Salmonella* sp. and *Staphylococcus aureus*. While the crewmembers are exceptionally healthy, dysfunction of their immune system has been repeatedly associated with spaceflight missions (Gueguinou et al. 2009), suggesting an increased susceptibility to infection. Other factors, such as the relatively crowded living conditions on flight vehicles, also increase the risk of infectious disease during spaceflight. Indeed, transfer of microbial flora between crewmembers has been demonstrated (Taylor 1974; Pierson et al. 1996). In addition, infections from the astronauts' own normal microbiological flora are still a risk, such as staphylococcal and streptococcal skin infections and urinary tract infections. Evaluations of the environmental microbiome aboard Mir and the International Space Station (ISS) indicated a predominance of common members of the environmental flora (Castro et al. 2004), although the appearance of medically significant organisms has been documented (Ott 2004). Moreover, increased antibiotic resistance for some bacteria during culture in spaceflight has been reported (Tixador et al. 1985; Kacena and Todd 1999), which could potentially compromise effective prophylactic treatment if a crew member were to acquire an in-flight infection from such an organism. Latent viruses also remain a risk to the astronauts (see Chap. 19) because of their ubiquity, the ineffective current preventive practices (e.g., quarantine), and their immunocompromised state (Pierson et al. 2007). Thus, the presence of opportunistic and obligate pathogens and the corresponding risk of infectious diseases cannot be completely prevented during spaceflight.

In order to fully understand the impact of spaceflight on infectious disease risks to the crew, it is critical to advance our knowledge of the effects of spaceflight on the human immune system in a synergistic approach with studies to characterize spaceflight-associated changes in microorganisms, alterations in the human and environmental microbiome, and the resulting impact on host–pathogen interactions. A wide variety of spaceflight experiments have been performed over the past 50 years demonstrating an extensive range of observed phenotypic and, recently, molecular genetic changes in microorganisms (Dickson 1991; Nickerson et al. 2004; Klaus and Howard 2006; Horneck et al. 2010); however, information elucidating the mechanism(s) behind these changes and how spaceflight affects microbial virulence has only recently begun to emerge.

18.1 Modeling Aspects of Spaceflight Culture on Earth

Our knowledge of spaceflight-induced alterations in microbial virulence has been enhanced by the use of ground-based spaceflight analog culture systems, such as the NASA-designed Rotating Wall Vessel (RWV) bioreactor (Fig. 18.1). The RWV is an optimized form of suspension culture in which cells are grown in cylindrical bioreactors, called high aspect ratio vessels (HARV) or slow turning lateral vessels (STLV) in physiologically relevant low fluid-shear conditions. The RWV consists of a hollow disk (HARV) or cylinder (STLV) that is completely filled with culture medium and rotates on an axis parallel to the ground (Klaus 2001; Nickerson et al.

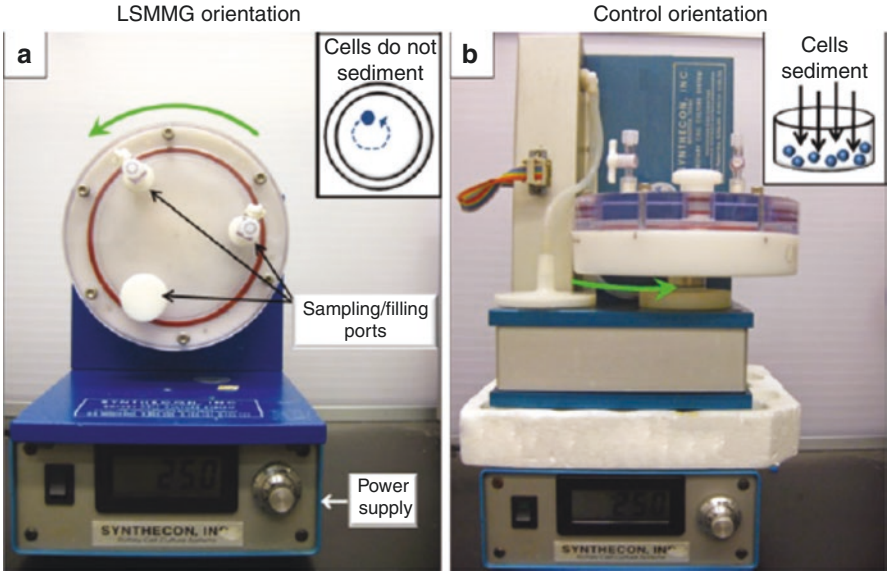


Fig. 18.1 The rotating wall vessel (RWV) bioreactor and power supply. The HARV RWV bioreactor is depicted in the (**a**) Low-shear modeled microgravity (LSMMG) and (**b**) control orientations. For both orientations, the cylindrical culture vessel is completely filled with culture medium through ports on the face of the vessel (indicated by black arrows in **a**) and operates by rotating around a central axis. Cultures are aerated through a hydrophobic membrane that covers the back of the reactor. In the LSMMG orientation (**a**), the axis of rotation of the RWV is perpendicular to the direction of the gravity force vector. In the control orientation (**b**), the axis of rotation is parallel with the gravity force vector. The direction of rotation is indicated by a green arrow in both orientations. The effect of RWV rotation on particle suspension is depicted for each orientation (insets). When the RWV is not rotating, or rotating in the control orientation, the force of gravity will cause particles in the apparatus to sediment and eventually settle on the bottom of the RWV (**b**, inset). When the RWV is rotating in the LSMMG position, particles are continually suspended in the media (**a**, inset). The result is a solid-body rotation of the medium and cells within the RWV, with the sedimentation of the particles/cells due to gravity being offset by the upward forces of rotation. The result is low fluid-shear aqueous suspension that is similar to what would occur in true microgravity

2004). The result is solid-body rotation of the medium and the cells within and a constant rotation perpendicular to the gravitational field that results in an environmental culture, which mimics aspects of the spaceflight environment (Nickerson et al. 2004). As a result, sedimentation of cells due to gravity is offset by the forces of the RWV rotation. The culture environment experienced by cells in the RWV is commonly referred to as low-shear modeled microgravity (LSMMG), modeled microgravity (MMG), or simulated microgravity (SMG). Interestingly, the low fluid-shear culture conditions in the RWV are relevant to those encountered by numerous microbial pathogens and commensals during their normal life cycles in the gastrointestinal, respiratory, and urogenital tracts (Nauman et al. 2007). A gas-permeable membrane on one side of the RWV (HARV) or a central core gas exchange membrane (STLV) allows constant air exchange during growth (Nickerson et al. 2004). Several studies culturing microbes in both the RWV and true spaceflight have focused on profiling molecular genetic (transcriptomic and proteomic), phenotypic (in vitro stress), and virulence responses (in vivo) to provide new insights into how microorganisms respond to culture in the microgravity environment of spaceflight. Notably, since pathogens encounter similar low fluid-shear regions in the human body, these studies have also revealed novel virulence strategies used by pathogens during the natural course of infection, and thus hold promise for the development of new strategies for treatment and prevention of infectious diseases on Earth.

18.2 Spaceflight Analog (LSMMG) Culture of *Salmonella enterica* Serovar Typhimurium

The first studies to demonstrate that culture of microbes in both LSMMG and true spaceflight conditions alters microbial virulence was performed using the obligate bacterial pathogen, *Salmonella enterica* serovar Typhimurium in a murine model of infection (Nickerson et al. 2000; Wilson et al. 2007). Indeed, *S.* Typhimurium remains the best characterized microorganism in response to spaceflight and spaceflight analog culture. As a common food-borne pathogen, *S.* Typhimurium was chosen as the model organism for these studies because (1) it has been extensively studied and well characterized, (2) it is a leading cause of intestinal and diarrheal disease in healthy individuals, and serious systemic illness in the immunocompromised, and (3) it is one of the five basic categories of organisms targeted by NASA for preflight monitoring of spaceflight food. Cultures of *S.* Typhimurium grown in the RWV environment (LSMMG) (Nickerson et al. 2004) displayed a significant increase in virulence as evidenced by a decreased time-to-death, decreased lethal dose 50 (LD_{50}), and increased tissue colonization (liver and spleen) in murine infections as compared to control cultures. The *S.* Typhimurium LSMMG cultures also displayed increased survival in cultured macrophages and increased resistance to acid stress, two key pathogenesis-related responses that are relevant to bacterial

virulence. In addition, this study was the first to demonstrate that the LSMMG environment elicits a global molecular genetic response in bacteria using 2-D protein gel electrophoresis to show that *S.* Typhimurium protein levels changed during LSMMG culture as compared to controls (Nickerson et al. 2000). This study established the paradigm that LSMMG can alter bacterial virulence and serve as a master signal to globally reprogram bacterial gene expression. Moreover, this work provided the first evidence that fluid-shear levels relevant to those encountered by *Salmonella* between the brush border microvilli of intestinal epithelial cells within the infected host act as a novel environmental signal that regulates the virulence, stress resistance, and gene expression of this pathogen (Nickerson et al. 2000, 2004).

To identify the *Salmonella* genes that changed expression in response to LSMMG culture, whole genome microarray analysis was performed using RNA harvested from *S*. Typhimurium cultures grown in LSMMG and control conditions (Wilson et al. 2002a). The results demonstrated that 163 genes globally distributed across the *S*. Typhimurium genome are either upregulated (97 genes) or downregulated (68 genes) during growth in LSMMG. These genes belonged to a variety of functional groups including protein secretion systems, lipopolysaccharide (LPS) synthesis, ribosomal subunits, starvation/stress response, virulence factors, transcriptional regulation, iron-utilization enzymes, and several of unknown functions. Interestingly, none of the upregulated genes corresponded to known virulence factors, even though LSMMG enhanced *S.* Typhimurium virulence. This suggests that LSMMG may alter *Salmonella* virulence by a previously uncharacterized mechanism(s) that could involve novel virulence functions. Alternatively, the increase in *Salmonella* virulence due to LSMMG may be the result of contributions of multiple genes of different functions that are regulated as part of the global reprogramming of *Salmonella* under LSMMG conditions. Secondary assays including RT-PCR and LPS gels were used to confirm the hits obtained from the microarray analysis. In addition, since the authors noticed that ferric uptake regulator (Fur) protein-binding sites were associated with many of the genes found in the analysis, they tested the ability of an *S.* Typhimurium *fur* mutant strain to increase acid stress resistance under LSMMG conditions (as previously observed with the wild-type strain). The *fur* mutant did not display this phenotype, thus indicating that the *fur* gene may play a role in the response of *S.* Typhimurium to LSMMG.

Given the global alterations in molecular genetic and phenotypic responses of *S.* Typhimurium to LSMMG culture, it was hypothesized that the *rpoS* gene was a likely candidate for playing a role in LSMMG signal transmission, as it is a master regulator of the stress and virulence responses in many bacteria (Hengge-Aronis 2000; Dong and Schellhorn 2010). Specifically, an *S.* Typhimurium strain containing an *rpoS* mutation was extensively and systematically compared to an isogenic wild-type strain for responses to LSMMG culture (Wilson et al. 2002b). This study provided key information regarding the bacterial LSMMG response:

(1) the *rpoS* gene is not required for *S.* Typhimurium to display LSMMG-induced phenotypes (analyzed in exponential phase of growth), (2) LSMMG alters resistance to other stresses besides acidic and intracellular macrophage survival, including osmotic, thermal, and oxidative stresses, and (3) cells grown in LSMMG in minimal media show a shorter lag phase and doubling time compared to control cultures.

A follow-up study demonstrated a progressive relationship between the applied fluid-shear in the RWV bioreactor and pathogenesis-related molecular genetic and phenotypic responses of *S.* Typhimurium (Nauman et al. 2007). When exposed to progressively increasing fluid-shear levels in the RWV, planktonic cultures of *S.* Typhimurium displayed corresponding progressive changes in acid and thermal stress responses and targeted gene expression profiles, including *rtsA*, a regulatory protein implicated in *Salmonella* intestinal invasion. This was the first study to provide evidence that incremental changes in fluid-shear can cause corresponding changes in biological responses in *S.* Typhimurium during the infection process and may lead to discovery of new targets for antimicrobial therapeutic development against *Salmonella* and other pathogens.

The initial studies investigating the impact of LSMMG (and later the spaceflight environment) on microbial virulence focused on *S.* Typhimurium strain χ3339, which causes gastroenteritis in humans. Interestingly, a subsequent study investigated the effect of LSMMG culture on a different *S.* Typhimurium strain (D23580), which is a multidrug-resistant clinical isolate of ST313 causing life-threatening systemic infections (Yang et al. 2016). Unlike classic gastrointestinal *Salmonella* strains (e.g., χ3339), gastroenteritis is often absent during ST313 clinical infections and isolates are most commonly recovered from blood, rather than from stool in patients—suggesting the possibility that these isolates may be routinely exposed to the higher fluid-shear conditions found in the blood stream—which in turn may shape their responses to different fluid shear forces. This study showed that D23580 does indeed respond to fluid shear forces; however, it does so in a distinctly different manner relative to classic *S.* Typhimurium strains that cause gastroenteritis. Specifically, exposure of D23580 to high fluid shear (relevant to those encountered in areas of the bloodstream) increased its virulence potential and enhanced resistance to select environmental stressors.

18.3 Spaceflight Culture of *Salmonella enterica* Serovar Typhimurium

To determine whether the true microgravity environment of spaceflight alters bacterial virulence and gene expression in a similar manner to that of spaceflight-analog (LSMMG) culture, a flight experiment designated as MICROBE was flown aboard Space Shuttle mission STS-115 (Wilson et al. 2007). MICROBE was the first experiment to examine the effect of spaceflight on the virulence of a pathogen, and the first to obtain the entire molecular genetic response (transcriptomic and proteomic) of a bacterium to spaceflight. In this experiment, split samples of *S.*

Typhimurium were grown in otherwise identical environmental conditions aboard the Shuttle during spaceflight and on the ground in the Orbital Environmental Simulator (OES) room at the Kennedy Space Center. Growth of *S.* Typhimurium was initiated in both settings after the Shuttle was established in microgravity conditions of orbit. A portion of the *S.* Typhimurium spaceflight cultures were preserved with fixative on orbit to preserve samples for RNA/protein analysis to measure gene expression changes (via microarray and proteomic assays); while the

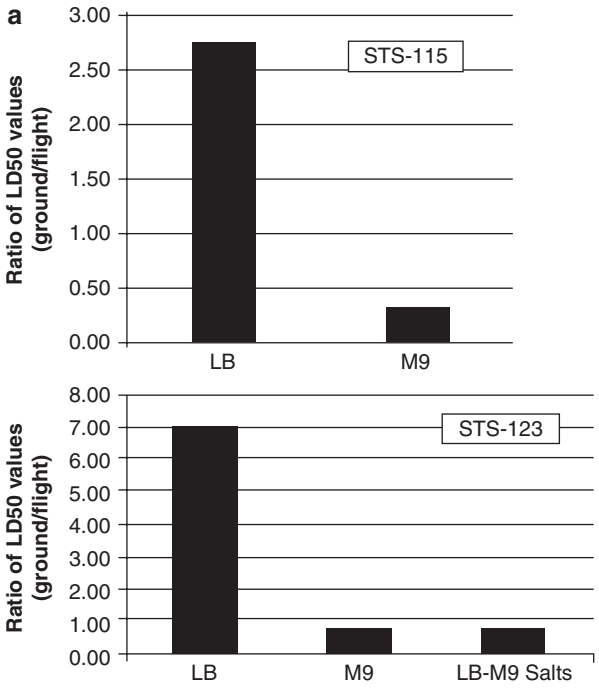
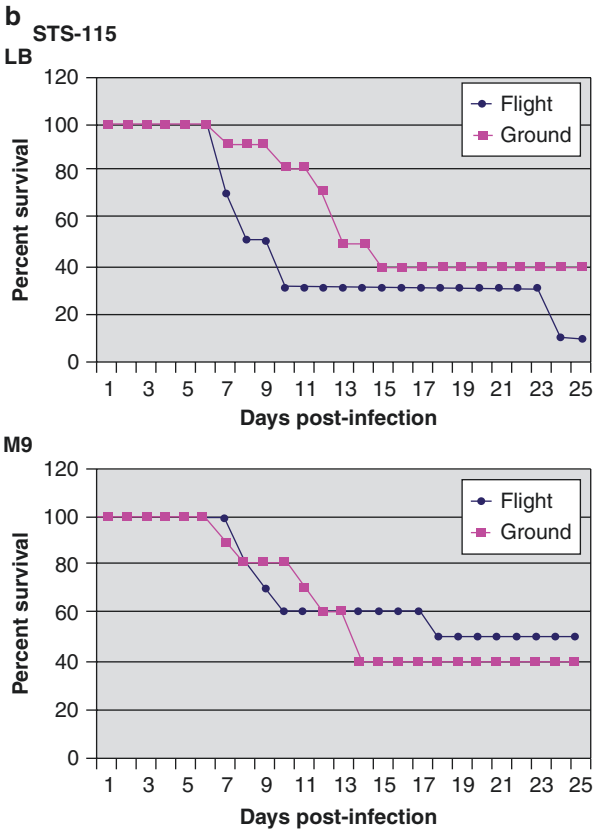


Fig. 18.2 *S.* Typhimurium virulence in LB, M9, and LB-M9 spaceflight cultures. (**a**) Ratio of LD_{50} values of *S.* Typhimurium spaceflight and ground cultures grown in LB (Lennox Broth), M9 (minimal carbon, high salt medium), or LB-M9 salts media (Lennox Broth supplemented with NaH_2PO_4, KH_2PO_4, NH_4Cl, NaCl, and $MgSO_4$) from STS-115 and STS-123 shuttle mission. Female Balb/c mice were perorally infected with a range of bacterial doses from either spaceflight or ground cultures and monitored over a 30-day period for survival. (**b**) Time-to-death curves of mice infected with spaceflight and ground cultures from STS-115 (infectious dosage: 10^7 bacteria for both media). (**c**) Time-to-death curves of mice infected with spaceflight and ground cultures from STS-123 (infectious dosage: 10^6 bacteria for LB and 10^7 bacteria for M9 and LB-M9 salts). Infectious dosages were selected such that the rates in time-to-death facilitated normalized comparisons across the different media. (**d**) SEM of spaceflight and synchronous ground control cultures of *S.* Typhimurium bacteria showing the formation of an extracellular matrix and associated cellular aggregation of spaceflight cells suggesting biofilm formation (magnification: ×3500) (Wilson et al. 2007)



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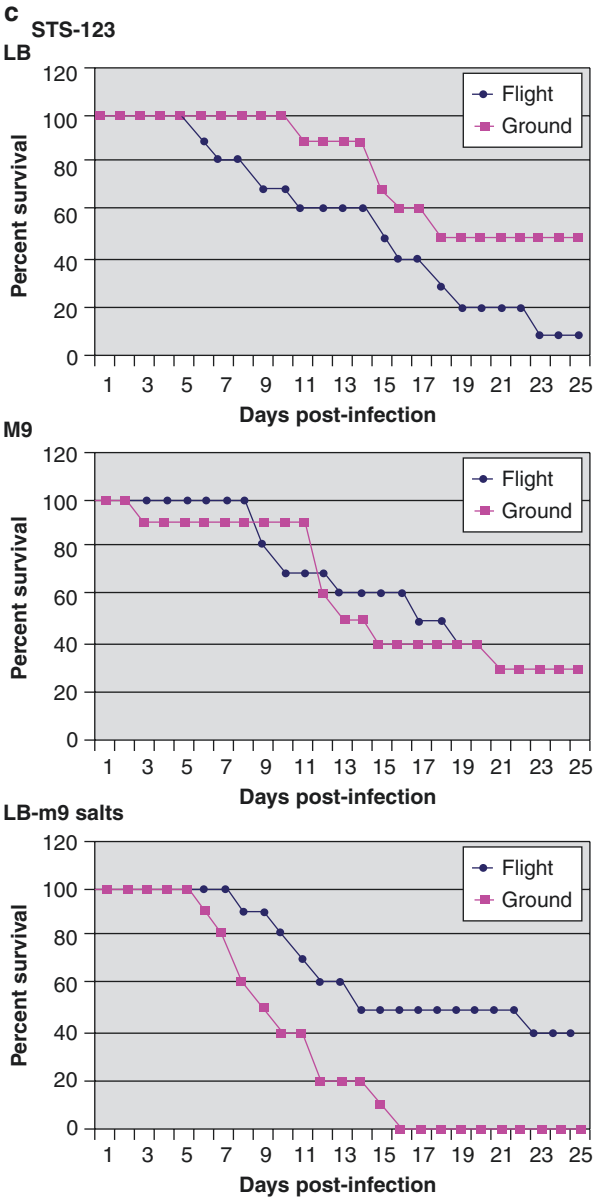

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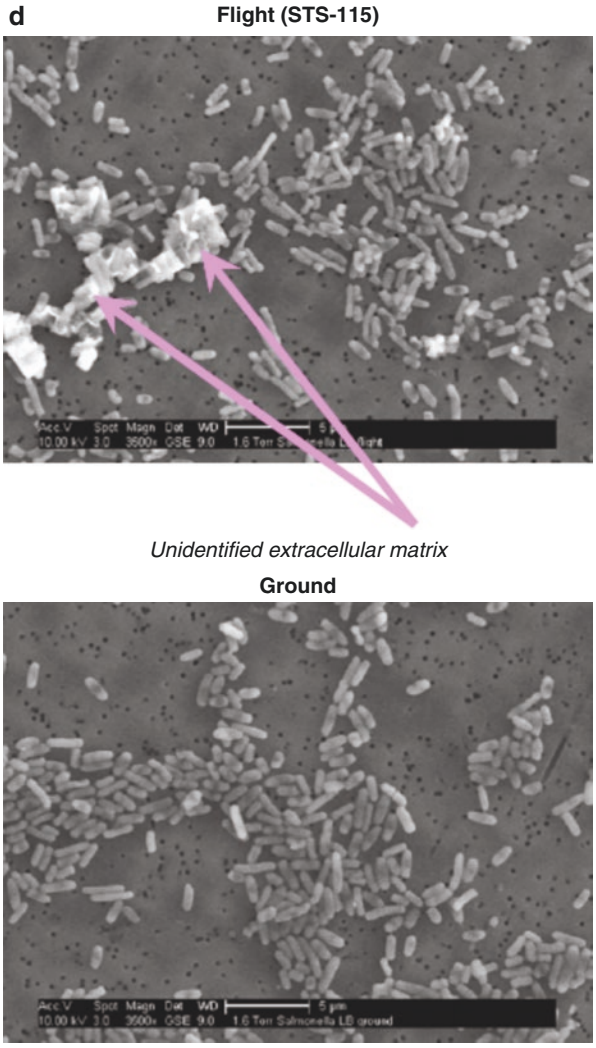

Fig. 18.2 (continued)

other portion of cultures were supplemented with fresh media and used (upon return to ground) for murine infections to measure virulence. Remarkably, the virulence assay results mimicked what was observed in LSMMG conditions in that the spaceflight cultures displayed increased virulence as measured by (1) decreased time to death, (2) decreased LD_{50}, and (3) increased percent mortality across multiple infectious dosages (given perorally) in murine infections as compared to ground controls (Fig. 18.2). In addition, 167 transcripts and 73 proteins were

Table 18.1 Genes of the Hfq-regulon of *S.* Typhimurium and *P. aeruginosa* differentially expressed in response to spaceflight culture

Salmonella spaceflight			*Pseudomonas* spaceflight		
Gene number	Gene name	Function	Gene number	Gene name	Function
Stress resistance/virulence					
STM0831	*dps*	Stress response protein	PA2300[a]	*chiC*	Chitinase
STM1070	*ompA*	Outer membrane porin	PA3479[a]	*rhlA*	Rhamnosyltransferase chain A
STM1572	*ompD*	Outer membrane porin	PA4944	*hfq*	RNA-binding protein Hfq
STM1652	*ynaF*	Putative universal stress protein			
STM2267	*ompC*	Outer membrane porin	*Microaerophilic/anaerobic metabolism*		
STM2638	*rseB*	Anti-αE factor	PA0518	*nirM*	Cytochrome c-551 precursor
STM2640	*rpoE*	SigmaE (α24) factor	PA0519	*nirS*	Nitrite reductase precursor
STM2884	*sipC*	Cell invasion protein	PA0524	*norB*	Nitric-oxide reductase subunit B
STM4361	*hfq*	RNA-binding protein Hfq	PA1557	PA1557	Probable cytochrome oxidase subunit (cbb3-type)
STM4561	*osmY*	Hyperosmotically inducible protein	PA5491	PA5491	Probable cytochrome
Plasmid transfer apparatus			*Hypothetical proteins*		
PSLT081	*traB*	Conjugative transfer	PA0200[a]	PA0200	Hypothetical protein
PSLT095	*traN*	Conjugative transfer	PA1123[a]	PA1123	Hypothetical protein
PSLT099	*trbB*	Conjugative transfer	PA2225	PA2225	Hypothetical protein
PSLT100	*traH*	Conjugative transfer	PA2453	PA2453	Hypothetical protein
PSLT101	*traG*	Conjugative transfer	PA2747	PA2747	Hypothetical protein
PSLT104	*traD*	Conjugative transfer	PA2753[a]	PA2753	Hypothetical protein
PSLT110	*traX*	Conjugative transfer	PA3369	PA3369	Hypothetical protein
SSL_T12	*traT*	Conjugative transfer	PA3520[a]	PA3520	Hypothetical protein
SSL_T20	*traK*	Conjugative transfer	PA3795	PA3795	Probable oxidoreductase
SSL_T24	*traF*	Conjugative transfer	PA4220	PA4220	Hypothetical protein
SSL_T3	*trbC*	Conjugative transfer	PA4351	PA4351	Probable acyltransferase
SSL_T5	*trbA*	Conjugative transfer	PA4352[a]	PA4352	Hypothetical protein

(continued)

Table 18.1 (continued)

Salmonella spaceflight			*Pseudomonas* spaceflight		
Gene number	Gene name	Function	Gene number	Gene name	Function
STM_PSLT085	*traR*	Conjugative transfer	PA4633	PA4633	Probable chemotaxis transducer
Small RNAs[b]			PA4739	PA4739	Hypothetical protein
STM_sRNA_α_RBS	*αRBS*	Small RNA			
STM_sRNA_CsrB	*csrB*	Small RNA			
STM4124	*oxyS*	Small RNA			
STM_sRNA-RFN	*RFN*	Small RNA			
STM_sRNA_RNaseP	*rnaseP*	Small RNA			
STM_sRNA_rne5	*rne5*	Small RNA			
STM_sRNA_tke1	*Tke1*	Small RNA			
Ribosomal proteins					
STM0469	*rpme2*	50 ribosomal protein L31	PA4433	*rplM*	50S ribosomal protein L13
STM3425	*rpsF*	30S ribosomal subunit protein S6			
STM3433	*rplP*	50S ribosomal subunit protein L16			
STM3436	*rpsS*	30S ribosomal subunit protein S19			
STM3439	*rplD*	50S ribosomal subunit protein L4			
STM3448	*rpsL*	30S ribosomal subunit protein S12			
STM4150	*rplA*	50S ribosomal subunit protein L1			
STM0469	*rpme2*	50S ribosomal protein L31			
Iron utilization/storage (Fur or Hfq-regulated)					
STM0596	*entE*	2,3-Dihydroxybenzoate-AMP ligase	PA3621	*fdxA*	Ferredoxin I
STM0831	*dps*	Stress response protein and ferritin	PA4880	PA4880	Probable bacterioferritin
STM1660[a]	*fnr*	Transcriptional regulator, Fe binding			
STM1732	*ompW*	Outer membrane protein W			
STM3443	*bfr*	Bacterioferrin, iron storage			

Biofilm formation					
STM1070	*ompA*	Outer membrane porin			
STM1172	*flgM*	Flagellar biosynthesis			
STM1916	*cheY*	Flagellar biosynthesis			
STM1925	*flhD*	Regulator of flagellar biosynthesis			
STM1962	*fliT*	Flagellar biosynthesis			
Various cellular functions					
–	*mysB*	Suppresses protein export mutants	PA1156	*nrdA*	Ribonucleotide-diphosphate reductase alpha subunit
STM0376	*sbmA*	ABC superfamily transporter	PA1183	*dctA*	C4-dicarboxylate transport protein
STM0536	*ppiB*	Peptidyl-prolyl isomerase B	PA1610	*fabA*	3-hydroxydecanoyl-ACP dehydratase
STM0665	*gltI*	ABC glutamate/aspartate transporter	PA1776	*sigX*	ECF sigma factor SigX
STM0833	*ompX*	Outer membrane protein	PA2003	*bdhA*	3-Hydroxybutyrate dehydrogenase
STM0943	*cspD*	Similar to CspA; not cold-induced	PA2247	*bkdA1*	2-Oxoisovalerate dehydrogenase (alpha subunit)
STM1066	*rmf*	Ribosome modulation factor	PA2248	*bkdA2*	2-Oxoisovalerate dehydrogenase (beta subunit)
STM1290	*gapA*	Glyceraldehyde dehydrogenase	PA2634	PA2634	Isocitrate lyase
STM1682	*tpx*	Thiol peroxidase	PA2851	*efp*	Elongation factor P
STM1749	*adhE*	Fe-dependent dehydrogenase	PA2966	*acpP*	Acyl carrier protein
STM1751	*hns*	DNA-binding protein	PA3686	*adk*	Adenylate kinase
STM1959	fliC	Flagellin, structural protein	PA4031	*ppa*	Inorganic pyrophosphatase
STM2282	*glpQ*	Glycerophosphodiesterase	PA4569	*ispB*	Octaprenyl-diphosphate synthase
STM2488	*nlpB*	Lipoprotein-34	PA5355	*glcD*	Glycolate oxidase subunit GlcD
STM2542	*nifU*	Fe-S cluster formation protein			
STM2665	*yfiA*	Ribosome-associated factor			
STM2801	*ygaC*	Putative cytoplasmic protein			
STM2802	*ygaM*	Putative inner membrane protein			

(continued)

Table 18.1 (continued)

Salmonella spaceflight			*Pseudomonas* spaceflight		
Gene number	Gene name	Function	Gene number	Gene name	Function
STM3060	*ygfE*	Putative cytoplasmic protein			
STM3285	*rbfA*	Ribosome-binding factor			
STM3648	*yiaG*	Putative transcriptional regulator			
STM3840	*rnpA*	RNase P, protein component			
STM3870	*atpE*	Membrane-bound ATP synthase			
STM3915	*trxA*	Thioredoxin 1, redox factor			
STM4231	*lamB*	Phage λ receptor protein			
STM4392	*priB*	Primosomal replication protein N			

Note: Both up- and downregulated genes with a differential expression value of more than twofold are included and are organized in major functional categories

[a]Also involved in microaerophilic/anaerobic metabolism

[b]Small RNAs were not included in the microarray analysis of *P. aeruginosa*. In addition, the RNA purification kit used for the *S*. Typhimurium microarray analysis had a 200 nucleotide cut-off, therefore additional smaller RNA species may have been inadvertently excluded in this work

identified to change expression in response to spaceflight, and these genes were globally distributed across the *S.* Typhimurium genome and belonged to a variety of functional groups. Of the genes identified in microarray analysis, a preponderance belonged to the Hfq regulon (including those encoding small regulatory RNAs, outer membrane proteins, ribosomal proteins, stress response proteins, plasmid transfer functions, iron metabolism, and ion transport) as well as the *hfq* gene itself (which was downregulated) (Table 18.1). Hfq is a highly conserved bacterial RNA chaperone protein that binds to small regulatory RNAs thereby facilitating their association with mRNAs, the result of which plays a diverse role in global regulation of prokaryotic gene expression, virulence, and physiology in response to stress (Gottesman 2004; Majdalani et al. 2005; Gottesman et al. 2006; Guisbert et al. 2007; Pfeiffer et al. 2007; Sittka et al. 2007, 2008). The spaceflight-induced Hfq regulon gene changes were up- or downregulated in correlation with a decrease in *hfq* gene expression. This finding corroborates previous microarray analysis during *S.* Typhimurium culture in LSMMG, where the *hfq* gene is also downregulated (Wilson et al. 2002a). Moreover, the number of downregulated genes (98) was larger than the number of upregulated genes (69) in response to spaceflight, another similarity to the LSMMG microarray results. Interestingly, Hfq also regulates expression of the Fur protein, which was found to play a role in the LSMMG-induced acid stress response in *S.* Typhimurium. Subsequent LSMMG ground-based studies using an isogenic *hfq* mutant strain of *S.* Typhimurium not only supported involvement of Hfq in the *S.* Typhimurium response to microgravity but also established the utility of using the RWV in the laboratory to confirm observations obtained from spaceflight experiments. Interestingly, electron microscopic evaluation of *S.* Typhimurium spaceflight samples revealed striking differences in cellular aggregation and clumping that was associated with the formation of an extracellular matrix reminiscent of biofilms as compared to the ground control cultures (Fig. 18.2d) (Wilson et al. 2007). This phenotypic observation was consistent with corresponding differences in the expression of genes associated with biofilm formation and may play a role in the enhanced virulence of the organisms grown in space.

Spaceflight studies of *Salmonella* demonstrate that microgravity culture impacts a wide range of microbial characteristics, including growth, morphology, survival, metabolism, and gene expression (Nickerson et al. 2004; Wilson et al. 2007, 2008). However, these experiments, as well as experiments with other microorganisms, have been done with pure cultures using relatively short-duration studies (typically $\leq$96 h). Long-term heritable changes, resulting from natural selection and microbial evolution, also need to be addressed particularly in the context of human exploration class missions (e.g., Mars mission) during which changes in the spacecraft and human microbiome would undoubtedly occur and could pose a risk to mission success. Indeed, the first experiment to look at the impact of long-duration spaceflight culture on microbial responses will launch to the ISS in late 2018. This study (entitled EVOLVES) is led by Principal Investigator Cheryl Nickerson from Arizona State University and will characterize the functional response of wildtype and mutant *Salmonella* strains to long-term multigenerational growth in the

chronic stress of microgravity by examining a range of genotypic, molecular genetic and phenotypic responses (https://humanresearchroadmap.nasa.gov/Tasks/task.aspx?i=436). These studies will provide clear evidence as to whether microgravity creates selective mutations that could impact human exploration of deep space.

18.4 Role of Ion Composition on Spaceflight-Induced Virulence

The results of the MICROBE experiment aboard STS-115 led to a follow-up experiment, designated MDRV, aboard STS-123. The goal of this experiment was to (1) confirm the experimental results from STS-115, and (2) determine if altering the ion composition of the growth medium could decrease the spaceflight-induced increase in virulence (Wilson et al. 2008). The hypothesis that manipulation of ion concentrations could counteract or inhibit the spaceflight-associated increase in *Salmonella* virulence was based on initial results from the MICROBE experiment, which suggested that *S.* Typhimurium cultures grown in a minimal carbon, high-salt medium called M9 did not respond with the same spaceflight-induced increase in virulence observed with Lennox broth (LB). In a rare opportunity to replicate a spaceflight result, the data from STS-123 fully supported the results from STS-115 in that (1) *S.* Typhimurium spaceflight cultures grown in LB medium displayed increased virulence in murine infection compared to ground controls, and (2) the spaceflight cultures grown in M9 did not display increased virulence compared to controls (Fig. 18.2). Moreover, the addition of similar concentrations of five key inorganic salts found in M9 medium (NaH_2PO_4, KH_2PO_4, NH_4Cl, NaCl, and $MgSO_4$) to the LB medium reversed the increase in virulence of spaceflight cultures grown in LB medium alone. Interestingly, although different virulence responses were observed in spaceflight cultures grown in the LB and M9 media, significant similarities in gene and protein expression profiles indicated involvement of the Hfq regulon in either media. Subsequent ground-based investigations using the RWV reinforced the flight data indicating an inhibitory effect of high ion concentrations in the growth medium on pathogenesis-related responses. By systematically adding different combinations of the inorganic salts to the LB medium in the RWV, phosphate ion (PO_4) was isolated as the key component to repressing this microbial pathogenesis-related response (Wilson et al. 2008).

New discoveries using *S.* Typhimurium as a model organism are continuing as a follow-up flight experiment to build upon results obtained from MICROBE and MDRV, and were flown on STS-131 (April, 2010). This experiment, designated as STL-IMMUNE, was the first experiment to conduct an in-flight infection of human cells (intestinal) with a microbial pathogen (*S.* Typhimurium). The data from this spaceflight experiment will provide insight into alterations in host–pathogen interactions that occur during spaceflight and will unveil the cellular and molecular

mechanisms behind those changes. This information has the potential to significantly change microbial risk assessment and operational requirements during a mission.

The discovery that spaceflight culture increased the virulence of *Salmonella*, yet genes known to be important for the virulence of this pathogen were not regulated as expected when this organism is grown on Earth, led to a follow-up experiment aboard Space Shuttle mission STS-135 in an effort to translate these research findings toward medical applications. Specifically, researchers investigated the impact of spaceflight culture on the protective immunogenicity and gene expression of live Recombinant Attenuated *Salmonella* Vaccine (RASV) strains, including those in clinical trials. These genetically engineered vaccine strains are used as carriers to infect the host and deliver protective antigens against different microbial pathogens to the immune system (Curtiss et al. 2009, 2010; Li et al. 2009; Shi et al. 2010a, b). The ultimate goal of this spaceflight vaccine initiative experiment was to accelerate the development of RASV strains carrying a protective antigen against *Streptococcus pneumonia* (or pneumococcus) by (1) enhancing their ability to safely induce a potent and protective immune response and (2) unveiling novel gene targets to develop new and improved existing vaccine strains. Pneumococcus causes life-threatening diseases, such as pneumonia, meningitis, and bacteremia, and kills over ten million people annually—and is particularly dangerous for newborns and the elderly, who are less responsive to current anti-pneumococcal vaccines. Experiments like these hold the potential to benefit astronauts on future exploration missions and the general public on Earth (Sarker et al. 2010).

18.5 The Response of *Pseudomonas aeruginosa* to Spaceflight and Spaceflight Analog Conditions: Similarities and Differences as Compared to *Salmonella*

As a versatile, ubiquitous bacterium that is occasionally part of the normal human flora, *P. aeruginosa* can also survive in extraterrestrial habitats, as evidenced by its isolation from the potable water system on the ISS and from Apollo crewmembers (Taylor 1974; Hawkins and Ziegelschmid 1975; Castro et al. 2004; Bruce et al. 2005). Astronaut cross-contamination with *P. aeruginosa* has been reported during short-term missions emphasizing the potential of this infectious agent to rapidly spread among crewmembers (Taylor 1974). Thus far, the presence of *P. aeruginosa* in the spaceflight vehicle has led to one reported incapacitating urinary tract infection in-flight (Taylor 1974). In addition to the importance for astronaut safety, studying the behavior of *P. aeruginosa* in the low fluid-shear conditions of microgravity provides insights into the role that low fluid-shear regions in the human body play in triggering virulence characteristics.

In a seminal spaceflight study, McLean et al., demonstrated that *P. aeruginosa* formed biofilms on polycarbonate membranes that were strongly resistant to mechanical disruption (McLean et al. 2001). More recent studies discovered that

the microgravity environment of spaceflight increased the formation of biofilms by *P. aeruginosa*, and resulted in a unique biofilm architecture, referred to as column-and-canopy by the authors (Kim et al. 2013b). Specifically, biofilms grown in spaceflight on cellulose ester membrane discs generated column-shaped structures overlaid by canopies (resembling mushroom-shaped biofilms typically observed in flow cells), while biofilms formed under ground control conditions were flat (commonly observed under static conditions). Since an increased oxygen supply abolished the observed differences between biofilms grown under microgravity and control conditions, oxygen limitation in microgravity conditions was proposed to play a role in the observations (Kim et al. 2013a). The spaceflight environment has also been shown to result in higher *P. aeruginosa* densities following 72 h of culture in modified artificial urine medium (Kim et al. 2013a). The authors proposed that phosphate and oxygen limitations under spaceflight growth conditions were the causative factors for the observed increased bacterial densities (Kim et al. 2013a). In a separate study, the susceptibility of *P. aeruginosa* to antibiotics was examined with cultures grown in spaceflight and phenotypic analysis done on Earth using antibiotic disc tests on solid media. A decreased susceptibility to the polymyxin antibiotic colistin was observed, as well as an increased susceptibility to cephalothin, polymyxin B, and rifampixin (Juergensmeyer et al. 1999).

Future research into the development and impact of biofilms during spaceflight is critical to protect crew health, vehicle systems/integrity, and mission success. However, biofilm formation and architecture has only been studied in spaceflight experiments using single, pure cultures of microorganisms. To better understand the impact of microgravity on the formation, architecture, disinfection sensitivity, and corrosion potential of polymicrobial biofilms, a new spaceflight study led by Robert McLean at Texas State University will investigate the development of biofilms created by co-cultures of *P. aeruginosa* and *Escherichia coli*, the ability of silver solutions to disinfect these biofilms, and the corrosion caused by these biofilms on stainless steel. These studies will provide new evidence as to whether current biofilm control is adequate for spacecraft during human exploration of deep space.

The transcriptional and proteomic responses of *P. aeruginosa* to spaceflight conditions were profiled as a part of the MICROBE experiment (Crabbé et al. 2011). Intriguingly, Hfq and a significant part of the Hfq regulon were differentially regulated by *P. aeruginosa* in spaceflight. As described above, Hfq was initially identified as a key regulator in the LSMMG and spaceflight response of *S.* Typhimurium (Wilson et al. 2002a, 2007, 2008). Hence, Hfq is the first transcriptional regulator ever shown to be involved in the spaceflight response across bacterial species. Among the genes with the highest fold inductions in spaceflight-grown *P. aeruginosa* were those encoding the lectins, LecA and LecB. Lectins play a role in the bacterial adhesion process to eukaryotic cells, and have clinically important cytotoxic effects (Gilboa-Garber et al. 1977; Bajolet-Laudinat et al. 1994; Chemani et al. 2009). Another virulence gene that was induced by *P. aeruginosa* in response to spaceflight culture conditions was *rhlA*, which encodes rhamnosyltransferase I

involved in rhamnolipid surfactant biosynthesis. Rhamnolipids are glycolipidic surface-active molecules with cytotoxic and immunomodulatory effects in eukaryotic cells (McClure and Schiller 1996; Davey et al. 2003; Pamp and Tolker-Nielsen 2007). Furthermore, spaceflight induced the expression of genes and proteins involved in the anaerobic growth of *P. aeruginosa*. Indeed, more limited oxygen availability could occur in spaceflight conditions due to low fluid-shear and thus, low mixing growth conditions. In a separate study, the gene expression profiles of *P. aeruginosa* and *S*. Typhimurium cultured in spaceflight were compared using a systems biology approach. Common pathways that were differentially regulated under spaceflight conditions in both organisms included pathways related to ribosome synthesis, RNA degradation, protein export, flagellar assembly, methane metabolism, toluene degradation, oxidative phosphorylation, TCA cycle, glycolysis, and purine and pyrimidine metabolism (Roy et al. 2016).

The cultivation of *P. aeruginosa* PAO1 in spaceflight analog conditions (LSMMG) in the RWV (28°C) in LB medium induced a transcriptomic and

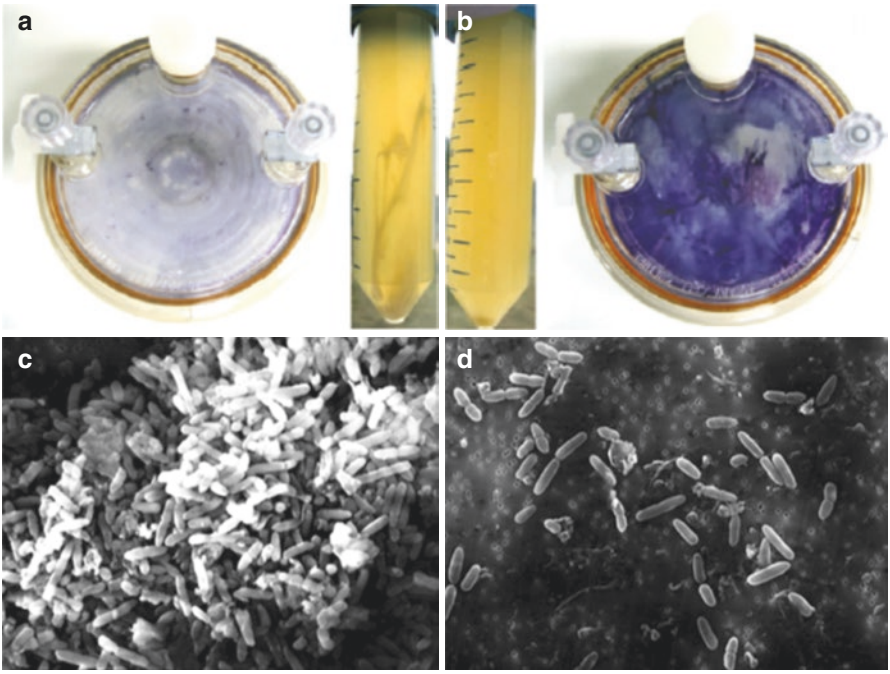

Fig. 18.3 Biofilm formation of *P. aeruginosa* in LSMMG (**a** and **c**) and higher shear (**b** and **d**) conditions using the RWV bioreactor. Panels **a** and **b** show *P. aeruginosa* PAO1 grown in LB medium and the gas-permeable membrane on the backside of the vessel was stained with Crystal Violet to detect biofilm formation. The decanted culture was collected in Falcon tubes (center). Panels **c** and **d** show SEM images of *P. aeruginosa* CF isolate PA39 grown in artificial sputum medium. Magnification = 4500× (Dingemans et al. 2016)

phenotypic profile related to virulence (Crabbé et al. 2010). More specifically, PAO1 produced higher amounts of the exopolysaccharide alginate when grown in LSMMG, as compared to the control. Alginate is an important virulence factor in *P. aeruginosa* since it restricts the diffusion of antimicrobial agents and confers resistance to immune defense mechanisms by avoiding phagocytic uptake, scavenging reactive oxygen species and suppressing leukocyte function (Learn et al. 1987; Pier et al. 2001). Accordingly, an increased oxidative stress resistance in LSMMG-grown *P. aeruginosa* was observed, as well as a higher transcription of the alternative sigma factor *algU*, essential for alginate production. As mentioned above, Hfq was also found to be an important regulator in the LSMMG response of *P. aeruginosa* (Table 18.1) (Wilson et al. 2002a, 2007, 2008; Crabbé et al. 2010, 2011). In addition, the *P. aeruginosa* LSMMG regulon (comprised of 134 genes) included genes involved in stress resistance, motility, and microaerophilic/anaerobic metabolism.

Another impact of low fluid-shear was demonstrated in a study where LSMMG-grown *P. aeruginosa* (37°C) formed dense self-aggregating biofilms in LB medium (Fig. 18.3a) (Crabbé et al. 2008) in contrast to membrane-attached biofilms formed in the higher fluid shear control orientation (Fig. 18.3b). Interestingly, the phenotypic and gene expression profiles grown in LSMMG showed similarities with those of *P. aeruginosa* found in lung secretions of cystic fibrosis (CF) patients (Lam 1980; Singh et al. 2000; Sriramulu et al. 2005; Bjarnsholt et al. 2009). In CF patients, the formation of drug-resistant and/or tolerant microcolonies by *P. aeruginosa* in the dense and viscous lung mucus is the major cause of mortality (Wagner and Iglewski 2008). Low fluid-shear zones are believed to be present in the lung mucus of CF patients due to the absence of mucociliary clearance, which represents the main shear-causing factor in the normal lung mucus (Blake 1973). Recently, LSMMG-induced self-aggregating biofilms were also reported for a highly adapted, transmissible *P. aeruginosa* CF isolate cultured in artificial sputum medium (Fig. 18.3c, d) (Dingemans et al. 2016). Using a *P. aeruginosa* strain and growth medium relevant for the CF lung environment resulted in the induction of additional pathways by LSMMG that are involved in the metabolism and virulence of this microorganism in the CF patient population (Dingemans et al. 2016).

18.6 How Universal Is the Microbial Response to Spaceflight and Spaceflight Analog Culture?

The studies described in the previous sections involving *S*. Typhimurium and *P. aeruginosa* provide key examples of two biomedically important human pathogens that exhibit a variety of similarities and differences in their responses to spaceflight and spaceflight-analog culture. While these organisms have received the most extensive degree of study, the response of other pathogens to these environments have also been investigated, and results from these studies

suggest both potential common mechanisms as well as a myriad of different responses.

For example, *Staphylococcus aureus* is a Gram-positive bacterium of particular importance to crew health due to its prevalence and reported transmission aboard spacecraft (Pierson et al. 1996; Castro et al. 2004). RWV-cultured *S. aureus* displayed slower growth and generally repressed virulence characteristics, including decreased carotenoid production (Rosado et al. 2010; Castro et al. 2011), decreased capacity to lyse red blood cells (Rosado et al. 2010), increased susceptibility to oxidative stress (Castro et al. 2011), reduced survival in whole blood (Castro et al. 2011), and intriguingly, increased formation of a biofilm phenotype (Castro et al. 2011). Furthermore, molecular genetic expression analysis revealed the downregulation of the RNA chaperone protein Hfq, which parallels the response of *S.* Typhimurium to LSMMG culture. This common association with Hfq in both Gram-positive and Gram-negative organisms suggests an evolutionarily conserved response to fluid-shear among structurally diverse prokaryotes (Castro et al. 2011). However, unlike *S.* Typhimurium and *P. aeruginosa*, these results suggest *S. aureus* responds to the RWV environment by initiating a biofilm phenotype with diminished virulence characteristics that may enable the organism to establish a long term commensal relationship with the host. Collectively, these comparisons may provide unique insight into key factors influencing the delicate balance between infection and colonization by *S. aureus* during the initial host–pathogen interaction.

In addition, other Enterobacteriaceae have been profiled in response to LSMMG culture to determine the conserved nature of this response (Pacello et al. 2012; Soni et al. 2014). For example, a study by Soni et al. evaluated various Enterobacteriaceae from different genera in a systematic "side-by-side" manner. Evaluations of *S.* Typhimurium, *E. coli*, *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens* revealed essentially identical growth kinetics in both the LSMMG and control orientation for each organism. Each species was also profiled for LSMMG-induced stress resistance at stationary phase, including acid and oxidative stress. These studies confirmed that culture in LSMMG altered the acid stress response of most of these microorganisms, however some became more sensitive while others became more tolerant. Notably, *C. freundii* did not display any change in acid stress response. When evaluated for changes in oxidative stress response, all cultures grown in LSMMG became more sensitive to oxidative stress. In addition, qRT-PCR analysis demonstrated that the molecular genetic response of these species to LSMMG is conserved across Enterobactericeae (e.g., *hfq*, *trpD*, and *ydcI*), but the direction of gene expression changes (i.e. up or down) can vary depending on the genus.

The number and variety of microorganisms that have been studied in spaceflight and spaceflight analog culture is extensive, and beyond the scope of this chapter. However, several other findings from experiments investigating the responses of medically significant microorganisms during culture in these environments are presented in Table 18.2.

Table 18.2 Other pertinent findings from experiments investigating medically significant microorganisms cultured in spaceflight and spaceflight analog conditions

Microorganism	Environment	Finding
E. coli	LSMMG	Increased resistance to a variety of pathogenesis-related stresses, including low pH, osmotic, alcohol, and thermal stress (Gao et al. 2001; Lynch et al. 2004, 2006; Allen et al. 2008).
	LSMMG	Acid and osmotic stress resistance was shown to be RpoS-independent in the exponential phase of culture (similar to the findings for *S.* Typhimurium cultured to exponential phase in the RWV (Wilson et al. 2002b)), but RpoS-dependent in stationary phase (Lynch et al. 2004).
	LSMMG	Dense biofilms that exhibited increased resistance to some environmental stresses and antibiotics were observed when *E. coli* was cultured to stationary phase under LSMMG conditions, as compared to control conditions, in the presence of glass microcarrier beads (Lynch et al. 2006).
	LSMMG	Carvalho et al. (2005) infected a 3-D model of colonic epithelium with either LSMMG or control-cultured Enteropathogenic *E. coli* (EPEC) or Enterohaemorrhagic *E. coli* (EHEC), respectively, when both the host and pathogen were simultaneously cultured in the RWV bioreactor. Formation of attaching and effacing lesions similar to that observed during the normal course of infection in vivo, and increased intimin expression were observed during EHEC infection of 3-D colon cells in the RWV.
	LSMMG	Increased toxin production observed in Enterotoxigenic *E. coli* (ETEC) cultured under LSMMG conditions as compared to control cultures, which correlated with increased fluid accumulation in mice infected with LSMMG-cultured ETEC (Chopra et al. 2006). Increased TNF production was observed in macrophages infected with EPEC cultured under LSMMG as compared to control cultures.
	LSMMG	Increased adherence of adherent-invasive *E. coli* to Caco-2 colonic epithelial cells when the bacteria were cultured under LSMMG; an effect which became even more pronounced with an *rpoS* mutant (Allen et al. 2008).
	LSMMG	Multigenerational growth of *E. coli* in LSMMG induced genetic changes compared to an unadapted control suggesting the microgravity analog environment may selectively adapt microorganisms over time (Tirumalai et al. 2017).
	LSMMG	A comparison was performed evaluating *E. coli* strain MG1655 transcriptional responses when cultures were grown in rich and minimal medium under LSMMG and control conditions. While reproducible patterns were identified under each condition, no specific genes were identified that would suggest a single gene that was consistently differentially regulated under every LSMMG condition (Tucker et al. 2007).
	LSMMG	Evaluation of several strains of *E. coli* O157:H7 cultured under LSMMG provided evidence that cells were increasing in size in a media dependent fashion (Kim et al. 2014).
	LSMMG	Evaluation of several strains of *E. coli* O157:H7 cultured under LSMMG indicated a decreased resistance to thermal stress (55°C) and higher membrane fluidity based on fatty acid content (Kim and Rhee 2016).
	LSMMG	*E. coli* cultured in LSMMG displayed a decreased susceptibility to ciprofloxacin compared to control cultures. One possible explanation was increased expression of efflux pump genes *acrAB-tolC* (Xu et al. 2015).

Microorganism	Environment	Finding
Streptococcus pneumoniae	LSMMG	Differential transcriptional regulation of 101 genes, including those involved in the adherence and invasion (Allen et al. 2007).
Saccharomyces cerevisiae	Apollo 16	Increased phosphate uptake observed during spaceflight culture (Berry and Volz 1979).
	Apollo 16	Increased survival in tissues following multiple infection routes in a mouse model of infection (intraperitoneal, tail vein, and epidermal) as compared to ground-based controls (Hiebel and Volz 1977; Volz 1990).
	LSMMG	Random budding pattern and tendency to self-aggregate and clump in response to LSMMG culture in contrast to normal bipolar budding normally observed in controls. Corresponding changes in expression of genes important for polarity, budding, and cell separation (Purevdorj-Gage et al. 2006).
	LSMMG	Microarray analysis showed differential regulation of *S. cerevisiae* genes including those important for environmental stress responses (Johanson et al. 2002; Sheehan et al. 2007).
Candida albicans	LSMMG	Increased frequency of filamentous forms in LSMMG cultures accompanied by changes in expression of *hwp1* and *ywp1*, associated with yeast-hyphal transition. The morphogenic switch from round, budding yeast to filamentous form is associated with enhanced virulence (Altenburg et al. 2008).
	Space Shuttle STS-115	The first global transcriptional profiling and phenotypic characterization of the fungal pathogen, *C. albicans*, grown in spaceflight conditions. Enhanced random budding of spaceflight-cultured cells was observed as opposed to bipolar budding patterns for ground controls (Fig. 18.4). Spaceflight differentially regulated 452 genes compared to ground controls, including those involved in antifungal agent and stress resistance, e.g., ABC transporters, ergosterol, and oxidative stress resistance (Crabbé et al. 2013).
Streptococcus mutans	LSMMG	Increased sensitivity to oxidative stress. Transcriptomic analysis showed differential expression of 247 genes compared to control cultures, including those involved in carbohydrate metabolism, translation, and stress responses (Orsini et al. 2017).
Mycobacterium marinum	LSMMG	LSMMG enhanced sensitivity to oxidative stress as compared to control conditions. Transcriptomic analysis demonstrated differential gene expression of 562 genes, including those involved in metabolism, lipid degradation, and chaperone and mycobactin expression. Role for SigH identified in LSMMG response (Abshire et al. 2016).
Salmonella enterica (various strains)	LSMMG	*S. enterica* strains were challenged with hydrogen peroxide to determine conservation of their oxidative stress response. All strains displayed enhanced resistance. In addition, the deletion of the genes encoding for the catalases KatG and KatN removed the enhanced resistance induced by LSMMg culture. Interestingly, deletion of Hfq, RpoE, RpoS or OxyR from strains did not affect the enhanced resistance phenotype (Pacello et al. 2012).
Klebsiella pneumoniae	LSMMG	*K. pneumoniae* grown under LSMMG formed thicker biofilms and higher production of cellulose compared to control orientation cultures. RNA seq transcriptomic analysis showed 171 diferentially regulated genes between the two growth conditions belonging to 15 functional categories (Wang et al. 2016).

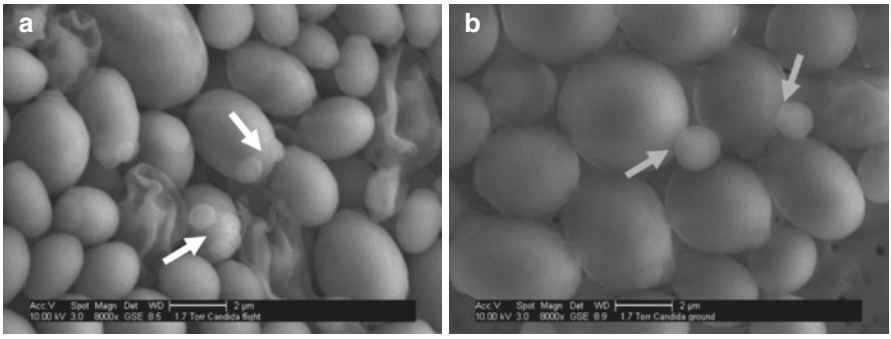

Fig. 18.4 Scanning electron microscopy image of *Candida albicans* cultured under spaceflight conditions aboard Space Shuttle STS-115 (**a**) or in ground control conditions (**b**). Random budding was only observed in cells cultured under spaceflight conditions (white arrows), while bipolar budding patterns were observed for ground controls (grey arrows). Magnification = 8000× (Crabbé et al. 2013)

18.7 Importance to Spaceflight and Life on Earth: The Future Has Started Now

As humans explore space, microorganisms will travel with them. Thus, understanding microbial responses to spaceflight will have a tremendous impact on how we design our spaceflight vehicles, build bioregenerative systems, grow food during a mission, and mitigate the risk of infectious disease to the crew. While several key studies have provided critical mechanistic insight into how microbial responses to the spaceflight environment may affect virulence, many questions still remain unanswered. Indeed, spaceflight-induced alterations in microbial virulence have just begun to be investigated. The impact of these changes on the host–pathogen interaction during spaceflight and corresponding clinical implications for a potentially susceptible crew is still unclear. Beyond prevention of exposure, antibiotics are the primary countermeasure to microbial infection during a spaceflight mission. Previous spaceflight experiments have identified increases in antibiotic resistance for organisms such as *E. coli* (kanamycin and colistin) and *S. aureus* (oxacillin, chloramphenicol, and erythromycin) in response to spaceflight culture (Tixador et al. 1985). The lack of conclusive information on changes in antibiotic resistance for a broad range of microorganisms and corresponding pharmacokinetics indicates a large knowledge gap in infectious disease control, although recent approaches may allow a better prediction (Sommer et al. 2017). Interestingly, the use of microgravity and space flight conditions and its distinct effects on microbial resistance may serve as an additional tool in this direction. Moreover, understanding the microbiota to which the crew will be exposed, and how spaceflight alters the microbial consortia and interactions with the crew, is one of the cornerstones of microbiological risk assessment during a mission. While microorganisms associated with the environment and food supply have been the focus of operational

monitoring efforts, very little is known about the changes in crew and spacecraft microbiome during a mission while multiple new investigations are under way. Likewise, little is known about mutation rates and heritable changes in all microorganisms associated with the crew and their environment during a mission—and nothing is known of long-term spaceflight-induced changes to either the host or pathogen. Alone or in combination, these factors could dramatically affect the impact of microorganisms on spaceflight mission success since the resistancies and the specific antibiotic' availabilities are unforeseeable variables. As we look to the future and introduction of commercial spaceflights with greater civilian participation, including spaceflight tourism, we need a greater understanding of the unique microbial risks associated with human spaceflight. Moreover, lessons learned from spaceflight studies have profound implications for the general public, in terms of expanding our knowledge of (1) the mechanisms of microbial pathogenesis, which hold potential for development of novel strategies to a point of care diagnosis, allowing optimized treatments, and—most efficiently—to prevent infectious disease, and (2) the human microbiome and how stressful environments (see Chap. 34) alter the relationship between host and commensal that determine the transition between normal homeostasis and disease progression (http://commonfund.nih.gov/hmp/).

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