Scientific Research Publishing

Advances in Microbiology, 2023, 13, 539-558
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Analysis of Growth Characteristics and Differentially Expressed Homologous Genes in *Rhodobacter sphaeroides* under Normal and Simulated Microgravity Conditions

Weerakkody Ranasinghe[1], Eduardo Gutierrez[1], Zelaya Alyson[1], Sabrina Vazquez[1], Ashleigh Ogg[1], Rajesh Prabhu Balaraman[1], Hyuk Cho[2], Madhusudan Choudhary[1*]

[1]Department of Biological Sciences, Sam Houston State University, Huntsville, Texas, USA
[2]Department of Computer Science, Sam Houston State University, Huntsville, Texas, USA
Email: *mchoudhary@shsu.edu

How to cite this paper: Ranasinghe, W., Gutierrez, E., Alyson, Z., Vazquez, S., Ogg, A., Balaraman, R.P., Cho, H. and Choudhary, M. (2023) Analysis of Growth Characteristics and Differentially Expressed Homologous Genes in *Rhodobacter sphaeroides* under Normal and Simulated Microgravity Conditions. *Advances in Microbiology*, **13**, 539-558.
https://doi.org/10.4236/aim.2023.1311035

Received: October 12, 2023
Accepted: November 27, 2023
Published: November 30, 2023

Abstract

The term "microgravity" is used to describe the "weightlessness" or "zero-g" circumstances that can only be found in space beyond earth's atmosphere. *Rhodobacter sphaeroides* is a gram-negative purple phototroph, used as a model organism for this study due to its genomic complexity and metabolic versatility. Its genome has been completely sequenced, and profiles of the differential gene expression under aerobic, semi-aerobic, and photosynthetic conditions were examined. In this study, we hypothesized that *R. sphaeroides* will show altered growth characteristics, morphological properties, and gene expression patterns when grown under simulated microgravity. To test that, we measured the optical density and colony-forming units of cell cultures grown under both microgravity and normal gravity conditions. Differences in the cell morphology were observed using scanning electron microscopy (SEM) images by measuring the length and the surface area of the cells under both conditions. Furthermore, we also identified homologous genes of *R. spheroides* using the differential gene expression study of *Acidovorax* under microgravity in our laboratory. Growth kinetics results showed that *R. sphaeroides* cells grown under microgravity experience a shorter log phase and early stationary phase compared to the cells growing under normal gravity conditions. The length and surface area of the cells under microgravity were significantly higher confirming that bacterial cells experience altered morphological features when grown under microgravity conditions. Differentially expressed homologous gene analysis indicated that genes coding for several COG and GO functions, such as metabolism, signal-transduction,

transcription, translation, chemotaxis, and cell motility are differentially expressed to adapt and survive microgravity.

Keywords

Simulated Microgravity, Differential Gene Expression, Bacteria, Gene Homology, Space Exploration

1. Introduction

Living organisms have evolved and developed under normal gravity conditions but with the developing interest in spaceflight and space explorations, it is important to understand how living organisms are affected under simulated microgravity. Bacteria have the ability to survive under a variety of unfavorable conditions like hot water springs, volcanoes, and glaciers [1] [2]. Space has many unfavorable conditions including microgravity, high vacuum, high radiation, and heat, and no oxygen [3] [4]. All microorganisms in spacecraft, regardless they are earth-born pollutants, experimental materials, or the normal microbiome of astronauts, are exposed to the stressful effects of space. Studying the effect of microgravity on microorganisms is important due to two major reasons. First, microbes are related to the health of astronauts and microgravity may alter the physiology of commensal bacteria making them pathogenic in space [5] [6] [7]. Second, microorganisms under microgravity may produce different secondary metabolites that can have potential applications in medicine, biotechnology, or health industries [8] [9] [10].

The term "microgravity" is used to describe the "weightlessness" or "zero-g" circumstances that can only be found in space beyond earth atmosphere. The "microgravity" level typically ranges from about 10^{-3} to 10^{-6} g and depends on the location in the spacecraft and how often vibrations occur [11] [12]. Over the past several decades, substantial progress has been made in the knowledge of the effects in space ambient variables, both actual and simulated [4]. Numerous investigations have demonstrated that bacterial cells have altered physiological properties, including cell division, pathogenicity, flagellar motility, resistance to antibiotics, and altered metabolism [13] [14] [15] [16] [17]. Several elements can alter bacterial metabolism, which can bring out different adaptive strategies to meet that cellular stress under these conditions. The frontier of space travel is hostile to all types of living things, including bacteria [4]. The stressful conditions that bacterial cells encounter during actual spaceflight and in a microgravity simulator, may alter the physiological properties and cellular metabolism.

It has been reported that various bacterial species respond to the stress imposed by microgravity in different ways. Both motile and non-motile species share some similar responses; however, majority of non-motile bacterial species demonstrate increased rate of cell division under microgravity compared to motile species [18] [19] [20] [21]. Some bacterial species exhibited reduced growth,

while many bacteria show enhanced growth under microgravity [19] [22]. Some bacteria like *Stenotrophomonas maltophilia* and *Escherichia coli* respond to microgravity by altering their primary metabolism, such as carbohydrate, protein, lipid, and nucleic acid metabolisms [23] [24]. Increased biofilm formation is another common response observed in opportunistic pathogenic bacteria like *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* [5] [22] [25] to survive under microgravity. Secondary metabolite production by bacteria is also significantly impacted by microgravity but the responses are unique to the bacterial species as well as their growth conditions. These include increased production of a wide range of secondary metabolites including antibiotics like Actinomycin D [10], polymers like Poly-b-hydroxybutyrate [26] and toxins like microcystin [27]. In contrast, some bacteria exhibited reduced production of certain secondary metabolites like Microcin B17 and β-lactam antibiotics [28], and Rapamycin [29].

Two different types of experiments have been used to study how microgravity affects bacterial physiology: 1) Experiments on real spacecraft like the International Space Station (ISS), and 2) Ground-based facilities (GBFs) on earth that simulate microgravity employing Clinostats and Rotating Wall Vessels (RWV) [4] [30]. Although there are some investigations carried out in a space environment using spacecraft and the space station, it is expensive to conduct microgravity experiments in space because of logistical and technological challenges. Therefore, replicating microgravity on Earth is inexpensive and much easier using various GBFs built using several physical principles [30] [31]. This study used rotating wall vessels originally developed by the NASA Johnson Space Center (Houston, TX) to investigate simulated microgravity [32]. This device is made up of a hollow disk or cylinder that rotates on one axis perpendicular to the gravity vector and is fully filled with a liquid medium, without any bubbles [32]. Similar to space microgravity, RWV analogs work by simulating low shear modeled microgravity (LSMMG), in which cells continue to fall freely after rotation. When the RWVs spin in solid-body rotation, the cells grown in a liquid medium are under unique culture conditions, creating an environment for cell growth that is continuous low-shear and low-turbulence corresponding to the microgravity environment in space [13].

In this study, we characterized growth kinetics, cellular characteristics, and differential gene expression of homologous genes in *R. sphaeroides* grown under normal and simulated microgravity conditions.

R. sphaeroides is a Gram-negative, facultative photosynthetic bacterium. The cells of *R. sphaeroides* generate a purple film on the surface of ponds and may survive in both freshwater and saltwater. *R. sphaeroides* has expanded metabolic abilities, photosynthesis, aerobic and anaerobic respiration, nitrogen-fixation, and the production of tetrapyrroles, heme, and vitamin B12. Its complex genome consist of two chromosomes (I: 3,139,278 bp and II: 968,108 bp) and five endogenous plasmids (A-E) [33] [34] [35], and several strains of this species have been completely sequenced [36]. This purple phototroph has been widely used

as a model organism for basic biochemical genetic investigations of photochemistry, metabolism, and gene regulation, and therefore as the first study, it would provide a model organism to study the effect of microgravity due to its high metabolic versatility and genetic suitability.

In this study we hypothesized that *R. sphaeroides* will show altered growth characteristics, cellular properties and altered gene expression when grown under simulated microgravity compared to normal gravity conditions. To test that we measured the optical density and colony forming units of *R. sphaeroides* cultures grown under microgravity and normal gravity conditions. We also examined the cell morphology using scanning electron microscopy (SEM) of the cells under both conditions. Furthermore, we also identified homologous genes of *R. sphaeriodes* using the differential gene expression study of *Acidovorax* under microgravity in our laboratory (unpublished research).

2. Materials and Methods

2.1. Bacterial Strains and Growth Media

Rhodobacter sphaeroides strain 2.4.1 (NCBI taxonomy ID: 272943) was utilized for the experiments involving growth kinetics, cellular morphology, and colony forming units (cfu). The bacterial strain was grown in Sistrom-minimal-medium [37]. *R. sphaeroides* was transferred from a frozen stock onto a SIS agar plate and incubated at 30˚C for four days. Standard aseptic practices were applied while culturing and handling bacteria to avoid potential contaminations. Simulated microgravity conditions were applied to bacteria using the Synthecon Rotary Cell-Culture System developed by the NASA Johnson Space Center, Houston Texas [14] which is a Rotating Wall Vessel (RWV) analogue that works on the same principle as RWVs using a different configuration.

2.2. Growth Kinetics and Colony Forming Units

A single bacterial colony was inoculated into 10 mL of 1× SIS broth and incubated at 30˚C in a rotary shaker for 3 - 4 days in dark until bacterial growth reached at late-log phase (0.6 - 0.8 Optical Density at 600 nm). 100 µL samples were transferred from the previously grown liquid culture into two sets of four fresh culture tubes, each containing 10 mL SIS minimal media. Four culture tubes were placed in a normal rotary shaker and incubated under normal gravity condition. Another set of four samples was incubated at 30˚C in dark under simulated microgravity condition using Synthecon Rotary Cell-Culture System.

Optical density (OD) of each sample was measured at 24, 48, 72 and 96 hr. time intervals using the VIS-UV spectrophotometer. Colony forming units were counted by plating out 100 µL of diluted culture along with beads at different dilution series, 10^{-4} to 10^{-8}. Four SIS-agar plates were used to plate out each culture, and then they were incubated at 30˚C under normal gravity or microgravity conditions for 3 - 4 days. Incubated plates of 10^{-8} dilution were selected for counting the colony forming units as they had about 300 colonies on each plate. Average CFUs in the original samples under each treatment (microgravity and

normal gravity) were extrapolated and the data was used to plot the graph.

2.3. Electron Microscopy and Cell Morphology

Cell culture grown under normal gravity, or microgravity condition were transferred into multiple sterilized centrifuge tubes and centrifuged for 10 minutes at 10,000 RPM to form cell pellet. The cell pellet was extracted by removing the supernatant from tubes using sterilized pipettes. The cell pellets were initially preserved by exposure to a 3% glutaraldehyde solution for 24 hours. First, they were rinsed three times with sterile distilled water. Next, the samples were dehydrated by submerging samples successively in ethanol solutions with incremental concentrations (30%, 50%, 70%, 80%, 90%, 95%, and 99.8%). Subsequently, the samples were allowed to air-dry for 24 hours at 50˚C. To improve the quality of the imaging process, the dehydrated samples were sputter-coated with a layer of gold, resulting in the formation of a conducting film approximately 5 nanometers thick [38] [39] [40] [41] and images were taken under the SEM. All the SEM images were captured at a magnification of 3000×, accelerating voltage of 25.0 kV and a working distance of 4.7 mm at room temperature. Length and width of 150 individual cells were measured from the SEM images. The area of the cells was estimated using cell length and width data and the formulae A = π((a/2) (b/2) where a is the major axis/diameter and b is the minor axis/diameter.

2.4. Protein Homology and COGs Analysis

In another experiment, the gene expression data was recently obtained for *Acidovorax* (Strain ID: 1608163) under normal and simulated microgravity conditions (unpublished result). The gene-expression data obtained from the above experiment was used to map the homologous genes and proteins of the *R. sphaeroides*' genome.

The accession numbers of each differentially expressed gene in *Acidovorax* under microgravity were used as queries to search the corresponding proteins in *R. sphaeroides* 2.4.1 genome through the NCBI website [42].

For the protein homology search, the BLASTp was used [43]. The protein homology criteria were as follows: max Score > 100, query coverage > 50%, E-Value < 10^{-3}, and amino acid identity > 30%. The levels of gene-expressions in *Acidovorax* for the homologous genes identified in *R. sphaeroides* were used for further gene expression analysis.

The OrthoDB database [44], which is readily accessible online, was used to assign COG categories and subcategories, as well as annotate the GO functions of the homologous genes identified in *R. sphaeroides.*

3. Results and Discussion

3.1. Growth Kinetics and Colony Forming Units of *R. sphaeroides* under Normal Gravity and Microgravity Conditions

Overall, growth of bacterial cultures under microgravity had higher OD (absor-

bance at 600 nm) compared to the cultures grown under normal gravity conditions indicating a higher number of cells at each time interval (Figure 1(a)). The stationary growth phase was observed at 72 hours (about 3 days) under microgravity, whereas under normal gravity conditions, the stationary phase reached after 96 hours of incubation indicating a shorter doubling time of cells under microgravity conditions. This indicates that cells enter a short log-phase and early stationary phase when grown under microgravity whereas the cells experience a long log-phase and late-stationary phase when grown under normal gravity conditions. The result clearly shows that the growth curves at log phase as well as stationary phase under microgravity have a steeper slope compared to the respective slope observed under normal gravity, which indicates reaching a higher growth rate at both log and stationary phases under microgravity condition.

Since OD indicates absorbance of both dead and live cells, CFUs were counted as they indicate only the number of viable cells in the bacterial cultures. The number of CFUs also shows the same pattern as the observed ODs from the corresponding cultures at different time intervals. Cultures grown under microgravity showed a significantly higher amount of CFUs compared to the cultures grown under normal gravity conditions at each time interval (Figure 1(b)). Aligning with the growth kinetic results, the highest number of CFUs (viable cells) was observed after 72 hours of incubation under microgravity conditions whereas, under normal gravity conditions, the highest CFUs were observed after 96 hours of incubation validating rapid bacterial growth with faster doubling (cell cycle) time under microgravity conditions (P = 0.011, two-sample t-test).

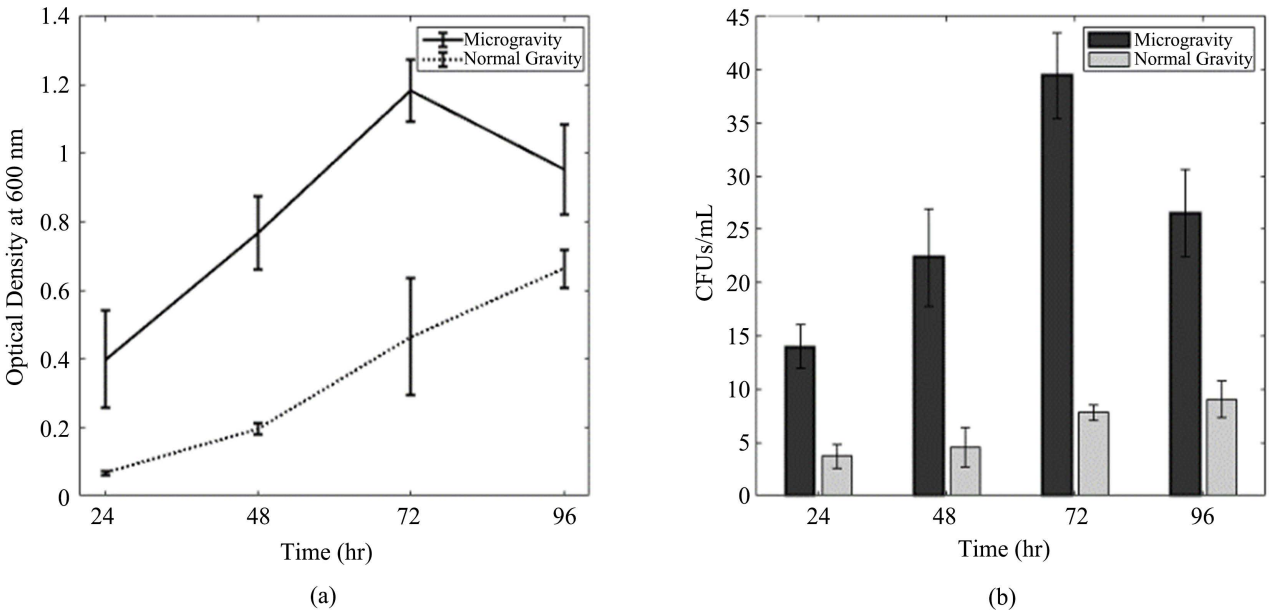

Figure 1. Growth characteristics of *Rhodobacter sphaeriodes* 2.4.1 under normal gravity and microgravity conditions. (a) Growth kinetics (OD at 600 nm) of *Rhodobacter sphaeroides* under normal gravity and microgravity conditions. (b) Colony Forming Units of *Rhodobacter sphaeroides* grown under normal gravity and microgravity condition.

These results also support the fact that cells under microgravity experience a short log-phase and early stationary phase compared to the cells growing under normal gravity conditions. Moreover, the number of viable cells at both log and stationary phases under microgravity is significantly higher than that of viable cells at the respective stages under normal gravity, which indicates microgravity has influenced the rate of cell division at both the phases. The result exhibits more viable cells at each time interval under microgravity compared to the normal gravity. However, to determine the cell survival time under microgravity, the study needs to be conducted for a prolonged time, beyond 96 hours.

Bacterial cells grown under microgravity experience diminished gravitational forces, leading to a notable absence of sedimentation, minimal shear stress, and reduced turbulence [26]. The absence of gravity-related forces and flows, such as buoyancy, sedimentation, and convection, results in the restriction of molecular movement to and from the cell primarily through diffusion [4] [13] [27]. It implies that the transfer of nutrients to cells and the removal of waste products from cells mainly rely on Brownian motion [28]. Resulted decrease in extracellular nutrient availability and the buildup of bacterial byproducts near the cell will significantly impact micro-organisms, particularly its cellular metabolism [4] [26] [28]. Our results indicated that *R. sphaeroides* experience a short log phase and early stationary phase showing a higher growth rate and cell density when grown under microgravity. Similar results have been found in various studies conducted under spaceflight microgravity conditions confirming that bacterial cells exhibit a shortened lag phase and an increase in cell density under reduced gravity conditions [28] [29] [30] [31] [32].

3.2. Scanning Electron Microscopic Observation and Analysis

The SEM images of the cells under microgravity were visually more spherical in shape and bigger in size compared to the cells grown under normal gravity where they are much smaller in size and wrinkled. Lots of actively dividing cells are visible in the images of cells under microgravity (**Figure 2(a)**) compared to the cells grown under normal gravity (**Figure 2(b)**) indicating faster bacterial cell division and reduced doubling time. To confirm these morphological differences of the cells grown under above these two conditions, cell length and cell surface area were further analyzed.

The length of the cells grown under microgravity were significantly higher than the cells grown under normal gravity ($n = 150$, $P = 1e{-}05$, two-sample t-test) supporting the visual observation of the cells been larger in size in the SEM images. Moreover, cellular surface area was also significantly higher in the cells grown under microgravity compared to the cells grown under normal gravity conditions ($n = 150$, $P = 3.7e{-}10$, two sample t-test) confirming the observed morphological changes of the SEM images. Overall, it can be concluded that the bacterial cells experience altered morphological features when grown under microgravity conditions (**Figure 3**).

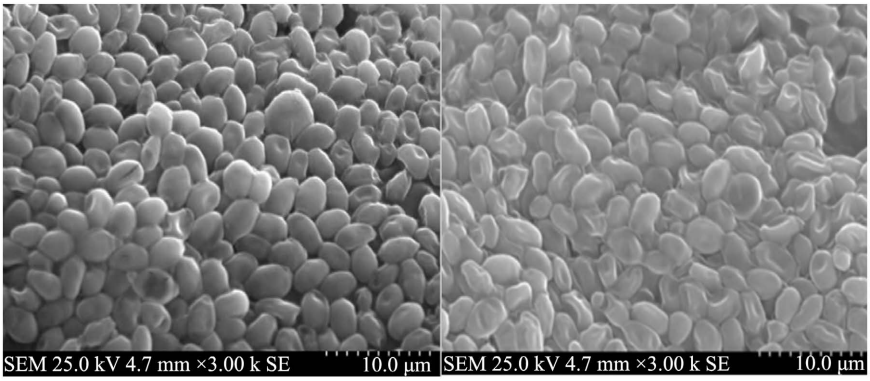

Figure 2. SEM images of *R. sphaeroides* cells. (a) Under microgravity. (b) Normal gravity.

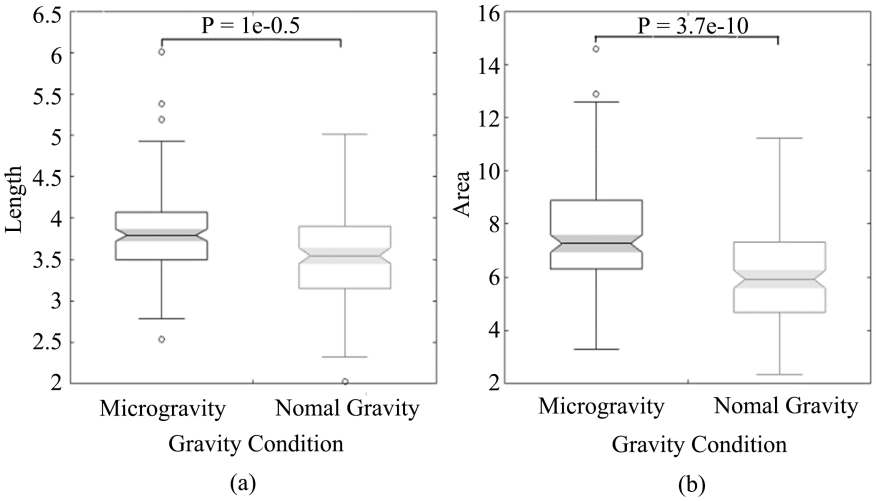

Figure 3. Cell morphology measurements of *R. sphaeroides*. (a) Box plots illustrating the cell length under normal gravity and microgravity. (b) Box plots illustrating the surface area of the cells under normal gravity and microgravity.

The enhanced growth of the cells was observed in the SEM images, showing larger cell length and surface area with more actively proliferating cells under microgravity. Several theories can be used to explain this phenomenon. One theory proposes that this phenomenon is a direct result of reduced gravity, leading to minor alterations in cellular machinery or the cell membrane [45]. These changes, in turn, affect the energy requirement of the cell and promote cell growth [46]. Another hypothesis is that the increased cell proliferation result from an indirect consequence of the absence of sedimentation under microgravity [24]. In the absence of sedimentation, bacteria can linger in proximity to their by-products within the calm environment. Among these by-products are enzymes or co-factors that could potentially promote cellular growth. Under conditions of minimal shear in microgravity, bacteria receive a continual supply of these advantageous by-products, thereby enhancing their cellular development [24]. Conversely, on Earth, cells settle away from these beneficial by-products, resulting in a lack of observable enhanced growth.

3.3. Differentially Expressed (DE) Homologous Genes in *R. sphaeroides*

To understand the gene-expression patterns, two different comparisons were made as shown in Figure 4. A comparison of normal gravity vs microgravity at the log phase, of the total 150 differentially expressed genes, 83 (55.33%) and 67 (44.67%) genes were up- and down-regulated, respectively, whereas at the stationary phase, of the total 91 differentially expressed genes, 11 (12.01%) and 80 (88.89%) genes were up- and down-regulated, respectively. Notably, there were only a few gene-functions like ABC transporters that overlap between both comparisons, however their gene IDs were different. While numbers of up- and down-regulated genes were not significantly different at the log phase of normal vs microgravity comparison, majority of genes (~89%) were down-regulated at the stationary phase of the normal vs microgravity comparison. Therefore, this result concludes that the major down-gene-regulation mechanism operates at the stationary phase in normal gravity vs microgravity comparison. In other words, most differentially regulated genes are downregulated at the stationary phase under simulated microgravity condition. It is also evident that the gene expression profiles at both the log phase and stationary phase are different under microgravity, compared to its expression under the normal gravity condition. This supports the growth kinetics results and further demonstrates that the gene expression of *R. sphaeroides* at both log and stationary phases are affected by microgravity.

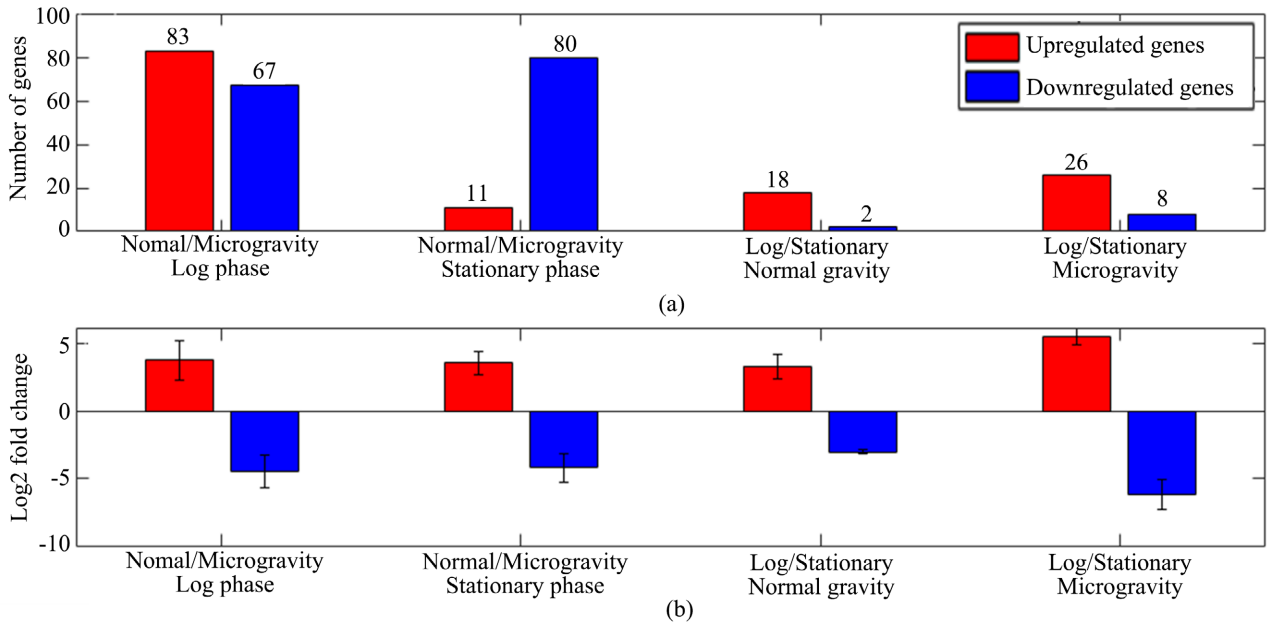

Figure 4. Differentially expressed homologous genes of *R. sphaeroides* under normal gravity and microgravity (MG) at the log- and stationary phase. (a) Number of differentially expressed genes at log- and stationary phase under normal gravity vs microgravity, and genes under normal gravity and microgravity at log vs stationary phase. (b) Log-fold expression changes of differentially expressed genes at log- and stationary phase under normal gravity vs microgravity, and genes under normal gravity and microgravity at log vs stationary phase. (c) Box plot of the log-fold expression changes of genes at log- and stationary phase under normal gravity vs microgravity, and genes under normal and microgravity at log vs stationary phase.

Another comparison was done between the log phase vs stationary phase separately for both normal and microgravity growth conditions. In the comparison of log phase vs stationary phase under normal gravity condition, of the total 20 genes, 18 (90%) and 2 (10%) were up- and downregulated respectively, while at the log vs stationary under microgravity, of the total 34 genes, 26 (76.47%) and 8 (23.53%) genes are up- and downregulated. Also, there was no overlap of up- and down-regulated genes for both comparisons, however some of specific gene-functions like ABC transporters were shared. These results clearly suggest that at log phase vs stationary phase comparison of both normal gravity and microgravity conditions, majority of differentially expressed genes were up regulated, and that demonstrates the differentially regulated genes are prevalently upregulated at both log and stationary phases of normal and microgravity growth conditions.

Comparing the log-fold expression change of the up-regulated and down-regulated genes, highest magnitude of upregulation and downregulation was observed between the log vs stationary phase comparison under microgravity suggesting that the magnitude of gene expression in *R. sphaeriodes* is highly affected by the gravity condition at both the log and stationary growth phases.

Our results demonstrated that differentially regulated genes are prevalently upregulated at the log phase under microgravity growth conditions, which validates previous findings that several genes are upregulated when grown under microgravity [27] [28] [29] [30] [31]. Since microgravity creates a starvation-stress environment inside the cell due to nutrient diffusion limitation [24], upregulation in the expression of genes involving metabolism including carbohydrates and amino acids metabolism and secondary metabolite synthesis and transport was seen in bacteria under microgravity [23]. It was also observed that most differentially expressed genes are downregulated at the stationary phase compared to the log phase under simulated microgravity condition. A study on *E. coli* claimed that the majority of the genes that are differentially expressed during the stationary phase promote long-term survival under stress [47]. This study was conducted under normal gravity and yet the nutrient deficiencies at the stationary phase were identified to cause stress and trigger the tight regulation of genes for the cell survival. Under microgravity, the stress level was much higher during the stationary phase, and this could lead to the much tight regulation of genes for the cell survival and proliferation during the stationary phase compared to the log phase.

3.4. COG Analysis of Differentially Expressed Genes in *R. sphaeroides*

COG Analysis as shown in **Figure 5** exhibits the frequency distribution of differentially expressed genes for four different comparisons: normal gravity vs microgravity at log phase, normal gravity vs microgravity at stationary phase, log phase vs stationary phase under normal gravity, and log phase vs stationary phase under microgravity.

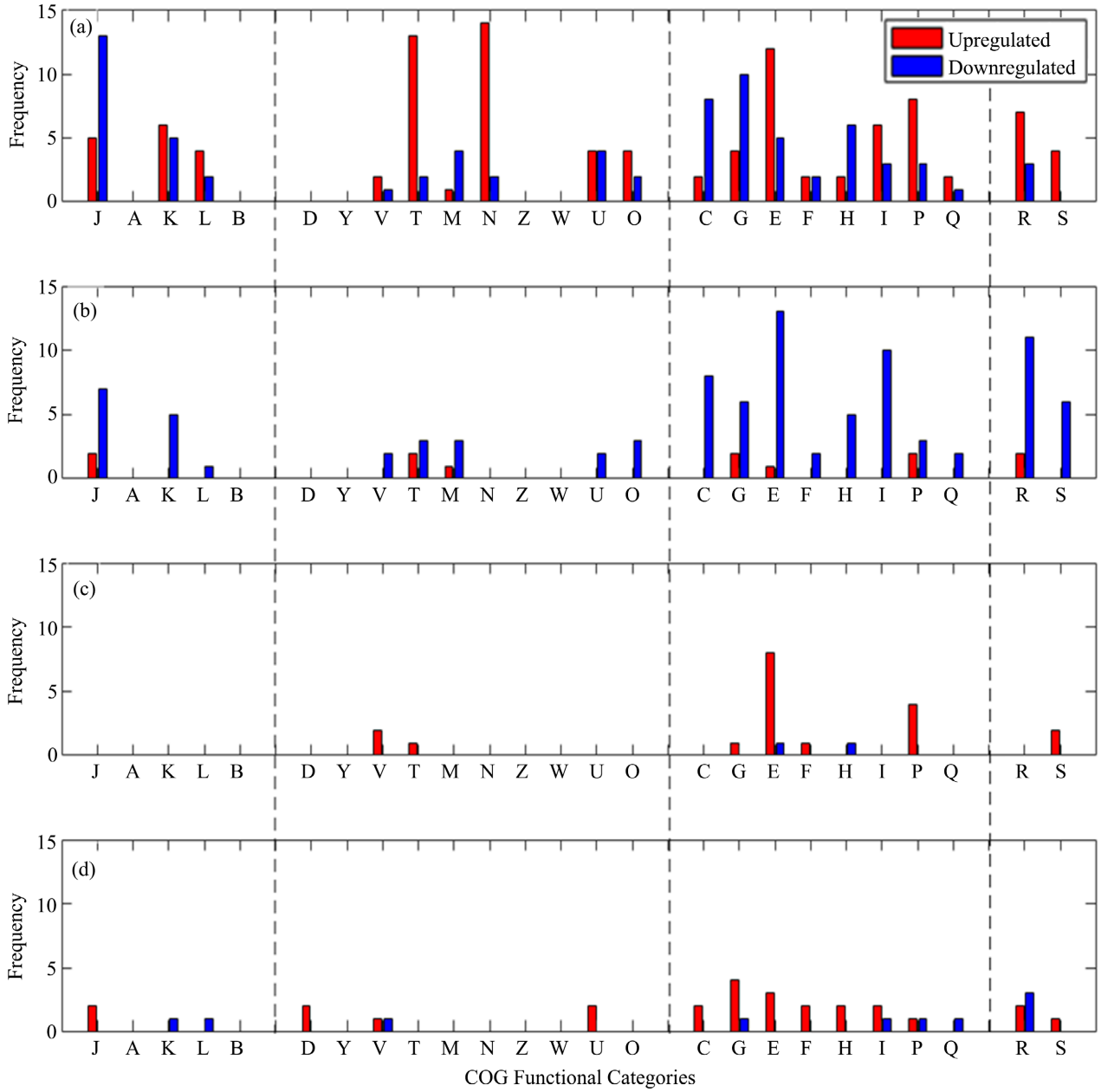

Figure 5. Distribution of Clusters of Orthologous Groups of proteins (COGs) functions of differentially expressed genes of *R. sphaeroides*. (a) At log-phase under normal gravity vs microgravity. (b) At stationary-phase under normal gravity vs microgravity. (c) In normal gravity at log phase vs stationary phase. (d) In microgravity at log-phase vs stationary phase.

In log-phase normal gravity vs microgravity, 59% of all the differentially expressed genes were upregulated whereas 41% of genes were downregulated under microgravity compared to normal gravity (**Figure 5(a)**), which suggests that microgravity affect the genes expression pattern during the growth at log phase. Differentially expressed genes belonging to information storage and processing group function was represented by three different subcategories, translation, ribosome structure and biogenesis (J), transcription (K), and DNA replication, recombination, and repair (L). Majority of the genes of the storage processing

category were downregulated (20) whereas only 14 genes were upregulated. Downregulated genes include 13 genes related to translation, ribosome structure and biogenesis (J), 05 genes of transcription (K) and 02 genes of DNA replication and repair (L). Genes that were upregulated includes three genes of DNA replication recombination and repair (L), six genes of transcription (K) and three genes of translation (J) subcategories. Cellular processes group function was represented by different subcategories, defense mechanisms (V), signal transduction mechanisms (T), cell motility and secretion (N), intracellular trafficking and secretion (U), posttranslational modification, protein turnover, chaperones (O), and cell envelope biogenesis, outer membrane (M). Most of the genes under this category were upregulated (37) whereas only 15 genes were downregulated. Signal transduction mechanisms (T) and cell motility and secretion (N) included the highest number of genes (13 in each subcategory) that were upregulated while more genes representing cell envelope biogenesis, and outer membrane (M) were downregulated. The number of up and down regulated genes of intracellular trafficking and secretion (U) remains the same. Metabolism group function was represented by different subcategories, amino acid transport and metabolism (E), lipid metabolism (I), inorganic ion transport and metabolism (P), RNA processing and modification (A), nucleotide transport and metabolism (F), energy production and conversion (C), carbohydrate transport and metabolism (G) and coenzyme metabolism (H). Overall, total number of upregulated genes (37) and the number of downregulated genes (35) did not differ significantly. The number of up and down-regulated genes in nucleotide transport and metabolism (F) were about the same. However, the more differentially expressed genes representing amino acid transport and metabolism (E), lipid metabolism (I), inorganic ion transport and metabolism (P) were upregulated, while more differentially regulated genes in energy production and conversion (C), carbohydrate transport and metabolism (G) and coenzyme metabolism (H) were down regulated.

In the stationary-phase normal vs microgravity comparison, there is a notable difference between the total number of differentially expressed genes where a majority of the differentially expressed genes were downregulated (73) and only 10 genes were up regulated under microgravity when compared with normal gravity conditions, which validates that genes expression during stationary growth phase is affected due to the microgravity environment. These genes belong to several subcategories (**Figure 5(b)**). Information storage and processing group function was represented by three different subcategories, translation, ribosome structure and biogenesis (J), transcription (K), and DNA replication, recombination, and repair (L). Most genes of these three subcategories are down regulated. Cellular processes group function was represented by different subcategories, defense mechanisms (V), signal transduction mechanisms (T), cell envelope biogenesis, outer membrane (M), intracellular trafficking and secretion (U) and posttranslational modification, protein turnover, chaperones (O). A majority of differentially expressed genes of all these above subcategories were

down regulated. Metabolism group function was represented by different subcategories, energy production and conversion (C), carbohydrate transport and metabolism (G), amino acid transport and metabolism (E), nucleotide transport and metabolism (F), coenzyme metabolism (H), lipid metabolism (I), inorganic ion transport and metabolism (P), and secondary metabolites biosynthesis, cellular transport, and catabolism (Q). Most genes of the above categories were down regulated (90%) whereas only a few genes (10%) were upregulated.

In normal gravity at log phase vs stationary phase comparison (**Figure 5(c)**), information storage and processing group function was not represented. Cellular processes group function was represented by two subcategories, defense mechanisms (V) and signal transduction mechanisms (T). All genes of both subcategories were upregulated. Metabolism group function was represented by subcategories, carbohydrate transport and metabolism (G), amino acid transport and metabolism (E), nucleotide transport and metabolism (F), and most genes of these subcategories were upregulated. Overall, most of the differentially expressed genes at normal gravity log vs stationary phase were upregulated (89%) and only a very few genes (12%) were down regulated.

In microgravity at log phase vs stationary phase comparison (**Figure 5(d)**), information storage and processing group function was represented by subcategories, translation, ribosome structure and biogenesis (J), transcription (K), and DNA replication, recombination, and repair (L). Most differentially expressed genes representing translation, ribosome structure and biogenesis (J) were upregulated while most differentially regulated genes representing transcription (K) and DNA replication, recombination, and repair (L) were down regulated. Cellular processes group function was represented by subcategories, cell division and chromosome partitioning (D), intracellular trafficking and secretion (U) and defense mechanisms (V). Most differentially regulated genes of cell division and chromosome partitioning (D), intracellular trafficking and secretion (U) were upregulated. Metabolism group function was represented by categories, energy production and conversion (C), carbohydrate transport and metabolism (G), amino acid transport and metabolism (E), nucleotide transport and metabolism (F), and coenzyme metabolism (H), lipid metabolism (I). Most of the differentially expressed genes under the above subcategories were up regulated. Overall, most of the differentially expressed genes in microgravity at log phase vs stationary phase comparison were upregulated (79%) whereas only a very few numbers of genes (21%) were downregulated.

Previous studies that studied bacterial differential expression under simulated gravity and microgravity report similar findings. Many studies reported that the majority of the total genes were upregulated under microgravity consistent with the findings of the log phase of this study [48] [49] [50] [51]. A study conducted on *Vibrio natriegens* also showed a high number of genes related to signal transduction and cell motility upregulated under microgravity conditions [52]. Bacteria employ sensory mechanisms to adjust diverse environmental conditions

using various gene-interactions, including signal transduction systems. Ultimately, these mechanisms can influence gene expression through cellular motility, allowing bacteria to adapt to changing environmental stress [53] [54]. This is supported by our results indicating an upregulation in the genes regulating signal transduction and cellular motility during the log phase. Our study also reported that the genes related to translation were downregulated during the log phase of growth under microgravity similar to the study on *Vibrio natriegens* [52]. A previous study conducted under simulated microgravity on *E. coli* K12 reported that genes controlling energy production were downregulated [45]. These results are consistent with the findings of our study suggesting that microgravity affect the expression of these genes enabling them to survive under the unfavorable condition. Another study conducted on *Stenotrophomonas maltophilia* showed up-regulation of genes responsible for carbohydrates and amino acid metabolism, as well as energy production under microgravity [23]. In contrast, our results indicated a downregulation of carbohydrate and energy metabolism under microgravity, but amino acid metabolic genes are upregulated similarly as observed in previous studies.

During the stationary phase of *R. sphaeroides* an opposite trend is exhibited where majority of the genes (88%) are downregulated, and few genes (12%) are upregulated under microgravity. However, similar to the log phase, genes of functional sub-categories including translation, energy production and conversion, lipid metabolism were constantly downregulated consistent with previous findings as mentioned above [45] [52] [55]. There is a greater reduction in the number of upregulated genes related to the cellular metabolism during the stationary phase compared to the log phase showing a tight regulation of metabolism and transportation of nutrients during the stationary phase. This change can be occurred due to much longer exposure time and starvation-stress environment created by the microgravity because of consuming many nutrients during the exponential growth phase of cells. Moreover, reduced metabolic activity in the stationary phase may be related to the limited diffusion of nutrients and reduced mass transport in microgravity environments, making it harder for bacteria to access and utilize nutrients. Therefore, it can be concluded that the exposure time of microgravity has a strong role to play on the differential expression of *R. sphaeriodes* genes.

The genes are classified in four major groups: information storage and processing (first column), cellular processes (second column), metabolism (third column), and poorly characterized (fourth column). A more detailed breakdown of the distribution of the genes is given based on different cellular functions represented in 25 COG sub-groups: translation, ribosomal structure and biogenesis (J); RNA processing and modification (A); transcription (K); DNA replication, recombination and repair (L); Chromatin structure and dynamics (B); cell division and chromosome partitioning (D); nuclear structure (Y); defense mechanisms (V); signal transduction mechanisms (T); cell envelope biogenesis,

outer membrane (M); cell motility and secretion (N); cytoskeleton (Z); extracellular structures (W); intracellular trafficking and secretion (U); posttranslational modification, protein turnover, chaperones (O); energy production and conversion (C); carbohydrate transport and metabolism (G); amino acid transport and metabolism (E); nucleotide transport and metabolism (F); coenzyme metabolism (H); lipid metabolism (I); inorganic ion transport and metabolism (P); secondary metabolites biosynthesis, transport and catabolism (Q); general function prediction only (R); and unknown function (S).

3.5. GO Analysis

GO (Gene Ontology) Analysis exhibits aims to identify those biological processes, cellular locations, and molecular functions that are significantly affected for four different comparisons: normal gravity vs microgravity at log phase, normal gravity vs microgravity at stationary phase, log phase vs stationary phase under normal gravity, and log phase vs stationary phase under microgravity.

Comparing the differentially expressed genes at the log phase under microgravity to the log phase of normal gravity, out of 67 down regulated genes, 56 genes are located on the primary chromosome (CI) while 11 genes were located on secondary chromosome (CII). However, of the total 83 upregulated genes, 65 and 14 genes are located on CI and CII, respectively. In addition, four genes are located on plasmid-A (PA). This indicates that a partnership of both CI, CII and PA is necessary for the *R. sphaeroides* to mediate the microgravity environment. A variety of molecular functions involving transcription, translation, cellular metabolism including carbohydrate, nucleic acid, and fatty acid, vitamin B12 synthesis, transport function, energy production, and cell motility are downregulated under microgravity conditions. Also, several similar molecular functions are upregulated. Notably, nine genes involving chemotaxis and cell motility are upregulated at the log phase under microgravity conditions. These findings validate several previous studies suggesting that motility genes and chemotaxis genes in bacteria are upregulated under microgravity environments [5] [45] [56].

At the stationary phase of microgravity to normal gravity comparison, of the total 80 downregulated genes, 66 and 11 genes are located on the primary and secondary chromosomes, respectively. In addition, two genes are located on plasmid-B, and a gene is located on plasmid plasmid-E. However, of the total 11 upregulated genes, nine were located on the primary chromosome, and one gene each on plasmid-A (PA) and Plasmid-E (PE). A variety of molecular functions, such as translation, transcription, metabolism, energy transfer, and transport are similarly represented in down-regulation category. However, more importantly, genes involving DNA replication and cell wall synthesis are notably down regulated. This can be explained by the fact that cells in the stationary phase may experience a nutrient depletion and limited diffusion under microgravity conditions. As a response, cells may try to halt their growth temporarily by decreasing cellular functions like DNA replication and cell wall synthesis.

It is observed that both the log and stationary phases under microgravity have an altered gene expression (up or down regulation relative to the expression under normal gravity), which controls several biological, cellular and molecular functions, and suggests the impact of microgravity affect the bacterial cells in different pathways at each growth phase.

Under the normal gravity of log phase vs stationary phase comparison, only two genes representing vitamin biosynthesis and carbohydrate metabolism, respectively are down regulated and they are located on the primary chromosome. While of the total 17 upregulated genes, the primary and secondary chromosomes have eight genes, and a gene is located on the plasmid-B. It is remarkably noted that seven genes of the upregulate category represents molecular functions involving transport.

Under the microgravity of log phase vs stationary phase comparison, a total of eight genes are down regulated, of which seven and one are located on the primary and secondary chromosomes, respectively. While of 26 upregulated genes, 20 are located on CI and six are located on CII. Several similar molecular functions are represented in both down and upregulated genes. Notably, two genes representing cell morphology regulation and oxygen processing are upregulated. The upregulation of cell morphology under microgravity can explain the altered morphological features observed in the cells during the SEM observation confirming that microgravity affects the cellular morphology.

4. Conclusion

Rhodobacter spharoides 2.4.1 shows altered growth kinetics, cellular morphology and gene expression patterns at both log and stationary phases grown under microgravity, which demonstrates that simulated microgravity has a major impact on bacterial growth. Most bacteria, including *R. sphaeroides*, adapt to the microgravity stress environment through various gene-regulation mechanisms by reducing the metabolic activity and preserving energy and nutrients to survive in the microgravity environment. The exposure time of the cells under microgravity plays a major role as the cells are affected differently during each growth phase mediated by differential expression of genes at log and stationary growth phases.

Acknowledgements

This study was supported by the NSF-REU grant (#2050232) for a stipend to Ashley Ogg, and the Office of Research and Sponsored Program (ORSP), Sam Houston State University. The authors thank Melissa Sims, Jenna Skinner, Quentin Figueroa, and Alice Jenks for their help through experiments in the laboratory.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Garcia-Lopez, E., Ruiz-Blas, F., Sanchez-Casanova, S., Peña Perez, S., Martin-Cerezo, M.L. and Cid, C. (2022) Microbial Communities in Volcanic Glacier Ecosystems. *Frontiers in Microbiology*, **13**, Article ID: 825632. https://doi.org/10.3389/fmicb.2022.825632

[2] Rupasinghe, R., Amarasena, S., Wickramarathna, S., Biggs, P.J., Chandrajith, R. and Wickramasinghe, S. (2022) Microbial Diversity and Ecology of Geothermal Springs in the High-Grade Metamorphic Terrain of Sri Lanka. *Environmental Advances*, **7**, Article ID: 100166. https://doi.org/10.1016/j.envadv.2022.100166

[3] Bijlani, S., Stephens, E., Singh, N.K., Venkateswaran, K. and Wang, C.C.C. (2021) Advances in Space Microbiology. *iScience*, **24**, Article ID: 102395. https://doi.org/10.1016/j.isci.2021.102395

[4] Horneck, G., Klaus, D.M. and Mancinelli, R.L. (2010) Space Microbiology. *Microbiology and Molecular Biology Reviews*, **74**, 121-156. https://doi.org/10.1128/MMBR.00016-09

[5] Crabbé, A., *et al.* (2010) Response of *Pseudomonas aeruginosa* PAO1 to Low Shear Modelled Microgravity Involves AlgU Regulation. *Environmental Microbiology*, **12**, 1545-1564. https://doi.org/10.1111/j.1462-2920.2010.02184.x

[6] Wilson, J.W., *et al.* (2007) Space Flight Alters Bacterial Gene Expression and Virulence and Reveals a Role for Global Regulator Hfq. *Proceedings of the National Academy of Sciences of the United States of America*, **104**, 16299-16304. https://doi.org/10.1073/pnas.0707155104

[7] Crabbé, A., *et al.* (2013) Spaceflight Enhances Cell Aggregation and Random Budding in *Candida albicans*. *PLOS ONE*, **8**, e80677. https://doi.org/10.1371/journal.pone.0080677

[8] Benoit, M.R., *et al.* (2006) Microbial Antibiotic Production aboard the International Space Station. *Applied Microbiology and Biotechnology*, **70**, 403-411. https://doi.org/10.1007/s00253-005-0098-3

[9] Lam, K.S., Mamber, S.W., Pack, E.J., Forenza, S., Fernandes, P.B. and Klaus, D.M. (1998) The Effects of Space Flight on the Production of Monorden by *Humicola fuscoatra* WC5157 in Solid-State Fermentation. *Applied Microbiology and Biotechnology*, **49**, 579-583. https://doi.org/10.1007/s002530051216

[10] Lam, K.S., *et al.* (2002) The Effect of Space Flight on the Production of Actinomycin D by *Streptomyces plicatus*. *Journal of Industrial Microbiology and Biotechnology*, **29**, 299-302. https://doi.org/10.1038/sj.jim.7000312

[11] Jules, K., McPherson, K., Hrovat, K., Kelly, E. and Reckart, T. (2004) A Status Report on the Characterization of the Microgravity Environment of the International Space Station. *Acta Astronautica*, **55**, 335-364. https://doi.org/10.1016/j.actaastro.2004.05.057

[12] Tryggvason, B.V., *et al.* (2001) The Vibration Environment on the International Space Station: Its Significance to Fluid-Based Experiments. *Acta Astronautica*, **48**, 59-70. https://doi.org/10.1016/S0094-5765(00)00140-5

[13] Huang, B., Li, D.-G., Huang, Y. and Liu, C.-T. (2018) Effects of Spaceflight and Simulated Microgravity on Microbial Growth and Secondary Metabolism. *Military Medical Research*, **5**, Article No. 18. https://doi.org/10.1186/s40779-018-0162-9

[14] Grimm, D., Wise, P., Lebert, M., Richter, P. and Baatout, S. (2011) How and Why Does the Proteome Respond to Microgravity? *Expert Review of Proteomics*, **8**, 13-27. https://doi.org/10.1586/epr.10.105

[15] Baker, P.W., Meyer, M.L. and Leff, L.G. (2004) *Escherichia coli* Growth under Modeled Reduced Gravity. *Microgravity Science and Technology*, **15**, 39-44. https://doi.org/10.1007/BF02870967

[16] Tirumalai, M.R., *et al.* (2017) The Adaptation of *Escherichia coli* Cells Grown in Simulated Microgravity for an Extended Period Is both Phenotypic and Genomic. *NPJ Microgravity*, **3**, Article No. 15. https://doi.org/10.1038/s41526-017-0020-1

[17] Crabb, A., De Boever, P., Van Houdt, R., Moors, H., Mergeay, M. and Cornelis, P. (2008) Use of the Rotating Wall Vessel Technology to Study the Effect of Shear Stress on Growth Behaviour of *Pseudomonas aeruginosa* PA01. *Environmental Microbiology*, **10**, 2098-2110. https://doi.org/10.1111/j.1462-2920.2008.01631.x

[18] Arunasri, K., Adil, M., Venu Charan, K., Suvro, C., Himabindu Reddy, S. and Shivaji, S. (2013) Effect of Simulated Microgravity on *E. coli* K12 MG1655 Growth and Gene Expression. *PLOS ONE*, **8**, e57860. https://doi.org/10.1371/journal.pone.0057860

[19] Benoit, M.R. and Klaus, D.M. (2007) Microgravity, Bacteria, and the Influence of Motility. *Advances in Space Research*, **39**, 1225-1232. https://doi.org/10.1016/j.asr.2006.10.009

[20] Senatore, G., Mastroleo, F., Leys, N. and Mauriello, G. (2018) Effect of Microgravity & Space Radiation on Microbes. *Future Microbiology*, **13**, 831-847. https://doi.org/10.2217/fmb-2017-0251

[21] Thévenet, D., D'ari, R. and Bouloc, P. (1996) The SIGNAL Experiment in BIORACK: *Escherichia coli* in Microgravity. *Journal of Biotechnology*, **47**, 89-97. https://doi.org/10.1016/0168-1656(96)01384-3

[22] Lynch, S.V., Mukundakrishnan, K., Benoit, M.R., Ayyaswamy, P.S. and Matin, A. (2006) *Escherichia coli* Biofilms Formed under Low-Shear Modeled Microgravity in a Ground-Based System. *Applied and Environmental Microbiology*, **72**, 7701-7710. https://doi.org/10.1128/AEM.01294-06

[23] Su, X., *et al.* (2021) Effects of Simulated Microgravity on the Physiology of *Stenotrophomonas maltophilia* and Multiomic Analysis. *Frontiers in Microbiology*, **12**, Article ID: 701265. https://doi.org/10.3389/fmicb.2021.701265

[24] Zea, L., *et al.* (2016) A Molecular Genetic Basis Explaining Altered Bacterial Behavior in Space. *PLOS ONE*, **11**, e0164359. https://doi.org/10.1371/journal.pone.0164359

[25] Castro, S.L., Nelman-Gonzalez, M., Nickerson, C.A. and Ott, C.M. (2011) Induction of Attachment-Independent Biofilm Formation and Repression of *hfq* Expression by Low-Fluid-Shear Culture of *Staphylococcus aureus*. *Applied and Environmental Microbiology*, **77**, 6368-6378. https://doi.org/10.1128/AEM.00175-11

[26] De Gelder, J., Vandenabeele, P., De Boever, P., Mergeay, M., Moens, L. and De Vos, P. (2009) Raman Spectroscopic Analysis of *Cupriavidus metallidurans* LMG 1195 (CH34) Cultured in Low-Shear Microgravity Conditions. *Microgravity Science and Technology*, **21**, 217-223. https://doi.org/10.1007/s12217-008-9037-0

[27] Xiao, Y., Liu, Y., Wang, G., Hao, Z. and An, Y. (2010) Simulated Microgravity Alters Growth and Microcystin Production in *Microcystis aeruginosa* (Cyanophyta). *Toxicon*, **56**, 1-7. https://doi.org/10.1016/j.toxicon.2010.01.026

[28] Fang, A., Pierson, D.L., Koenig, D.W., Mishra, S.K. and Demain, A.L. (1997) Effect of Simulated Microgravity and Shear Stress on Microcin B17 Production by *Escherichia coli* and on Its Excretion into the Medium. *Applied and Environmental Microbiology*, **63**, 4090-4092. https://doi.org/10.1128/aem.63.10.4090-4092.1997

[29] Fang, A., Pierson, D.L., Mishra, S.K. and Demain, A.L. (2000) Growth of *Streptomyces hygroscopicus* in Rotating-Wall Bioreactor under Simulated Microgravity Inhibits Rapamycin Production. *Applied Microbiology and Biotechnology*, **54**, 33-36. https://doi.org/10.1007/s002539900303

[30] Herranz, R., *et al.* (2013) Ground-Based Facilities for Simulation of Microgravity: Organism-Specific Recommendations for Their Use, and Recommended Terminology. *Astrobiology*, **13**, 1-17. https://doi.org/10.1089/ast.2012.0876

[31] Nickerson, C.A., *et al.* (2003) Low-Shear Modeled Microgravity: A Global Environmental Regulatory Signal Affecting Bacterial Gene Expression, Physiology, and Pathogenesis. *Journal of Microbiological Methods*, **54**, 1-11. https://doi.org/10.1016/S0167-7012(03)00018-6

[32] Schwarz, R.P. and Wolf, D.A. (1991) Rotating Bio-Reactor Cell Culture Apparatus. United States Patent. 4988623.

[33] Aizawa, S.-I. (2014) *Rhodobacter sphaeroides*—A Resourceful Little Bug. In: Aizawa, S.-I., Ed., *The Flagellar World*, Elsevier, Amsterdam, 66-68. https://doi.org/10.1016/B978-0-12-417234-0.00021-9

[34] Zeilstra-Ryalls, J., Gomelsky, M., Eraso, J.M., Yeliseev, A., O'Gara, J. and Kaplan, S. (1998) Control of Photosystem Formation in *Rhodobacter sphaeroides*. *Journal of Bacteriology*, **180**, 2801-2809. https://doi.org/10.1128/JB.180.11.2801-2809.1998

[35] Choudhary, M., Zanhua, X., Fu, Y.X. and Kaplan, S. (2007) Genome Analyses of Three Strains of *Rhodobacter sphaeroides*: Evidence of Rapid Evolution of Chromosome II. *Journal of Bacteriology*, **189**, 1914-1921. https://doi.org/10.1128/JB.01498-06

[36] Mackenzie, C., *et al.* (2001) The Home Stretch, a First Analysis of the Nearly Completed Genome of *Rhodobacter sphaeroides* 2.4.1. *Photosynthesis Research*, **70**, 19-41.

[37] Sistrom, W.R. (1962) The Kinetics of the Synthesis of Photopigments in *Rhodopseudomonas spheroides*. *Journal of General Microbiology*, **28**, 607-616. https://doi.org/10.1099/00221287-28-4-607

[38] Czerwińska-Główka, D. and Krukiewicz, K. (2021) Guidelines for a Morphometric Analysis of Prokaryotic and Eukaryotic Cells by Scanning Electron Microscopy. *Cells*, **10**, Article No. 3304. https://doi.org/10.3390/cells10123304

[39] Czerwińska-Główka, D., *et al.* (2020) Bacterial Surface Colonization of Sputter-Coated Platinum Films. *Materials*, **13**, Article No. 2674. https://doi.org/10.3390/ma13122674

[40] Czerwińska-Główka, D., *et al.* (2021) Electrically-Responsive Antimicrobial Coatings Based on a Tetracycline-Loaded Poly(3,4-ethylenedioxythiophene) Matrix. *Materials Science and Engineering*: *C*, **123**, Article ID: 112017. https://doi.org/10.1016/j.msec.2021.112017

[41] Skorupa, M., Więcławska, D., Czerwińska-Główka, D., Skonieczna, M. and Krukiewicz, K. (2021) Dopant-Dependent Electrical and Biological Functionality of PEDOT in Bioelectronics. *Polymers*, **13**, Article ID: 1948. https://doi.org/10.3390/polym13121948

[42] Sayers, E.W., *et al.* (2022) Database Resources of the National Center for Biotechnology Information. *Nucleic Acids Research*, **50**, D20-D26.

[43] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. and Lipman, D.J. (1990) Basic Local Alignment Search Tool. *Journal of Molecular Biology*, **215**, 403-410. https://doi.org/10.1016/S0022-2836(05)80360-2

[44] Waterhouse, R.M., Tegenfeldt, F., Li, J., Zdobnov, E.M. and Kriventseva, E.V. (2013) OrthoDB: A Hierarchical Catalog of Animal, Fungal and Bacterial Orthologs. *Nucleic Acids Research*, **41**, D358-D365. https://doi.org/10.1093/nar/gks1116

[45] Topolski, C., Divo, E., Li, X., Hicks, J., Chavez, A. and Castillo, H. (2022) Phenotypic and Transcriptional Changes in *Escherichia coli* K12 in Response to Simulated Microgravity on the EagleStat, a New 2D Microgravity Analog for Bacterial Studies. *Life Sciences in Space Research*, **34**, 1-8. https://doi.org/10.1016/j.lssr.2022.04.003

[46] Brown, R.B., Klaus, D. and Todd, P. (2002) Effects of Space Flight, Clinorotation, and Centrifugation on the Substrate Utilization Efficiency of *E. coli*. *Microgravity Science and Technology*, **13**, 24-29. https://doi.org/10.1007/BF02881678

[47] Chang, D.-E., Smalley, D.J. and Conway, T. (2002) Gene Expression Profiling of *Escherichia coli* Growth Transitions: An Expanded Stringent Response Model. *Molecular Microbiology*, **45**, 289-306. https://doi.org/10.1046/j.1365-2958.2002.03001.x

[48] Morrison, M.D., Fajardo-Cavazos, P. and Nicholson, W.L. (2019) Comparison of *Bacillus subtilis* Transcriptome Profiles from Two Separate Missions to the International Space Station. *NPJ Microgravity*, **5**, Article No. 1. https://doi.org/10.1038/s41526-018-0061-0

[49] Ott, E., *et al.* (2017) Proteometabolomic Response of *Deinococcus radiodurans* Exposed to UVC and Vacuum Conditions: Initial Studies Prior to the Tanpopo Space Mission. *PLOS ONE*, **12**, e0189381. https://doi.org/10.1371/journal.pone.0189381

[50] Li, J., *et al.* (2014) Genomic and Transcriptomic Analysis of NDM-1 *Klebsiella pneumoniae* in Spaceflight Reveal Mechanisms Underlying Environmental Adaptability. *Scientific Reports*, **4**, Article No. 6216. https://doi.org/10.1038/srep06216

[51] Senatore, G., Mastroleo, F., Leys, N. and Mauriello, G. (2020) Growth of *Lactobacillus reuteri* DSM17938 under Two Simulated Microgravity Systems: Changes in Reuterin Production, Gastrointestinal Passage Resistance, and Stress Genes Expression Response. *Astrobiology*, **20**, 1-14. https://doi.org/10.1089/ast.2019.2082

[52] Yin, M., *et al.* (2020) Changes in *Vibrio natriegens* Growth under Simulated Microgravity. *Frontiers in Microbiology*, **11**, Article No. 2040. https://doi.org/10.3389/fmicb.2020.02040

[53] Hazelbauer, G.L., Falke, J.J. and Parkinson, J.S. (2008) Bacterial Chemoreceptors: High-Performance Signaling in Networked Arrays. *Trends in Biochemical Sciences*, **33**, 9-19. https://doi.org/10.1016/j.tibs.2007.09.014

[54] Dufrêne, Y.F. and Persat, A. (2020) Mechanomicrobiology: How Bacteria Sense and Respond to Forces. *Nature Reviews Microbiology*, **18**, 227-240. https://doi.org/10.1038/s41579-019-0314-2

[55] Mastroleo, F., *et al.* (2013) Modelled Microgravity Cultivation Modulates N-Acylhomoserine Lactone Production in *Rhodospirillum rubrum* S1H Independently of Cell Density. *Microbiology*, **159**, 2456-2466. https://doi.org/10.1099/mic.0.066415-0

[56] Tucker, D.L., *et al.* (2007) Characterization of *Escherichia coli* MG1655 Grown in a Low-Shear Modeled Microgravity Environment. *BMC Microbiology*, **7**, Article No. 15. https://doi.org/10.1186/1471-2180-7-15