Evaluation of an ATP Assay to Quantify Bacterial Attachment to Surfaces in Reduced Gravity

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Abstract

Aim: To develop an assay to quantify the biomass of attached cells and biofilm formed on wetted surfaces in variable-gravity environments.

Methods and Results: Liquid cultures of *Pseudomonas aeruginosa* were exposed to 30–35 brief cycles of hypergravity (≤ 2-g) followed by free fall (i.e., reduced gravity) equivalent to either lunar-g (i.e., 0.17 normal Earth gravity) or micro-g (i.e., < 0.001 normal Earth gravity) in an aircraft flying a series of parabolas. Over the course of two days of parabolic flight testing, 504 polymer or metal coupons were exposed to a stationary-phase population of *P. aeruginosa* strain ERC1 at a concentration of $1{\cdot}0 \times 10^5$ cells per milliliter. After the final parabola on each flight test day, half of the material coupon samples were treated with either 400 $\mu g\ L^{-1}$ ionic silver fluoride (microgravity-exposed cultures) or 1% formalin (lunar-gravity-exposed cultures). The remaining sample coupons from each flight test day were not treated with a fixative. All samples were returned to the laboratory for analysis within 2 hours of landing, and all biochemical assays were completed within 8 hours of exposure to variable gravity. The intracellular ATP luminescent assay accurately reflected cell physiology compared to both cultivation-based and direct-count microscopy analyses. Cells exposed to variable gravity had more than twice as much intracellular ATP as control cells exposed only to normal Earth gravity.

Keywords: ATP, microgravity, parabolic flight, *Pseudomonas aeruginosa.*

Introduction and Background

The effects of exposure to reduced gravity on the pattern of gene expression, morphology, physiology, and ecology of bacterial populations have been studied by a variety of molecular and cellular approaches in environments ranging from ground-based, low-shear modeled microgravity to reduced-gravity flights on aircraft and spaceflight experiments in low Earth orbit (Benoit and Klaus 2007; McLean *et al.* 2001; Nickerson *et al.* 2003; Sule *et al.* 2009). In addition to studies of the fundamental effects of exposure to microgravity on cell physiology, there is increasing interest in applied studies to improve control of microbial populations in life support systems in closed environments. Water is a critical life support element, representing 65% of the daily mass input for crew. Long-term human missions beyond low Earth orbit require regenerative life support technologies to collect, store, recycle, and disinfect water for use and reuse. In addition to maintaining water quality for crew use, these systems must minimize mass, power, and resupply requirements. The technologies NASA currently employs for microbial control of spacecraft potable-water systems use a residual chemical biocide, such as iodine or either ionic or colloidal silver, and one or more physical disinfection devices to reduce the microbial burden at the point of use. None of these microbial control treatments are completely effective against all microorganisms and have limitations for long-term use because they do not provide an absolute barrier to microbial growth, are inactivated over time by chemical degradation or interaction with material surfaces, require repeated application, or pose risks to human health with prolonged use (Aoki *et al.* 2009; Boer *et al.* 2007; Stowe *et al.* 2001). NASA is supporting research to develop safe and effective technologies for microbial control and monitoring of potable-water systems in closed-loop life support systems.

Traditional methods for microbial detection and enumeration in spacecraft during mission operations typically require collection of a sample on-orbit and transportation back to the ground for analysis (Castro *et al.* 2004; Ferguson *et al.* 1975; Kawamura *et al.* 2001; Koenig and Pierson 1977; Taylor *et al.* 1977).The availability of sample return from even low Earth orbit missions, such as the International Space Station (ISS), is severely limited and results in delays of several weeks between sample collection and analysis. In addition, most microbial and detection enumeration methods for spacecraft still rely upon cultivation-based approaches that fail to detect all of the cells present in a sample (Buckstein *et al.* 2008; La Duc *et al.* 2004). As a result, microbial contamination of spacecraft often cannot be prevented, reliably detected, or quickly mitigated. One potential technology for the real-time detection and quantification of bacteria and fungi in spacecraft life support systems uses a third-generation bioluminescence assay targeting ATP (Chappelle and Levin 1968; Stanley 1989; Venkateswaran *et al.* 2001).

ATP-based assays are routinely used for bacterial detection and hygiene monitoring during food, beverage, cosmetic, and pharmaceutical processing (Griffiths 2004; Hara and Mori 2006; Hara *et al.* 2009; Thompson 2004; Whitehead *et al.* 2008) and as semi-quantitative or quantitative assays for measuring eukaryotic cell proliferation (Crouch *et al.* 1993) or unattached bacterial and biofilm growth (Sule *et al.* 2009) or for monitoring biocide efficacy (Junker and Clardy 2007; Romanova *et al.* 2007). ATP is a suitable target for cell detection and cell quantification because it is ubiquitous in viable cells, its concentration does not vary with growth rate in individual cells, and it is relatively constant across different growth conditions for the same species (Schneider and Grouse 2004). More recently, ATP has been used to measure cell attachment to surfaces during biofilm formation (Hong and Brown 2009; Junker and Clardy 2007; Sule *et al.* 2009). Bacterial adhesion to material surfaces and biofilm formation are of

particular importance in microgravity environments where physical changes affect the metabolic activity and physiology of each cell, resulting in changes in cell attachment and biofilm formation (Altenburg *et al.* 2008; Benoit and Klaus 2007; Chen *et al.* 2009; DeGelder *et al.* 2009; Leys *et al.* 2009; Liu *et al.* 2008; McLean *et al.* 2001; Nickerson *et al.* 2003; Rosado *et al.* 2010; Vukanti *et al.* 2008; Wilson *et al.* 2007).

The potential for bacterial adhesion to wetted surface materials is an important consideration in the design of potable-water systems for spacecraft because biofilms can accelerate material corrosion, reduce the mean time between failures for critical life support flight hardware, and enable microorganisms to evade antimicrobial control measures. If the material is conducive to increased metabolic activity, then the ability of the bacteria to adhere to the surface is also improved and vice versa (Hong and Brown 2009). Therefore, it is vital to use the least-responsive material that suits the engineering specifications. The focus of this study combines the evaluation of the natural antimicrobial properties of multiple materials with the use of an ATP assay for rapid quantification of attached bacteria.

Materials and Methods

Five materials were selected for efficacy testing in reduced gravity based on their potential application in potable-water systems for NASA spacecraft. Two metal coupon types, 316 stainless steel and titanium, were provided by BioSurface Technologies (Bozeman, Montana) and correspond to the 316 stainless steel and titanium 6Al4V base-lined for use in the NASA *Orion* Crew Exploration Vehicle (Pierson 2003). Each metal coupon was approximately 12.7 mm × 3 mm (diameter × thickness). Three polymer coupon types, polycarbonate, low-density polyethylene, and polydimethylsiloxane, were provided to NASA by Sharklet

Technologies (Denver, Colorado) for testing. The polycarbonate and low-density polyethylene coupons were approximately 12 mm × 0.2 mm (diameter × thickness). The polydimethylsiloxane coupons were 12 mm × 0.1 mm (diameter × thickness).

For reduced-gravity flight tests, coupons were affixed to the bottom of 60 mm Petri dish fixation units (PDFUs) mounted in Biological Research in Canisters-Light Emitting Diode (BRIC-LED) flight hardware. Coupons were sterilized by ethylene oxide treatment prior to integration with autoclave-sterilized flight hardware. A more detailed description of the flight hardware and previous flight experiments in the BRIC hardware is available online <http://www.nasa.gov/mission_pages/station/science/experiments/BRIC.html>. In brief, the BRIC-LED class I flight hardware is an anodized-aluminum container with two layers of biological containment housing six PDFUs and electrical connections for LEDs. LEDs were not activated during these tests. In addition to a single 60 mm × 15 mm Petri dish, each PDFU contained a single- or dual-chamber fluid reservoir, which could be emptied by depressing a plunger. The standard Petri dish fixation unit (SPDFU) contained a single-chamber reservoir for addition of a single fluid pulse during flight operations. The modified Petri dish fixation unit (MPDFU) contained a dual-chamber reservoir for addition of up to two fluids, typically one containing a growth medium, with or without inoculum, for PDFU activation and another containing a cell fixative or biocide for PDFU deactivation.

Before each experiment, seven replicate sterile test coupons were aseptically attached with adhesive to the bottom of 60 mm Petri dishes (BD Biosciences, Franklin Lakes, New Jersey), forming the base of a single PDFU, and positioned in the BRIC-LED hardware. Either silver fluoride (Sigma-Aldrich, St. Louis, Missouri) (400 μg L^{-1} AgF) or 1% formalin (Sigma-Aldrich) was aseptically loaded into the fixation reservoir in the MPDFUs.

For each flight day, three BRIC units, each containing six single-reservoir SPDFUs, and another three BRIC units, each containing six dual-reservoir MPDFUs, were loaded into a shuttle mid-deck locker assembly for attachment to the plane floor. On each flight test day, a series of 35 parabolas was scheduled to replicate either lunar gravity or microgravity. Material coupons exposed to reduced gravity in SPDFUs were placed on ice and returned to the laboratory for analysis, without addition of any fixative. Material coupons exposed to microgravity in MPDFUs were fixed with 400 $\mu g\ L^{-1}$ ionic silver (as AgF) after the final parabola, whereas coupons exposed to lunar gravity in MPDFUs were fixed in 1% formalin immediately after completion of the last parabola. For ground controls performed on each flight day, replicate samples were processed without fixation in SPDFUs or fixed in either 400 $\mu g\ L^{-1}$ ionic silver (microgravity flight day) or 1% formalin (lunar-gravity flight day) and were sampled hourly for up to 6 h of coupon exposure. The duration between inoculation and harvest for each of the unfixed flight samples was approximately 4.5 h. The duration between fixation and sample analysis was one week.

Coupons and the bulk fluid from each of the Petri dishes were sampled and diluted as required for heterotrophic plate count (HPC) on R2 Agar (R2A) (BD Biosciences), assay with the Live/Dead BacLight Bacterial Viability Kit (Invitrogen, Carlsbad, California) (L/D), ATP quantification with the Quench-Gone Aqueous test kit (LuminUltra Technologies, Fredericton, New Brunswick), and Acridine Orange (AO) direct count (AODC) (Sigma-Aldrich) solution. Replicate results from each assay were averaged and the standard deviation was calculated for the sample data. An agar streak plate of the test microorganism, *Pseudomonas aeruginosa* strain ERC1 (ATCC 700888), was maintained on R2A. The bacterial inoculum for reduced-gravity testing was prepared as a broth culture in Trypticase Soy Broth (TSB) (BD Biosciences, San

Jose, California) from the agar streak plate ≥ 12 h before flight each flight day, loaded into the flight hardware, and incubated overnight at 37°C. From the overnight broth culture, the *P. aeruginosa* was serially diluted to a final concentration of $1{\cdot}0 \times 10^5$ cells per milliliter in a 10% TSB solution and loaded into the Petri dishes contained within the flight hardware immediately before the first of the parabolas during the reduced-gravity flights. Inoculum cellular concentrations were verified by optical density, AODC, and HPC.

The HPC test, previously called the standard plate count method, provided an estimate of the total number of bacteria in each sample that developed into colonies during incubation on a nutrient-rich agar (e.g., R2A) (Bartram *et al.* 2003). During this experiment, the plates were incubated at 37°C for 20–24 h until individual colonies could be seen with accuracy. This method can detect a broad group of bacteria, including non-pathogens, pathogens, and opportunistic pathogens, but often does not accurately represent all of the bacteria in the water sample examined. For example, bacterial biofilms, injured bacteria, and viable but non-culturable bacteria may not form colonies on the selected nutrient medium.

For direct-count microscopy of cells, the samples were sonicated and/or diluted into 0.2 μm-filtered deionized water as needed, stained with either AO or 1.5 μL of 3.34 mM Syto 9 and 3 μL of 20 mM propidium iodide (L/D), and filtered onto 25 mm × 0.2 μm (diameter × thickness) black polycarbonate filters (Millipore, Billerica, Massachusetts) for enumeration and visual evaluation on an Axioskop 2 epifluorescent microscope (Carl Zeiss, Thornwood, New York) at 1000× oil immersion magnification (Bloem 1991; Hobbie *et al.* 1977). The Live/Dead BacLight Bacterial Viability Kit was used to determine the percentage of viable and total count of organisms in the samples (Boulos *et al.* 1999; Gregori *et al.* 2001). Viability as determined by BacLight assay equates the visualization of the green fluorescent dye with cells whose

membranes are intact, and this method can overestimate living cells in some environmental matrices.

For the bulk fluid samples, the Quench-Gone Aqueous test kit was used because of its applicability in water-based samples with low extraneous solid-material content. The coupons were sampled with a modified Deposit Surface Analysis test kit (LuminUltra), which is specific for detecting microorganisms that have adhered to surfaces. Once samples were processed through the ATP test kits, 100 μL was loaded into a 96-well, black and white isoplate (Perkin Elmer, Shelton, Connecticut) in triplicate. A control sample was added to the plate that contained 1 ng mL^{-1} of ATP, and finally 100 μL of Luminase (LuminUltra) was added to every well containing sample. Luminescence was quantified on a Victor 2 Plate Reader (Perkin Elmer).

Results and Discussion

Each of the testing analysis methods used, except ATP luminescence assay, had limitations that prevented its application to all sample types. The HPCs could be used for enumerating cells in the bulk fluid and on the coupons, but only without the addition of a fixative. The low number of cells that adhered to the coupons was below the lower limit of detection for the microscopy methods (AODC and L/D), so they were used only for the bulk fluid samples. Plus, the addition of a fixative to any of the bulk samples prevented the use of the L/D assay.

Analysis methods indicated that the flight hardware performed nominally and that there was minimal variability between flight and ground experiments (Table 1). However, the short duration of the microgravity and lunar-gravity parabolic flights was not conducive to the formation of advanced biofilm. The microgravity flight had an increase of $\log_{10}$ colony-forming units per milliliter (CFU mL^{-1}) = 0.33, the lunar-gravity flight had an increase of $\log_{10}$ CFU mL^{-}

^{1} = 0.38, and the ground testing had an increase of $\log_{10}$ CFU mL^{-1} = 0.41 between inoculation and harvest for the bulk fluid. The microscopy data supported the results found from the plate counts for the bulk fluid samples, demonstrating minimal variability between flight experiments. During the BacLight L/D assay, it was found that 88% to 92% of the cells found in the bulk fluid samples were alive. This result was optimal. The L/D total counts were similar to those found during HPC analysis. There were slight variations between the AO bulk fluid samples, based on fixation method.

A standard curve was generated using the LuminUltra Quench-Gone Aqueous test kit with the *P. aeruginosa* strain ERC1 (ATCC 700888) at a high range of cellular density and at a low range of cellular density, and both showed a positive correlation between the number of cells and the amount of ATP per milliliter of sample (Fig. 1). ATP analysis of the bulk fluid samples showed that after 1 week the microgravity flight produced a 1.6 log reduction after silver fixation, whereas the lunar-gravity flight produced a 2.3 log reduction after formalin fixation. This pattern was repeated to a lesser degree during ground testing, where there was approximately a 1 log reduction in picograms of ATP per milliliter with 1% formalin fixation and less than a 0.5 log reduction with 400 $\mu g\ L^{-1}$ ionic silver fixation (Fig. 2). As was expected, these results indicate that formalin has a greater negative effect on cellular metabolism than does silver. However, no differences were observed in the bulk fluid analyses based on material type.

The benchmark for healthy cells exposed to *Escherichia coli* in terrestrial gravity is approximately 1 fg of ATP per cell (Crombrugge and Waes 1991). This indicates that the amount of ATP in the *P. aeruginosa* cells during ground testing resembled the metabolic state of the benchmark samples. There was more ATP per cell in the samples exposed to short-term microgravity than in the ground-tested samples (Table 2). This effect was seen in a study with

plants in which cells exposed to short-term microgravity had an increased metabolic rate and then, after long-term exposure to microgravity, went into a relaxed metabolic state, even lower than the state experienced in terrestrial gravity (Hampp *et al.* 1997). In the cells that were not exposed to a fixative, there appeared to be more ATP per cell in the lunar-gravity flight samples than in those that experienced microgravity.

During the ATP analysis of the coupons, the unfixed cells on the coupons exposed to microgravity had 82.77% more ATP per square millimeter than the cells exposed to lunar-gravity parabolic-flight conditions (Fig. 3). This response was opposite the response observed in the bulk fluid samples. One explanation for this is that the surface proton concentration for the bacteria on the coupons can be significantly different than in the bulk fluid sample, which means that the pH would be different in the periplasmic space and would affect ATP levels (Hong and Brown 2010). In addition, the fixed cells had 47.38% less ATP per square millimeter than the unfixed cells. The samples fixed with 400 μg L^{-1} ionic silver had 10.12% more ATP per square millimeter than the cells exposed to 1% formalin. The only exception to this condition was found in the polycarbonate samples. Titanium coupons exerted the most influence in cells not exposed to fixation, the ATP per millimeter found in the microgravity samples was more than six times that found in the lunar-gravity flight samples. Similarly, for the polycarbonate samples, cells not exposed to fixation exhibited more than twice as much ATP per square millimeter in the microgravity samples as in the lunar-gravity flight samples. The large increased response for the titanium coupons was also observed in the fixed cells.

Further evaluation will be conducted to optimize the ATP method for additional coupon materials and to confirm whether the increased ATP found in the lunar-gravity flight over the microgravity flight is specific to the flight conditions or time-dependent. Additional experiments

at longer durations of reduced gravity are necessary to extrapolate the microgravity and 2-g segments of the parabolic data and to develop a better understanding of biofilm formation in correlation to ATP. New antimicrobial materials will be developed and tested in 1-g environments using ASTM methods to improve the antimicrobial response of materials.

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References

1. Altenburg, S.D., Nielsen-Preiss, S.M. and Hyma, L.E. (2008) Increased filamentous growth of *Candida albicans* in simulated microgravity. *Genomics Proteomics Bioinformatics* **6**, 42–50.
2. Aoki, S.K., Webb, J.S., Braaten, B.A. and Low, D.A. (2009) Contact-dependent growth inhibition causes reversible metabolic down regulation in *Escherichia coli. J Bacteriol* **191**, 1777–1786.
3. Bartram, J., Cotruvo, J., Exner, M., Fricker, C. and Glasmacher A. (2003) Heterotrophic plate counts and drinking-water safety: The significance of HPCs for water quality and the human health. WHO.
4. Benoit, M.R. and Klaus D.M. (2007) Microgravity, bacteria, and the influence of motility. *Adv Space Res* **39**, 1225–1232.
5. Bloem, J. 1995. Fluorescent staining of microbes for total direct counts. *Mol Micro Ecol Man* **4.1.8**, 1–12.
6. Boer, V.M., Crutchfield, C.A., Bradley, P.H., Botstein, D. and Rabinowitz, J.D. (2007) Growth-limiting intracellular metabolites in yeast growing under diverse nutrient limitations. *Mol Biol Cell* **21**, 198–211.
7. Boulos, L., Prevost, M., Barbeau, B., Callier, J. and Desjardins, R. (1999) Live/Dead BacLight: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. *J Microbiol Methods* **37**, 77–86.
8. Buckstein, M.H., He, J. and Rubin, H. (2008) Characterization of nucleotide pools as a function of physiological state in *Escherichia coli. J Bacteriol* **190**, 718–726.

9. Castro, V.A., Thrasher, A.N., Healy, M., Ott, C.M. and Pierson, D.L. (2004) Microbial diversity aboard spacecraft: Evaluation of the International Space Station. *Microb Ecol* **47**, 119–126.

10. Chappelle, E.W. and Levin, G. V. (1968) Use of the firefly bioluminescence reaction for rapid detection and counting of bacteria. *Biochem Med* **2**, 41–52.

11. Chen, G., Hong, Y. and Walker, S.L. (2009) Colloidal and bacterial deposition: Role of gravity. *Langmuir* **26**, 314–319.

12. Crombrugge, J. and Waes, G. (1991) ATP method. In *Methods for assessing the bacteriological quality of raw milk from the farm* ed. Heeschen, W. pp. 53–60, Brussels: Intl Dairy Fed.

13. Crouch, S.P., Kozlowski, M.R., Slater, K.J. and Fletcher, J. (1993) The use of ATP bioluminescence as a measure of cell proliferation and cytotoxicity. *J Immunol Methods* **160**, 81–88.

14. 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 Sci Technol* **21**, 217–223.

15. Ferguson, J.K., Taylor, G.R. and Mieszkuc, B.J. (1975) Microbiological investigations: Biomedical results of Apollo. NASA: Scientific and Technological Information Office. pp. 83–103.

16. Gregori, G., Citterio, S., Ghiani, A., Labra, M., Sgorbati, S., Brown, S. and Denis, M. (2001) Resolution of viable and membrane-compromised bacteria in freshwater and marine waters based on analytical flow cytometry and nucleic acid double staining. *Appl Environ Microbiol* **67**, 4662–4670.

17. Griffiths, M.W. (2004) ATP bioluminescence: Application in hygiene monitoring. In *Encyclopedia of Food Microbiology* ed. Robinson, R.K., Batt, C.A. and Patel, P. pp. 94–101. San Diego: Academic Press.

18. Hampp, R., Hoffmann, E., Schonherr, K., Johann, P. and Filippis, L. (1997) Fusion and Metabolism of Plant Cells as Affected by Microgravity. *Planta* **203**, S42–S53.

19. Hara, K.Y. and Mori, H. (2006) An efficient method for quantitative determination of cellular ATP synthetic activity. *J Biomol Screen* **11**, 310–317.

20. Hara, K.Y., Shimodate, N., Ito, M., Baba, T., Mori, H. and Mori, H. (2009) Systematic genome-wide scanning for genes involved in ATP generation in *Escherichia coli. Metab Eng* **11**, 1–7.

21. Hobbie, J.E., Daley, R.J. and Jasper, S. (1977) Use of Nucleopore filters for counting bacteria by fluorescence microscopy. *Appl Environ Microbiol* **33**, 1225–1228.

22. Hong, Y. and Brown, D.G. (2009) Variation in Bacterial ATP Level and Proton Motive Force Due to Adhesion to a Solid Surface. *Appl Environ Microbiol* **25**, 2346–2353.

23. Hong, Y. and Brown, D.G. (2010) Alteration of bacterial surface electrostatic potential and pH upon adhesion to a solid surface and impacts to cellular bioenergetics. *Biotechnol Bioeng* **105**, 965–72.

24. Junker, L.M., and Clardy, J. (2007) High-throughput screens for small-molecule inhibitors of *Pseudomonas aeruginosa* biofilm development. *Antimicrob Agents Chemother* **51**, 3582–3590.

25. Kawamura, Y., Li, Y., Liu, H., Huang, X., Li, Z. and Ezaki, T. (2001) Bacterial population in Russian Space Station, "MIR." **45**, 819–828.

26. Koenig, D.W. and Pierson, D.L. (1977) Microbiology of the space shuttle water system. *Water Sci Technol* **35**, 59–64.

27. La Duc, M.T., Sumner, R., Pierson, D., Venkat, P. and Venkateswaran, K. (2004) Evidence of Pathogenic Microbes in the ISS Drinking Water: Reason for Concern? *Habitation* **10**, 39–48.

28. Leys, N., Baatout, S., Rosier, C., Dams, A., s'Heeren, C., Wattiez, R. and Mergeay, M. (2009) The response of *Cupriavidus metallidurans* CH34 to spaceflight in the International Space Station. Antonie van Leeuwenhoek. **96**, 227–245.

29. Liu, H-Z., Wang, Q., Liu, X-Y. and Tan, S-S. (2008) Effects of spaceflight on polysaccharides of Saccharomyces cerevisiae cell wall. *Appl Microbiol Biotechnol* **81**, 543–550.

30. McLean, R.J., Cassanto, J.M., Barnes, M.B. and Koo, J.H. (2001) Bacterial biofilm formation under microgravity conditions. *FEMS Microbiol Lett* **195**, 115–119.

31. Nickerson, C.A., Ott, C.M., Wilson, J.W., Ramamurthy, R., LeBlanc, C.L., Honer zu Bentrup, K., Hammond, T. and Pierson, D.L. (2003) Low-shear modeled microgravity: a global environmental regulatory signal affecting bacterial gene expression, physiology, and pathogenesis. *J Micro Methods* **54**, 1–11.

32. Pierson, D.L. (2001) Microbial contamination in spacecraft. *Gravit Space Biol Bull* **14**, 1–6.

33. Romanova, N.A., Gawande, P.V., Brovko, L.Y. and Griffiths, M.W. (2007) Rapid methods to assess sanitizing efficacy of benzalkonium chloride to *Listeria monocytogenes* biofilms. *J Micro Methods* **71**, 231–237.

34. Rosado, H., Doyle, M., Hinds, J. and Taylor, P.W. (2010) Low-shear modelled microgravity alters expression of virulence determinants of *Staphylococcus aureus*. *Acta Astronaut* **66**, 408–413.

35. Schneider, D.A. and Grouse, R.L. (2004) Relationship between growth rate and ATP concentration in *Escherichia coli*. *J Biol Chem* **279**, 8262–8268.

36. Stanley, P.E. 1989. A review of bioluminescent ATP techniques in rapid microbiology. *J Biolumin Chemilumin* **4**, 375–380.

37. Stowe, R.P., Mehta, S.K., Ferrando, A.A., Feeback, D.L. and Pierson, D.L. (2001) Immune responses and latent herpes virus reactivation is spaceflight. *Aviat Space Environ Med* **72**, 884–889.

38. Sule, P., Wadhawan, T., Carr, N.J., Horne, S.M., Wolfe, A.J. and Prub, B.M. (2009) A combination of assays reveals biomass differences in biofilms formed by *Escherichia coli* mutants. *Lett App Microbiol* **49**, 299-304.

39. Taylor, G.R., Graves, R.C., Brockett, R.M., Ferguson, J.K. and Mieszkuc, B.J. (1977) Skylab environmental and crew microbiological studies: Biomedical results from Skylab. NASA: Scientific and Technical Information Office. pp. 53–63.

40. Thompson, A. (2004) ATP bioluminescence: Application in beverage microbiology. In *Encyclopedia of Food Microbiology* ed. Robinson, R.K., Batt, C.A. and Patel, P. pp. 101–109. San Diego: Academic Press.

41. Venkateswaran, K., Hattori, N., La Duc, M.T. and Kern, R. (2002) ATP as a biomarker for viable microorganisms in clean-room facilities. *J Microbiol Methods* **52**, 367–377.

42. Vukanti, R., Mintz, E. and Leff, L. (2008) Changes in gene expression of *E. coli* under conditions of modeled reduced gravity. *Microgravity Sci Technol* **20**, 41–57.

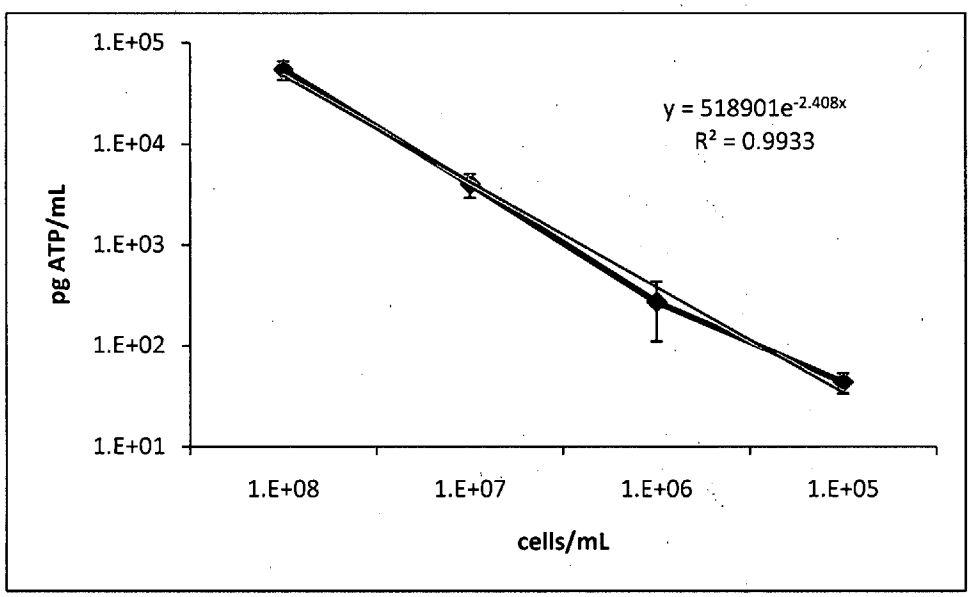
43. Whitehead, K.A., Smith, L.A. and Verran, J. (2008) The detection of food soils and cells on stainless steel using industrial methods: UV illumination and ATP bioluminescence. *Int J Food Microbiol* **127**, 121–128.

44. Wilson, J., Ott, M., Honer zu Bentrup, K., Ramamurthy, R., Quick, L., Porwollik, S., Cheng, P., McClelland, M., Tsaprailis, G., Radabaugh, T., Hunt, A., Fernandez, D., Richter, E., Shah, M., Kilcoyne, M., Joshi, L., Nelman-Gonzalez, M., Hing, S., Parra, M., Dumars,P., Norwood, K., Bober, R., Devich, J., Ruggles, A., Goulart, C., Rupert, M., Stodieck, L., Stafford, P., Catella, L., Schurr, M.J., Buchanan, K., Morici, L., McCracken, J., Allen, P., Baker-Coleman, C., Hammond, T., Vogel, J., Nelson, R., Pierson, D.L., Stefanyshyn-Piper, H.M. and Nickerson, C.A. (2007) Space flight alters bacterial gene expression and virulence and reveals a role for global regulator Hfq. *Proc Natl Acad Sci USA* **104**, 16299–16304.

Table 1. Recovered bulk fluid HPC results

Bulk fluid samples	$\log_{10}$ CFU mL^{-1}
Ground (n = 3)	5.63 ± 0.07
Lunar gravity (n = 18)	5.89 ± 0.18
Microgravity (n = 18)	5.70 ± 0.12

A. High cellular concentration ATP standard curve (n=9).



B. Low cellular concentration ATP standard curve (n=9).

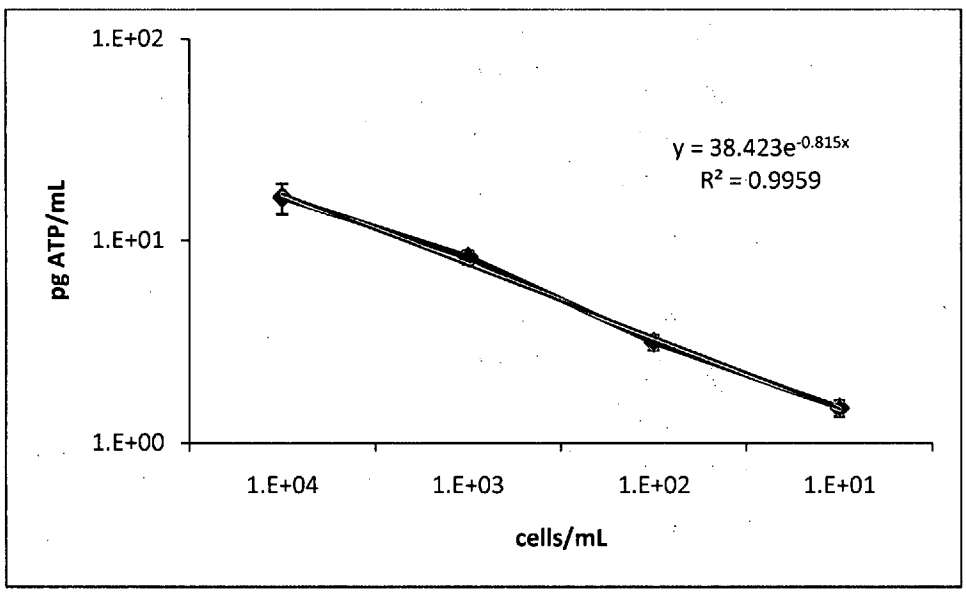


Fig. 1 Luminescent assay ATP standard curves

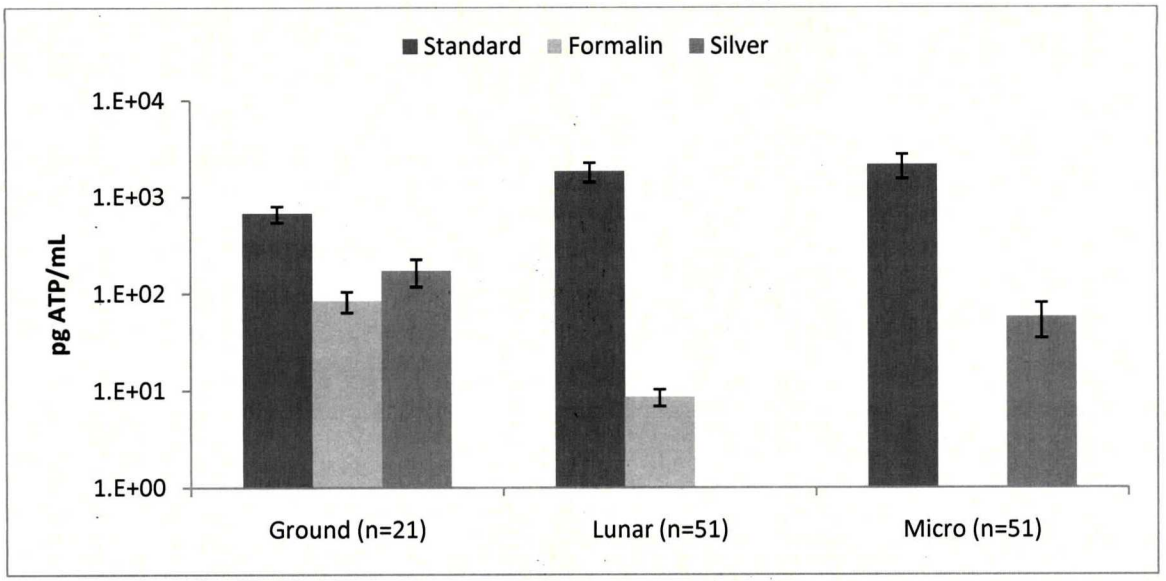
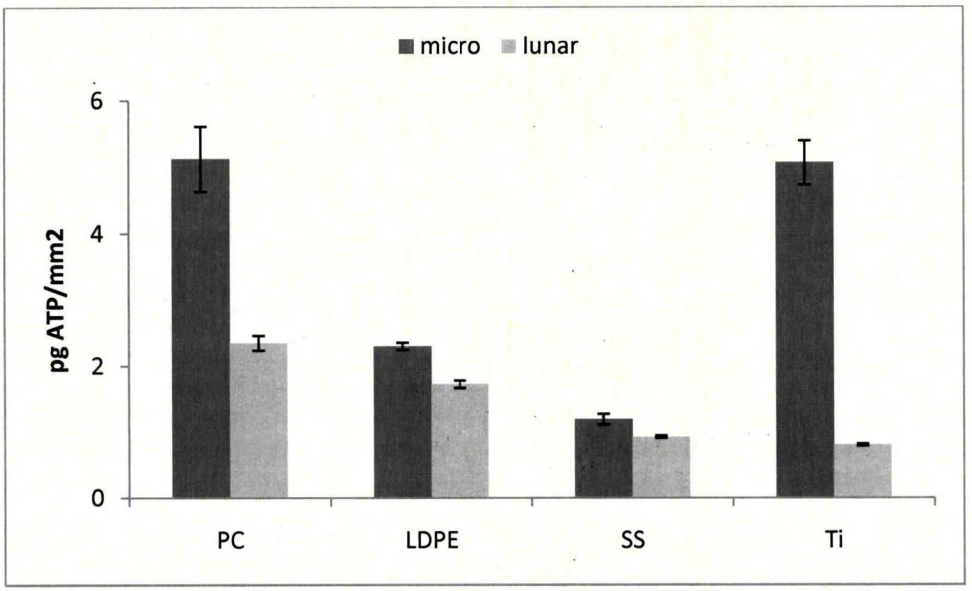


Figure 2. Bulk fluid ATP results with and without fixation. Standard samples were processed immediately following the ground or flight processes. Fixed samples were processed after one week of storage.

Table 2 Bulk fluid ATP/cell results

Bulk fluid samples	Standard	Formalin (1%)	Silver (400 $\mu g\ L^{-1}$)
Microgravity (n = 51)	2.02E-03 ± 5.75E-04	N/A	2.30E-05 ± 9.07E-06
Lunar gravity (n = 51)	2.70E-03 ± 6.05E-04	2.82E-05 ± 5.57E-06	N/A
Earth gravity (n = 21)	1.10E-03 ± 3.81E-04	1.38E-04 ± 3.31E-05	2.81E-04 ± 8.83E-05

A. Standard samples with unfixed cells processed at the completion of each parabolic flight (n=9).



B. Samples that were fixed and then stored for 1 week (n=9). Microgravity samples were fixed with 400 μg L^{-1} ionic silver. Lunar-gravity samples were fixed with 1% formalin.

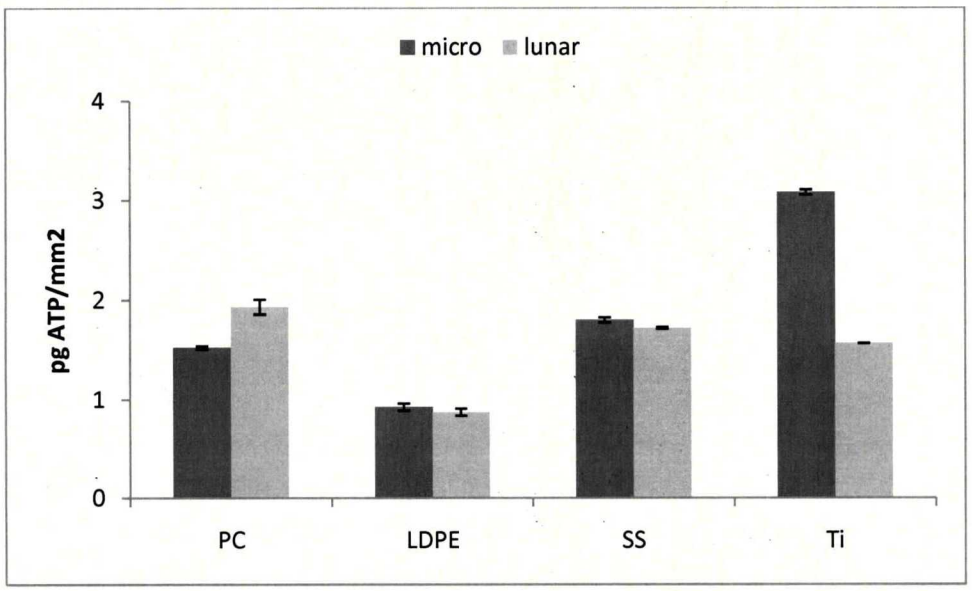


Fig. 3 Coupon ATP results by material type.