**Topical: Microbiology of Horizontal Gene Transfer and Microgravity Biofilms**

Authors:

Velez-Justiniano, Yo-Ann1

 The University of Alabama in Huntsville

 yj0015@uah.edu

Doerfert, Sebastian N1

The University of Alabama in Huntsville

 doerfert@gmail.com

Sysoeva, Tatyana1

The University of Alabama in Huntsville

tatyana.sysoeva@uah.edu

**Abstract**

Structured microbial communities - biofilms - have been found on multiple surfaces in space systems, mainly in the International Space Station (ISS) (Zea, 2018). Due to biofilm interactions with the materials and systems, they pose risks to the flight systems and thus are of interest to study in preparation for long-term crewed missions to the Moon and Mars. Many strategies for biofilm elimination have been proposed, studied, and used with some degree of success. However, many physiological processes of biofilms grown under reduced gravity conditions remain uncharacterized, hindering our success in biofilm mitigation. On Earth, biofilms are known to be “hot spots” for gene exchange among microbes through the processes of horizontal gene transfer (HGT). HGT in bacteria mainly occurs via transformation, transduction, and plasmid conjugation. All three processes appear to be affected by the extracellular polymeric substances (EPS) in the matrix of the biofilm as well as by the dynamics of biofilm maturity. Thus, the HGT rates cannot yet by predicted, but they certainly contribute to spreading of antibiotic resistance genes, virulence and metabolic cassettes. This publication aims to describe the previously identified connections between HGT and bacterial biofilm lifestyle, highlight existing knowledge gaps, and outline suggested studies as they relate to the space exploration.

**Factors that contribute to HGT in biofilms**

Conjugation in biofilms has been tested in several groups of studies (Hausner & Wuertz, 1999; Ghigo, 2001; Krol et al., 2011; Stalder & Top, 2016). One group of studies show increased HGT outcomes in bacterial biofilms in comparison with planktonically growing cells. Conjugation in biofilms has been extensively tested (reviewed in Stalder & Top, 2016). Studies show that close proximity of the bacterial cells and their tight interactions in the biofilm structure allow for faster transmission of conjugative plasmids (Hausner & Wuertz, 1999; Madsen et al., 2012; Krol et al., 2011). In addition, it has been shown that integrative conjugative element ICE*Bs1* moves faster within the chains of *Bacillus subtilis* cells than between separated cells or between the chains (Babic et al., 2011*)*. This observation is potentially consistent with the notion that the physical proximity of cells to one another contributes to higher conjugation rates (Gama et al., 2020). Biofilm matrix of many bacteria and some fungal species was shown to contain extracellular DNA (eDNA), that sometimes plays a structural role, for example in *Staphylococcus aureus* biofilms (Flemming & Wingender, 2010; Das & Manefield, 2013). In addition, biofilm structures often include wrinkles and internal channels that allow for the movement of eDNA and other molecules. Therefore, it is possible that natural transformation might be heightened within some biofilms. Additionally, *Pseudomonas aeruginosa* was recently shown to become competent for DNA uptake within the context of eDNA containing biofilms while previously it was thought to be not naturally transformable (Nolan et al., 2020).

On the other hand, there are studies that report unchanged HGT rates or factors that are not conducive to the fast transfer. This way, cells within the biofilm structure become less metabolically active than their planktonic counterparts, which might lead to slow transmission of plasmids (Madsen et al., 2012). In addition, other studies have argued that biofilm extracellular polymeric substances (EPS) form a barrier that may protect the internal communities of cells from external factors such as antibiotics. Such EPS then may regulate externally initiated transfer of genes (Goodman et al., 2011; Hu et al., 2019), despite high eDNA in their polymeric matrix composition (Das, 2013). There are also quantitative studies showing that careful accounting for donor and recipient populations within the biofilm indicates that conjugation is not increased in biofilms of multiple *E. coli* strains (Krol et al., 2013). This controversy suggests that either different strains and plasmids have very different HGT rates or we need to work on establishing better consensus on defining the HGT rates (Lopatkin, Sysoeva & You, 2016) comparable among studies (Stalder & Top, 2016).

Regulation of transfer operon(s) in conjugative plasmids is studied to a great extent in the model F plasmid of *E. coli* and in a few other models (Frost & Koraimann, 2010; Virolle et al., 2020; Kohler et al., 2019). A complex network of regulators is at play in all of them and tasked to modulate when the transfer will be activated. This network often involves stress regulators that are known to be upregulated within biofilm environment. Nevertheless, to date, it is not well characterized how transfer genes are expressed and function in the biofilm context. Overall, it has been observed that plasmid transfer is often affected by the growth stage of the donor and recipient cells (Frost & Koraimann, 2010; Sysoeva et al., 2020; Thomas, 2006; Smets et al., 1993). For example, classic F plasmid is strongly repressed in the stationary phase. At the same time, broad host range plasmids RP4 and R388 appear to be efficient in self-transfer regardless of the growth phase. Most of the identified regulated plasmids though are more efficiently transferred from exponentially or fast-growing donor cells. This condition cannot be met in a biofilm environment where logarithmic growth is not feasible and thus creates more open questions on how conjugation is regulated in such bacterial populations.

Conversely, various effects of HGT on biofilm formation rates and intercellular interactions were also recorded. For example, conjugative plasmids carrying drug resistance genes confer to the cells carrying them a heightened ability to form *E. coli* biofilms (Ghigo, 2001; May & Okabe, 2008; Gama et al., 2020). It is possible that presence of conjugative pili on these bacteria allow for additional adhesion properties that can play role in initial biofilm nucleation events or at the biofilm maturation stage. In addition, *Vibrio cholerae* was shown to have interconnected regulation between biofilm formation via quorum sensing and surface attachment and intercellular competition via Type 6 secretion system (T6SS) (Borgeaud et al., 2015)

**Microgravity Effects on HGT**

In 2017, a scientific report by Lawrence Livermore National Laboratory (Urbaniak et al., 2018) indicated that, based on the antibiotic resistance genes found shared in their study which used different bacterial species, HGT may be occurring in ISS organisms. It is also expected from first principles of microbial interactions. To compare how HGT in space differs from that on Earth, several studies addressed effect of microgravity. In 2007 the Mobilisatsia/Plasmida (Мобилизация/Плазмида) experiment flown in the Soyuz module garnered several measurements for conjugation in gram-positive bacteria and it was noted that plasmid exchange occurs more readily in microgravity than in-ground experiments (Boever, et al., 2007). However, the same could not be said for experiments with the tested gram-negative organisms, which contained a similar organism to those used in biofilm experiments for in-space water recovery systems, *Cupriavidus metallidurans* (Diaz et al., 2019). A few years later a group, related to the NASA Genelab, went on to present bioinformatic analysis indicating HGT activity in the ISS microbial communities based on metagenomic analyses of the curated samples (Bense et al., 2019). Most recently (Urbaniak et al., 2021), a study was published indicating that microgravity has an overall positive impact on HGT, and for the first time demonstrated a higher efficiency of HGT in gram-negative bacteria.

Various biofilm aspects change in microgravity. For example, the EPS production by *Micrococcus luteus* has been observed to increase (Mauclaire & Egli, 2010) but the correlation to HGT rates has not been studied. Although there is an increased knowledge of microgravity effects on HGT, the data on the metagenomes affected by microgravity, and the causative agent for the positive impact of spaceflight on HGT are scarce.

|  |
| --- |
|  **Table 1. Factors that affect/are affected by HGT as observed by transcriptomic studies.** |
| **Condition** | Ground | Microgravity |
| **Metabolism** | There are transferomes of mobile metabolically relevant genes that lead to prokaryotic evolution and metabolism enrichment which aid in species survival (Whitaker, McConkey & Westhead, 2009).  | Changes in cell metabolism have been observed in microgravity through our time (Zea et al., 2017; Aunins et al., 2018; Su et al., 2021). |
| **Proliferation** | HGT affects bacterial proliferation, as seen in toxin-antitoxin studies, which have affected bacterial and phage proliferation (Bustamante, Tello & Orellana, 2014; Song & Wood 2020), but other changes have been studied as well in association with HGT. Transformation and conjugation have been observed to occur after growing cells divide and before they move onto a nongrowing phase (Headd & Bradford, 2020; Utnes et al., 2015) | Growth curve study results of bacterial cells under microgravity are varied, with some finding no changes, and some finding changes in log/lag ratios, and some speculation on method of microgravity simulation influencing the results (reviewed in Huang et al., 2018). |

**Microgravity Biofilms in Relationship to HGT and Antimicrobial Resistance**

Metabolism and growth affect HGT under normal gravity and studies in microgravity show significantly altered growth and metabolism (Table 1). This combination argues that microgravity should also strongly affect HGT, but it is unclear whether it will be increased as found in the preliminary studies described above. In addition, diversity of HGT modes and regulation mechanisms certainly argues that the observed increase of HGT rates in microgravity might not be generalizable and has to be assessed for the microorganisms of interest on a case-by-case basis. By learning the conditions and transcriptomic changes induced by microgravity in biofilms of HGT-capable cells we will identify the causes for the observed HGT increase (or on occasion, static state). By understanding the cause for HGT change, more targeted approaches can be engineered to tackle the challenges to the astronaut health and space colonization, that are posed by the HGT spread phenotypic trends, such as antibiotic resistance, biofilm formation, and virulence factors. The following sections outline three directions that can take us further in assessing and addressing HGT in microgravity biofilms and associated phenotypes of antibiotic resistance. Those are also summarized in Table 2.

|  |
| --- |
|  **Table 2. Knowledge Gaps** |
| Genotypes and transcriptome associated with changes in HGT under microgravity |
| 1. Plasmidome of current space habitats such as the ISS
 |
| 1. Resistome and its feasible monitoring methods
 |
| 1. Activity of other HGT modes like DNA uptake or transduction
 |
| 1. Treatments for in space heightened antimicrobial resistance and virulence through either biochemical or biotechnological means. *E.g.*, novel drugs or transformation and conjugation as means of probiotic delivery.
 |
| 1. Single species and multispecies community differences in the above gaps.
 |

**Plasmid Transcriptome in Microgravity**

Plasmids in biofilms are known to be conserved due to the low metabolic rate of cells living in these communities (Roder, 2021). In plasmids, although the molecular structure remains complete, the transcribed genes may be different depending on the conditions under which they are living. In space, gravity and radiation are both factors that can affect biophysical systems. Transcriptomic studies in microgravity have been performed, that point to overexpression of genes related to reduced extracellular mass transport (Zea et al., 2016) as an explanation for some bacterial behavioral changes in space that may impact crewed missions. Some of these are, among others, increased antibiotic resistance and virulence. Reduced transport is associated with cell starvation and supports the hypothesis of limited transport of nutrients and waste in microgravity, to a diffusion-only model. However, despite limitations of molecular transport, the impact on HGT is not understood under conditions of spaceflight (Boever et al., 2007; Urbaniak et al., 2021).

Other transcriptomic studies in space (Aunins et al, 2018) have found that bacterial cells showed an increased expression of stress response genes, and that this overexpression is related to the increased antibiotic resistance of microorganisms in space. This is of high importance because medical attention during spaceflight is limited by flight payloads and crews cannot depend on a broad range of antibiotics being available. As it is known and studied on Earth, antibiotic resistance genes are generally transferred by means of HGT. A scenario in which both HGT genes and stress response pathways have increased transcription rates would be detrimental to human space health. The combination of all resistance genes is defined as the resistome. Some assessments related to the ISS resistome are discussed in the next section. Thus, with the current knowledge that microgravity strongly alters metabolic and stress pathways, one can conclude that HGT has to be affected as well, consistent with the few available HGT rates in space measurements. ***Therefore, the transcriptional changes to HGT-related genes in microgravity have to be assessed. This will be the first step in gaining understanding of HGT rates in microgravity biofilms.***

**Antibiotic Resistance in Microgravity**

Biofilm environment, HGT, and stress responses of bacteria in microgravity are intrinsically related to bacterial antibiotic resistance. One type of resistance is considered to be phenotypic and develops without acquiring or developing a true antibiotic resistance gene, as described in the mid-20th century (Hobby, Meyer & Chaffee, 1942; Bigger, 1944). This resistance is thought to develop through the slow metabolic state of so called ‘persister’ cells (Balaban et al., 2019). The cells achieve this by surviving treatment in a dormant state, and they have been described as:

1. having minimal active cellular processes (Barrett et al., 2019)
2. SOS response inducing (Barrett et al., 2019)
3. phenotypically resistant (Fisher et al., 2017)
4. existing, in the majority, within biofilms (Singh et al., 2009; Lewis, 2010)
5. being precursors to antibiotic resistance by providing a reservoir of viable cells that can increase survival and mutation rates (Windels et al., 2019)
6. drug-tolerant (Cabanos and Hata, 2021)

The existence of persister cells within biofilms complicates the surveillance of antibiotic resistance, as precursory resistance cannot be genotypically quantified and their phenotypes may be similar to cells already carrying resistance genes. Redundant mechanisms related to cellular SOS response have been found in biofilm formation, mutagenesis, and HGT (Podlesek and Bertok, 2020). The survivability of cells through a treatment may increase the chances of having horizontal gene transfer in recipient cells. ***However, stress response genes have been observed to have higher rates of transcription in space (Aunins et al., 2018), and while the trigger for persister cell formation is linked to nutrient limitation stresses (Amato et al., 2013), the rate of persister cell formation in microgravity has not been fully understood.*** Aside from this, when it comes to space travel, systems may endure dormant periods (Zea et al., 2020) without crew operations during which cells may persist through time and treatments. Examples of biological endurance previously observed in the space environment are bacterial and fungal spores, and plant seeds (Novikova et al., 2015). Another participant in the development of the resistome is EPS. When bacteria form biofilms, the matrix EPS contains polysaccharides, proteins, eDNA, lipids. These EPS are involved in adhesion, aggregation (Das, Sehar & Manefield, 2010), and keeping the biofilm structure, but also in preserving cells and symbiotic life within the biofilm (Balcazar et al., 2015). It also provides protection from small chemicals assaults such as penetration of antibiotics resulting in phenotypic resistance of the biofilm subpopulation. Other chemistries have been observed to influence the resistome, such as lower organic carbon, leading to abundance of bacterial antibiotic resistance genes and maintenance of bacterial antibiotic resistome (Wan et al., 2019). Space systems such as the Water Processor Assembly (WPA), where lower organic carbon is sought in an effort to purify water for consumption, also employs a variety of filters that have been found to host biofilms (Weir et al., 2012). In summary, many studies have pointed to abundant existence of antibiotic resistance in ISS-populating microorganisms. It occurs through genotypic resistance such as carrying resistance genes on chromosome or plasmids, that are often mobile; or through phenotypic resistance that comes from biofilm or persister formation (Yang et al., 2021).

**Biotechnological Challenges and Opportunities**

Long-duration missions outside our planet will face the challenges of deteriorating astronaut health (as previously mentioned), which heightens the need to understand a space system’s resistome, virulome, and how to combat them. The challenges of medicine in space are increased by the restrictions of the mission payloads and ground assistance (Duda et al., 2017). Because of this, detection devices, illness prevention, and treatments are all under the constraints of their mass and volume. Science in space also faces its own challenges, *e.g.* the methods for transcriptome sequencing *in situ*. Such sequencing includes complex RNA extraction procedures, large number of instruments and reagents, and elaborate computational analysis. All of these steps could benefit from simplified pipelines (Parra et al., 2017). If multidrug resistant pathogens were to arise during a faraway long-duration mission, treatment and identification methods would be scarce without available ground support. The efforts of maintaining a healthy human microbiome are critical (Voorhies et al., 2019) as it was shown that (1) astronaut’s immune system weakens due to a variety of physiological changes in the human body in space, and (2) the physicochemical properties of available drugs change after a certain duration in spaceflight (Agota et al., 2021). Aside from tackling those challenges, there are also opportunities for biotechnological applications in microgravity. Such opportunities are in, for example, synthetic biology for nutrient production utilizing *in situ* resources (ISRU), which are already underway (Ball et al., 2020). Another example of the space environment harboring opportunities is found in the enhanced recombinant protein production under microgravity. This enhancement can represent a cost-effective path to industrial production of proteins of interest (Chen, Li & Liu, 2021). Finally, enhanced HGT in space, if present, can potentially lead to improved treatments using probiotic plasmids for combating resistance (Lazdins et al., 2020).

**Conclusions**

Conjugation, transformation, and transduction related activity in microgravity is not fully understood. Studying the changes that the space environment causes on HGT is of importance to crew health and space missions. Aside, from helping in crewed space exploration, HGT knowledge may serve in developing in-space manufacturing and production.

**References**

Abe, K., Nomura, N., & Suzuki, S. (2020). Biofilms: hot spots of horizontal gene transfer (HGT) in aquatic environments, with a focus on a new HGT mechanism. FEMS microbiology ecology, 96(5), fiaa031. <https://doi.org/10.1093/femsec/fiaa031>

Amato SM, Orman MA, Brynildsen MP. Metabolic control of persister formation in Escherichia coli. Mol Cell. 2013 May 23;50(4):475-87. doi: 10.1016/j.molcel.2013.04.002. Epub 2013 May 9. PMID: 23665232.

Aunins, T. R., Erickson, K. E., Prasad, N., Levy, S. E., Jones, A., Shrestha, S., Mastracchio, R., Stodieck, L., Klaus, D., Zea, L., & Chatterjee, A. (2018). Spaceflight Modifies Escherichia coli Gene Expression in Response to Antibiotic Exposure and Reveals Role of Oxidative Stress Response. Frontiers in microbiology, 9, 310. <https://doi.org/10.3389/fmicb.2018.00310>

Babic, A., Berkmen, M. B., Lee, C. A., & Grossman, A. D. (2011). Efficient Gene Transfer in Bacterial Cell Chains. mBio, 2(2). doi:10.1128/mbio.00027-11

Balaban, N. Q., Helaine, S., Lewis, K., Ackermann, M., Aldridge, B., Andersson, D. I., … Zinkernagel, A. (2019). Definitions and guidelines for research on antibiotic persistence. Nature Reviews Microbiology. doi:10.1038/s41579-019-0196-3

Balcázar, J. L., Subirats, J., & Borrego, C. M. (2015). The role of biofilms as environmental reservoirs of antibiotic resistance. Frontiers in microbiology, 6, 1216. <https://doi.org/10.3389/fmicb.2015.01216>

Ball, N., Hogan, J., Hindupur, A., Kagawa, H., Levri, J., Sims, K. (2020) BioNutrients-1: Development of an On-Demand Nutrient Production System for Long-Duration Missions. 50th International Conference on Environmental Systems. Online. <https://hdl.handle.net/2346/86343>

Barrett, T.C., Mok, W.W.K., Murawski, A.M. et al. (2019) Enhanced antibiotic resistance development from fluoroquinolone persisters after a single exposure to antibiotic. Nat Commun 10, 1177 (2019). https://doi.org/10.1038/s41467-019-09058-4

Bense, N., Singh, N., Lee M., Beheshti, A., Cekanaviciute, E., Costes, S., Galazka, J.M., Venkateswaran, K. (2019) NASA GeneLab Computomics Reveal Horizontal Gene Transfer on International Space Station Environmental Metagenomes. American Society for Gravitational and Space Research (ASGSR). (2019) Denver, CO.[https://ntrs.nasa.gov/searc h?q=genelab%20HGT](https://ntrs.nasa.gov/search?q=genelab%20HGT)

Bigger, J. (1944). TREATMENT OF STAPHYLOCOCCAL INFECTIONS WITH PENICILLIN BY INTERMITTENT STERILISATION. The Lancet, 244(6320), 497–500. doi:10.1016/s0140-6736(00)74210-3

Borgeaud, S., Metzger, L. C., Scrignari, T., & Blokesch, M. (2015). The type VI secretion system of Vibrio cholerae fosters horizontal gene transfer. Science, 347(6217), 63–67. doi:10.1126/science.1260064

Bustamante, P., Tello, M., & Orellana, O. (2014). Toxin-antitoxin systems in the mobile genome of Acidithiobacillus ferrooxidans. PloS one, 9(11), e112226. https://doi.org/10.1371/journal.pone.0112226

Cabanos, H. F., & Hata, A. N. (2021). Emerging Insights into Targeted Therapy-Tolerant Persister Cells in Cancer. Cancers, 13(11), 2666. <https://doi.org/10.3390/cancers13112666>

Chen, X., Li, C., & Liu, H. (2021). Enhanced Recombinant Protein Production Under Special Environmental Stress. Frontiers in Microbiology, 12, 512. <https://doi.org/10.3389/fmicb>. 2021.630814

Das T, Sehar S, Manefield M. (2013) The roles of extracellular DNA in the structural integrity of extracellular polymeric substance and bacterial biofilm development. Environ Microbiol Rep. 2013 Dec;5(6):778-86. doi: 10.1111/1758-2229.12085. Epub 2013 Jul 25. PMID: 24249286.

De Boever, P., Mergeay, M., Ilyin, V. et al. (2007) Conjugation-mediated plasmid exchange between bacteria grown under space flight conditions. Microgravity Sci. Technol 19, 138. <https://doi.org/10.1007/BF02919469>

Diaz, A., Li, W., Calle, L., Callahan, M., Irwin, T. (2019) Investigation of Biofilm Formation and Control for Spacecraft - An Early Literature Review. 49th International Conference on Environmental Systems 2019. Boston, MA. <https://ttu-ir.tdl.org/handle/2346/84479>

Duda, Z., J. Gaffney, C. Graves, Q. Moore, J. Watkins, & J. Nagel. (2017). Medical sterilization system for NASA Space Exploration Missions. 2017 Systems and Information Engineering Design Symposium (SIEDS), 277–282. https://doi.org/10.1109/SIEDS.2017.7937731

Fisher RA, Gollan B, Helaine S. (2017) Persistent bacterial infections and persister cells. Nat Rev Microbiol. 2017 Aug;15(8):453-464. doi: 10.1038/nrmicro.2017.42. Epub 2017 May 22. PMID: 28529326.

Flemming, HC., Wingender, J. The biofilm matrix. Nat Rev Microbiol 8, 623–633 (2010). <https://doi.org/10.1038/nrmicro2415>

Frost LS, Koraimann G. Regulation of bacterial conjugation: balancing opportunity with adversity. Future Microbiol. 2010 Jul;5(7):1057-71. doi: 10.2217/fmb.10.70. PMID: 20632805.

Gama, J. A., Fredheim, E. G. A., Cléon, F., Reis, A. M., Zilhão, R., & Dionisio, F. (2020). Dominance Between Plasmids Determines the Extent of Biofilm Formation. Frontiers in Microbiology, 11, 2070. <https://doi.org/10.3389/fmicb.2020.02070>

Ghigo, JM. Natural conjugative plasmids induce bacterial biofilm development. Nature 412, 442–445 (2001). <https://doi.org/10.1038/35086581>

Goodman, S., Obergfell, K., Jurcisek, J. et al. (2011) Biofilms can be dispersed by focusing the immune system on a common family of bacterial nucleoid-associated proteins. Mucosal Immunol 4, 625–637 (2011). <https://doi.org/10.1038/mi.2011.27>

Hausner, M., & Wuertz, S. (1999). High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. Applied and environmental microbiology, 65(8), 3710–3713. <https://doi.org/10.1128/AEM.65.8.3710-3713.1999>

Headd, B., & Bradford, S. A. (2020). The Conjugation Window in an Escherichia coli K-12 Strain with an IncFII Plasmid. Applied and environmental microbiology, 86(17), e00948-20. <https://doi.org/10.1128/AEM.00948-20>

Hobby GL, Meyer K, Chaffee E. Observations on the Mechanism of Action of Penicillin. Proceedings of the Society for Experimental Biology and Medicine. 1942;50(2):281-285. doi:10.3181/00379727-50-13773

Hu, X., Kang, F., Yang, B., Zhang, W., Qin, C., & Gao, Y. (2019). Extracellular Polymeric Substances Acting as a Permeable Barrier Hinder the Lateral Transfer of Antibiotic Resistance Genes. Frontiers in Microbiology, 10, 736. <https://doi.org/10.3389/fmicb.2019.00736>

Huang, B., Li, DG., Huang, Y. et al. (2018) Effects of spaceflight and simulated microgravity on microbial growth and secondary metabolism. Military Med Res 5, 18 (2018). <https://doi.org/10.1186/s40779-018-0162-9>

Kohler, V., Keller, W., & Grohmann, E. (2019). Regulation of Gram-Positive Conjugation. Frontiers in Microbiology, 10, 1134. <https://doi.org/10.3389/fmicb.2019.01134>

Król JE, Nguyen HD, Rogers LM, Beyenal H, Krone SM, Top EM. Increased transfer of a multidrug resistance plasmid in Escherichia coli biofilms at the air-liquid interface. Appl Environ Microbiol. 2011 Aug;77(15):5079-88. doi: 10.1128/AEM.00090-11. Epub 2011 Jun 3. PMID: 21642400; PMCID: PMC3147451.

Król, J. E., Wojtowicz, A. J., Rogers, L. M., Heuer, H., Smalla, K., Krone, S. M., & Top, E. M. (2013). Invasion of E. coli biofilms by antibiotic resistance plasmids. Plasmid, 70(1), 110–119. doi:10.1016/j.plasmid.2013.03.003

Lazdins A, Maurya AP, Miller CE, Kamruzzaman M, Liu S, et al. (2020) Potentiation of curing by a broad-host-range self-transmissible vector for displacing resistance plasmids to tackle AMR. PLOS ONE 15(1): e0225202. <https://doi.org/10.1371/journal.pone.0225202>

Lewis, K. (2010). Persister Cells. Annual Review of Microbiology, 64(1), 357–372. <https://doi.org/10.1146/annurev.micro.112408.134306>

Lopatkin, A. J., Sysoeva, T. A., & You, L. (2016). Dissecting the effects of antibiotics on horizontal gene transfer: Analysis suggests a critical role of selection dynamics. BioEssays, 38(12), 1283–1292. doi:10.1002/bies.201600133

Madsen, J. S., Burmølle, M., Hansen, L. H., & Sørensen, S. J. (2012). The interconnection between biofilm formation and horizontal gene transfer. FEMS Immunology & Medical Microbiology, 65(2), 183–195. doi:10.1111/j.1574-695x.2012.00960.x

Mauclaire L, Egli M. (2010) Effect of simulated microgravity on growth and production of exopolymeric substances of Micrococcus luteus space and earth isolates. FEMS Immunol Med Microbiol. 2010 Aug;59(3):350-6. doi: 10.1111/j.1574-695X.2010.00683.x. Epub 2010 Apr 14. PMID: 20482631.

May, T., & Okabe, S. (2008). Escherichia coli Harboring a Natural IncF Conjugative F Plasmid Develops Complex Mature Biofilms by Stimulating Synthesis of Colanic Acid and Curli. Journal of Bacteriology, 190(22), 7479–7490. doi:10.1128/jb.00823-08

Nolan LM, Turnbull L, Katrib M, Osvath SR, Losa D, Lazenby JJ, Whitchurch CB. Pseudomonas aeruginosa is capable of natural transformation in biofilms. Microbiology (Reading). 2020 Oct;166(10):995-1003. doi: 10.1099/mic.0.000956. PMID: 32749953; PMCID: PMC7660920.

Novikova, N., Deshevaya, E., Levinskikh, M., Polikarpov, N., Poddubko, S., Gusev, O., & Sychev, V. (2015). Study of the effects of the outer space environment on dormant forms of microorganisms, fungi and plants in the “Expose-R” experiment. International Journal of Astrobiology, 14(01), 137–142. doi:10.1017/s1473550414000731

Parra, M., Jung, J., Boone, T. D., Tran, L., Blaber, E. A., Brown, M., Chin, M., Chinn, T., Cohen, J., Doebler, R., Hoang, D., Hyde, E., Lera, M., Luzod, L. T., Mallinson, M., Marcu, O., Mohamedaly, Y., Ricco, A. J., Rubins, K., … Almeida, E. A. C. (2017). Microgravity validation of a novel system for RNA isolation and multiplex quantitative real time PCR analysis of gene expression on the International Space Station. PLOS ONE, 12(9), e0183480. <https://doi.org/10.1371/journal.pone.0183480>

Podlesek, Z., & Žgur Bertok, D. (2020). The DNA Damage Inducible SOS Response Is a Key Player in the Generation of Bacterial Persister Cells and Population Wide Tolerance. *Frontiers in Microbiology*, *11*, 1785.<https://doi.org/10.3389/fmicb.2020.01785>

Røder, H.L., Trivedi, U., Russel, J. et al. Biofilms can act as plasmid reserves in the absence of plasmid specific selection. npj Biofilms Microbiomes 7, 78 (2021). https://doi.org/10.1038/s41522-021-00249-w

Rooney, L.M., Amos, W.B., Hoskisson, P.A. et al. Intra-colony channels in E. coli function as a nutrient uptake system. ISME J 14, 2461–2473 (2020). <https://doi.org/10.1038/s41396-020-0700-9>

Singh R, Ray P, Das A, Sharma M. (2009) Role of persisters and small-colony variants in antibiotic resistance of planktonic and biofilm-associated Staphylococcus aureus: an in vitro study. J Med Microbiol. 2009 Aug;58(Pt 8):1067-1073. doi: 10.1099/jmm.0.009720-0. Epub 2009 Jun 15. PMID: 19528167.

Simon Á, Smarandache A, Iancu V, Pascu ML. (2021) Stability of Antimicrobial Drug Molecules in Different Gravitational and Radiation Conditions in View of Applications during Outer Space Missions. Molecules. 2021 Apr 12;26(8):2221. doi: 10.3390/molecules26082221. PMID: 33921448; PMCID: PMC8069917.

Smets B F, Rittmann B E, & Stahl D A. (1993). The specific growth rate of Pseudomonas putida PAW1 influences the conjugal transfer rate of the TOL plasmid. Applied and Environmental Microbiology, 59(10), 3430–3437. <https://doi.org/10.1128/aem.59.10.3430-3437.1993>

Song, S., & Wood, T. K. (2020). A Primary Physiological Role of Toxin/Antitoxin Systems Is Phage Inhibition. Frontiers in Microbiology, 11, 1895. <https://doi.org/10.3389/fmicb.2020.01895>

Stalder, T., Top, E. Plasmid transfer in biofilms: a perspective on limitations and opportunities. npj Biofilms Microbiomes 2, 16022 (2016). <https://doi.org/10.1038/npjbiofilms.2016.22>

Sysoeva, T. A., Kim, Y. L., Rodriguez, J., Lopatkin, A. J., & You, L. (2019). Growth‐Stage‐Dependent Regulation of Conjugation. AIChE Journal. doi:10.1002/aic.16848

Su, X., Guo, Y., Fang, T., Jiang, X., Wang, D., Li, D., Bai, P., Zhang, B., Wang, J., & Liu, C. (2021). Effects of Simulated Microgravity on the Physiology of Stenotrophomonas maltophilia and Multiomic Analysis. Frontiers in Microbiology, 12, 2393. <https://doi.org/10.3389/fmicb.2021.701265>

Thomas, C. M. (2006). Transcription regulatory circuits in bacterial plasmids. Biochemical Society Transactions, 34(6), 1072–1074. doi:10.1042/bst0341072

Urbaniak C, Grams T, Mason CE, Venkateswaran K. Simulated Microgravity Promotes Horizontal Gene Transfer of Antimicrobial Resistance Genes between Bacterial Genera in the Absence of Antibiotic Selective Pressure. Life (Basel). 2021 Sep 13;11(9):960. doi: 10.3390/life11090960. PMID: 34575109; PMCID: PMC8468678.

Urbaniak, C., Sielaff, A.C., Frey, K.G. et al. (2018) Detection of antimicrobial resistance genes associated with the International Space Station environmental surfaces. Sci Rep 8, 814 (2018). <https://doi.org/10.1038/s41598-017-18506-4>

Utnes, A. L., Sørum, V., Hülter, N., Primicerio, R., Hegstad, J., Kloos, J., Nielsen, K. M., & Johnsen, P. J. (2015). Growth phase-specific evolutionary benefits of natural transformation in Acinetobacter baylyi. The ISME journal, 9(10), 2221–2231. <https://doi.org/10.1038/ismej.2015.35>

Virolle, C., Goldlust, K., Djermoun, S., Bigot, S., & Lesterlin, C. (2020). Plasmid Transfer by Conjugation in Gram-Negative Bacteria: From the Cellular to the Community Level. Genes, 11(11), 1239. doi:10.3390/genes11111239

Voorhies, A.A., Mark Ott, C., Mehta, S. et al. (2019) Study of the impact of long-duration space missions at the International Space Station on the astronaut microbiome. Sci Rep 9, 9911 (2019). https://doi.org/10.1038/s41598-019-46303-8

Wan, K., Zhang, M., Ye, C., Lin, W., Guo, L., Chen, S., & Yu, X. (2019). Organic carbon: An overlooked factor that determines the antibiotic resistome in drinking water sand filter biofilm. Environment International, 125, 117–124. <https://doi.org/10.1016/j.envint.2019.01.054>

Wang, L., Li, Y., Wang, L., Zhang, H., Zhu, M., Zhang, P., et al. (2017). Extracellular polymeric substances affect the responses of multi-species biofilms in the presence of sulfamethizole. Environ. Pollut. 235, 283–292. doi: 10.1016/j.envpol.2017.12.060

Weir, N., Wilson, M., Yoets, A., Molina, T., Bruce, R., & Carter, L. (2012). Microbiological Characterization of the International Space Station Water Processor Assembly External Filter Assembly S/N 01. 42nd International Conference on Environmental Systems. doi:10.2514/6.2012-3595

Whitaker, J. W., McConkey, G. A., & Westhead, D. R. (2009). The transferome of metabolic genes explored: analysis of the horizontal transfer of enzyme encoding genes in unicellular eukaryotes. Genome biology, 10(4), R36. <https://doi.org/10.1186/gb-2009-10-4-r36>

Windels, E.M., Michiels, J.E., Fauvart, M. et al. (2019) Bacterial persistence promotes the evolution of antibiotic resistance by increasing survival and mutation rates. ISME J 13, 1239–1251 (2019). <https://doi.org/10.1038/s41396-019-0344-9>

Yang, J., Barrila, J., Mark Ott, C. et al. (2021) Longitudinal characterization of multispecies microbial populations recovered from spaceflight potable water. npj Biofilms Microbiomes 7, 70 (2021). <https://doi.org/10.1038/s41522-021-00240-5>

Zea, L., Larsen, M., Estante, F., Qvortrup, K., Moeller, R., Dias de Oliveira, S., … Klaus, D. (2017). Phenotypic Changes Exhibited by E. coli Cultured in Space. Frontiers in Microbiology, 8. doi:10.3389/fmicb.2017.01598

Zea, L., McLean, R. J. C., Rook, T. A., Angle, G., Carter, D. L., Delegard, A., Denvir, A., Gerlach, R., Gorti, S., McIlwaine, D., Nur, M., Peyton, B. M., Stewart, P. S., Sturman, P., & Velez Justiniano, Y. A. (2020). Potential biofilm control strategies for extended spaceflight missions. Biofilm, 2, 100026. <https://doi.org/10.1016/j.bioflm.2020.100026>

Zea, L., Nisar, Z., Rubin, P., Cortesão, M., Luo, J., McBride, S. A., Moeller, R., Klaus, D., Müller, D., Varanasi, K. K., Muecklich, F., & Stodieck, L. (2018). Design of a spaceflight biofilm experiment. Acta astronautica, 148, 294–300. <https://doi.org/10.1016/j.actaastro.2018.04.039>

Zea L, Prasad N, Levy SE, Stodieck L, Jones A, et al. (2016) A Molecular Genetic Basis Explaining Altered Bacterial Behavior in Space. PLOS ONE 11(11): e0164359. <https://doi.org/10.1371/journal.pone.0164359>