



# The Effect of Modeled Microgravity on Microbial Gene Expression

George E. Fox [UH] / Richard C. Willson [UH] / Duane L. Pierson [NASA-JSC] / Don L. Tucker [UH]

## Abstract

Contrary to popular belief, bacterial virulence does not decrease in response to the microgravity of space. In fact, recent evidence indicates increased *Salmonella* virulence in response to modeled microgravity. It is possible that bacterial growth, virulence, resistances to stress and antibiotics, and cell survival may change in response to microgravity. A high aspect rotating vessel (HARV) bioreactor has allowed us to analyze bacterial physiology in a modeled microgravity environment. Researchers are attempting to identify changes in bacterial (*E. coli*) physiology and the corresponding changes in gene transcription and regulation, using DNA macroarray technology, to the low-shear modeled microgravity (LSMMG) environment experienced in HARV bioreactors. Potential changes are being analyzed in antibiotic and stress resistances in response to LSMMG.

A number of LSMMG regulated genes have been identified that respond in a significant way. These include genes that are substantially up-regulated as well as those that are down-regulated. Although many are genes of unknown function, a number have known or putative functions. Among the up-regulated genes are those associated with cell motility, acid tolerance response, and the chaperone *hdeA*. In contrast to previously published results in *Salmonella*, little variation in antibiotic and stress resistance has been identified in *E. coli* grown in LSMMG. Preliminary results indicate that the modeled microgravity and not low-shear are inducing the changes in LSMMG gene transcription. Research with HARV grown cultures is continuing to verify the modeled microgravity induced gene regulation and determine gene regulators and physiological responses to LSMMG.

**T**HE ABILITY TO ADAPT TO ADVERSE ENVIRONMENTS IS extremely important to pathogenic bacteria such as *E. coli*. An entirely unique environment is the microgravity experienced by bacteria during space flight. A large body of whole-organism-based research has demonstrated that prolonged exposure to microgravity has significant effects at a basic, cellular level.<sup>1</sup> The analysis of bacteria under microgravity has received considerably less attention because of the expense and difficulty of performing in-flight experiments onboard the shuttle or space station. In order to overcome this limitation, investigators have taken advantage of the partial simulation of microgravity obtained by growing bacterial cultures in High Aspect Rotating Vessels (HARV) developed by NASA.<sup>2</sup> For example, a recent study showed that *Salmonella enterica* serovar Typhimurium grown under

low-shear modeled microgravity (LSMMG) appeared to have increased virulence potential in a murine model system.<sup>3</sup> A follow-up study revealed that a significant number of the genes are transcriptionally regulated in response to LSMMG. Researchers identified increased resistance to antibiotics and low pH.<sup>4</sup> In addition to immediate changes in behavior, bacterial strains will possibly evolve during long-term space flight, potentially modifying virulence, resistances, and rate of culture survival. Our goal is to develop a more general and deeper understanding of LSMMG on bacterial gene expression.

## Technical Plan and Equipment

The HARV bioreactor was originally developed to minimize fluid motion for tissue culture differentiation while maintaining culture aeration through a gas permeable membrane. The rotation of the HARV also has the effect of randomizing the gravity vector by rotating in the plane of gravity, producing the LSMMG environment. To obtain this environment, the HARV device is rotated at a speed sufficient to maintain cell suspension in the media, completely filled, thereby preventing gas bubbles from causing solution turbulence (i.e., shear). The HARV apparatus approximates the physiological and transcriptional changes occurring in space flight due to microgravity, while allowing Earth-based culturing. Used in conjunction with commercially available functional genomics technology (Panorama Gene Arrays, Sigma-Genosys), the HARV makes it possible to study microbial gene expression on a genome-wide basis under LSMMG.

## Experimental Activity

The availability of the complete genomic sequence, commercially produced genomic arrays and the well-characterized knowledge of its metabolism and gene regulation, led to the choice of *E. coli* as our first model system for the initial bacterial functional genomic (gene expression) analysis in LSMMG. Previously, we had compared mid-log LSMMG gene expression in minimal glucose media with control cultures (1 × g HARV, static flasks, and 250 rpm shaken cultures) under aerobic conditions. The 1 × g control HARV is treated in a manner identical to the LSMMG HARV (no bubbles, same rotation speed), but the angle of rotation is perpendicular to gravity, allowing the effects of gravity to act on the culture. Proteomics, analysis of media composition during growth, post-LSMMG resistances (antibiotic, stress), and molecular biology techniques are being employed for comparison to the functional genomic results for further identification and elucidation of the genes and operons regulated by LSMMG. A number of over-expressed LSMMG genes and gene knockouts in *E. coli* will be used to further elucidate their roles in modeled microgravity transcription and growth. These methods of analysis are being repeated in cultures

**Dr. George E. Fox, Professor of Biology and Biochemistry at the University of Houston, accesses frozen RNA samples refrigerated at -70°C.**



**RESEARCH**—Don Tucker, a member of the biological/biochemistry research team, studies the virulence of bacteria in the vacuum of space. Formerly a UH Post-Doctoral Aerospace Fellow, Dr. Tucker assumed full-time research activity in June 2004 in the NASA-JSC microbiological laboratories.



**FROZEN AT -70°C**—Dr. Tucker withdraws *RNA* samples from refrigeration for use in expression studies. Samples are kept at the coldest temperature (-70°C) to maintain their integrity.



**INSTRUMENTATION**—Estimates are that it takes \$1 million for a university to equip a research laboratory in its initial stages. Scientists expend considerable time writing proposals to gain local and federal funding.

grown in complete Luria-Bertani (LB) media and under anaerobic conditions.

## Results

Our analysis of the *E. coli* LSMMG culture physiology has encompassed growth rate, final culture yield, cell morphology, length of lag and exponential phases, media composition, and stress survival. Interestingly, little change in culture physiology has been observed between the LSMMG, 1 × g, and static cultures. Only when comparisons are made to 250 rpm shaken cultures are there significant differences. The shake flask cultures exhibit an increased growth rate and greater final culture density compared to the other cultures. To an extent, this was expected because of the increased aeration, availability of nutrients, and removal of wastes present in the shaken cultures. An overall oxygen content decrease in aerated HARV culture media was identified in conjunction with increased culture density, but a significant and likely sufficient level of oxygen was maintained for continuous aerobic growth. Additionally, growth in LSMMG has been found to have little effect on the ability of *E. coli* to survive a number of post-LSMMG stresses (antibiotics, acid, base, osmotic, heat, and free radicals) compared to 1 × g HARV control.

Expression studies conducted on *E. coli* grown under LSMMG revealed a substantial number of genes that were either up-regulated or down-regulated relative to controls in replicate experiments. While the majority of these genes are currently of unknown function, some of the genes with increased transcription in response to LSMMG are involved in the *E. coli* acid tolerance response system (transcriptional gene regulators [*yhiE*, *yhiF*] and the chaperone [*hdeA*]), or are involved in cell motility (many *flg* and *fli* genes). The *yhi* and *hde* genes identified are possibly involved in a general *E. coli* stress response system which is also activated by LSMMG. These identified changes in bacterial LSMMG gene expression could lead to increased cell survival, virulence, and antibiotic resistances, indicating serious potential problems during long-term space flight.

Although growth in the HARV bioreactor has a significant effect on bacterial gene expression in multiple strains, it remains to be determined to what extent the effects seen correlate with the different properties of the HARV environment. Preliminary results indicate that transcriptional regulation is induced by modeled microgravity (i.e., randomized gravity vector) of the HARV and not the low-shear fluid effects present in the rotating bioreactor. This question is being addressed by growing *E. coli* cultures in HARVs with various perturbed solution flows within the vessels. This perturbation of the low-shear phenomenon has, so far, been achieved through the addition of a small non-spherical plastic bead to the HARV culture media. Small air bubbles will be introduced into the HARV. Functional genomic analysis of these perturbed (i.e., shear) samples are helping to separate the low shear effects from the randomized gravity vector (modeled microgravity) present in the HARV.

It is interesting to note that while *Salmonella* and *E. coli* are closely related, there is limited comparability between the LSMMG expressed genes reported in *Salmonella*<sup>4</sup> and those

we have identified in *E. coli*. The reasons for these differences in gene expression are currently unknown and will require additional research.

The post-LSMMG stress results also differ between *E. coli* and *Salmonella*.<sup>4</sup> In this regard, it will be of special interest to replicate the media (already in progress) and other environmental conditions used in the earlier *Salmonella* work as exactly as possible in order to increase the comparability of the results. In addition, the LSMMG environment will be extended to the Gram-positive, spore forming bacterium *Bacillus subtilis*.

## Acknowledgments

We would like to thank Dr. Yuriy Fofanov in the Department of Computer Science at the University of Houston and the various members of his group (Tong-Bin Li, Chetan Belapurkar, Lulu Shi, R. Luo, and J. Wang) for their advice and assistance in performing statistical analyses on the microarray data.

## References

- <sup>1</sup>J. M. Jessup and N. R. Pellis. "NASA Biotechnology: Cell Science in Microgravity," *In Vitro Cell Dev. Biol. Anim.* 37 (2001): 61-63.
- <sup>2</sup>T. L. Prewett, T. J. Goodwin, and G. F. Spaulding. "Three-Dimensional Modeling of T-24 Human Bladder Carcinoma Cell Line: A New Simulated Microgravity Culture Vessel," *J. Tiss Cult Methods* 15 (1993): 29-36.
- <sup>3</sup>C. A. Nickerson, C. M. Ott, S. J. Mister, B. J. Morrow, L. Burns-Kelisher, and D. L. Pierson. "Microgravity as a Novel Environmental Signal Affecting *Salmonella enterica* serovar Typhimurium Virulence," *Infect. Immun.* 68 (2000): 3147-52.
- <sup>4</sup>J. W. Wilson, R. Ramamurthy, S. Porwollik, M. McClelland, T. Hammond, P. Allen, C. M. Ott, D. L. Pierson, and C. A. Nickerson. "Microarray Analysis Identifies *Salmonella* Genes belonging to the Low-Shear Modeled Microgravity Regulon," *Proc., Natl. Acad. Sci. U. S. A.* 1999 (2002): 13807-12.

## Publications

- Balan, S., J. C. Murphy, I. Galaev, A. Kumar, G. E. Fox, B. Mattiasson, and R. C. Willson, "Metal Chelate Affinity Precipitation of RNA and Purification of Plasmid DNA," *Biotechnology Letters* 25 (2003): 1111-16.
- DeWalt, B., J. C. Murphy, G. E. Fox, and R. C. Willson. "Compaction Agent Clarification of Microbial Lysates," *Protein Expression and Purification* 28(2003): 220-23.
- Kourentzi, K. D., G. E. Fox, and R. C. Willson. "Hybridization-Responsive Fluorescent DNA Probes Containing the Adenine Analog 2-AminoPurine," *Analytical Biochemistry* 322 (2003): 124-26.
- Martin, K. A., J. L. Siefert, S. Yerrapragada, Y. Lu, T. Z. McNeil, P. A. Moreno, G. M. Weinstock, W. R. Widger, and G. E. Fox. "Cyanobacterial Signature Genes," *Photosynthesis Research* 75 (2003): 211-21.
- Murphy, J. C., G. E. Fox, and R. C. Willson. "Enhancement of Anion-Exchange Chromatography of DNA Using Compaction Agents," *J. Chromatogr. A* 984 (2003): 215-21.

## At NASA Laboratories in Building 37



**DNA**—Dr. Mark Ott checks results in the genetic analyzer that sequences DNA. The equipment identifies genus and species of bacteria.



**FINGERPRINTS**—The computer screen shows DNA fingerprints from bacteria isolated aboard the International Space Station.



**MICROGRAVITY**—Dr. Don Tucker utilizes high aspect rotating vessels designed for modeling bacteria in a microgravity situation.



**DNA SEQUENCING**—In the Microbiology Laboratory at the Johnson Space Center, NASA scientists engaged in sequencing DNA from fungus and bacteria. Above are Victoria Castro, microbiologist (*l.*) who focuses on environmental lead and Sondra Fontenot, microbiologist (*r.*).



**PROJECT**—Crystal Kalk, senior at Clear Creek High School, conducts research at the nation's finest laboratory near her home school.

- Murphy, J. C., D. L. Jewell, K. I. White, G. E. Fox, and R. C. Willson. "Nucleic Acid Separations Using Immobilized Metal Affinity Chromatography," *Biotechnology Progress* 19 (2003): 982-86.
- Nagaswamy, U. and G. E. Fox. "RNA Ligation and the Origin of tRNA," *Orig Life Evol Biosph.* 33 (2003): 199-209.

### Presentations

- Belapurkar, C., T. B. Li, G. E. Fox, R. C. Willson, and Y. Fofanov. "Improved R-Q Set Operations Facilitate Subsequence Analysis of Genomes," 20th Annual Meeting of Houston Society for Engineering in Medicine and Biology, Houston, TX, April 3-4, 2003.
- DeWalt, B., J. C. Murphy, T. Cano, J. Zijffer, G. E. Fox, and R. C. Willson. "Compaction Agent Clarification of Microbial Lysates," Annual Meeting American Chemical Society, New Orleans, LA, March 23-27, 2003.
- D'Souza, L. M., M. Larios-Sanz, R. A. Setterquist, R. C. Willson, and G. E. Fox. "Numerous Primary Sequences can be Incorporated into Artificial Stable RNAs," Environmental Biotechnology Session, Annual Meeting American Chemical Society, New Orleans, LA, March 23-27, 2003.
- Huang, H.-C., U. Nagaswamy, and G. E. Fox. "Structural Bioinformatics on Ribosomal RNA," Eighth Annual Structural Biology Symposium, Sealy Center for Structural Biology, Galveston, TX, May 2-4, 2003.
- Larios-Sanz, M., K. D. Kourentzi, G. E. Fox, and R. C. Willson. "Microbial Identification Using Signature Probes," Annual Meeting American Chemical Society, New Orleans, LA, March 23-27, 2003.
- Larios-Sanz, M., K. Kourentzi, Z. Zhang, R. C. Willson, D. L. Pierson, D. L. Tucker, and G. E. Fox. "Molecular Tools To Monitor Microbial Ecosystems During Long-Term Exploration Class Missions," 103rd General Meeting of the American Society for Microbiology, Washington, D.C., May 18-22, 2003.
- Nagaswamy, U. and G. E. Fox. "Transfer RNA May Have Arisen by RNA Ligation," NASA Astrobiology General Meeting 2003, Phoenix, AZ, Feb. 10-12, 2003.
- Shi, L., T. B. Li, D. Tucker, F. Karouia, R. C. Willson, G. E. Fox, and Y. A. Fofanov. "Pair-Wise Correlation Analysis Applied to Gene Expression Data from Two *Escherichia coli* Strains," 20th Annual Meeting of the Houston Society for Engineering in Medicine and Biology, Houston, TX, April 3-4, 2003 (*poster presentation*).
- Tucker, D. L., C. M. Ott, D. L. Pierson, R. C. Willson, and G. E. Fox. "Functional Genomic Analysis of *E. coli* in a Low-Shear Modeled Microgravity Environment," 103rd General Meeting of the American Society Microbiology, Washington, D.C., May 18-22, 2003.
- Wang, J., U. Nagaswamy, and G. E. Fox. "A Pair of Partially Identical Ribosomal Proteins," Eighth Annual Structural Biology Symposium, Sealy Center for Structural Biology, Galveston, TX, May 2-4, 2003.
- Wang J., Y. Luo, T. B. Li, D. L. Tucker, F. Karouia, G. E. Fox, R. C. Willson, and Y. Fofanov. "ImageAnalyzer: A flexible Application for Microarray Image Analysis," Eighth Annual Structural Biology Symposium, Sealy Center for

- Structural Biology, Galveston, TX, May 2-4, 2003.
- Willson, R. C. and G. E. Fox. "New Approaches to Nucleic Acid Purification: Compaction Precipitation and Metal-chelate Affinity," International Symposium on Biorecognition and Affinity Technology, Cambridge, UK, Aug. 2003.
- Yerrapragada, S., J. L. Siefert, K. A. Martin, and G. E. Fox. "Cyanobacterial Signature Genes," NASA Astrobiology General Meeting 2003, Phoenix, AZ, Feb. 10-12, 2003.

### Funding and proposals

- Fox, G. E. "Chiral-Selective Planetary Chemistries as a Marker for Life." NASA ASTID Program, requested for two years, \$429,920 (*submitted*).
- . "Evolution of Genomes and Cellular Processes in Astronomically Reasonable Environments." NASA Astrobiology Institute, June 1, 2003-May 31, 2008, \$7,188,349 (*not funded*). (The project was to have involved 15 laboratories at five institutions.)
- . "The Origins of Translation and Early Life." NASA Astrobiology Program, July 1, 2002-June 30, 2005, \$343,177.
- Fox, G. E. and R. C. Willson. "Microorganisms in the Spacecraft Environment." National Space Biomedical Research Institute, Oct. 1, 2000-Feb. 29, 2004, \$966,040.
- Fox, G. E. and R. C. Willson. "Microorganisms in the Spacecraft Environment." Office of Biological and Physical Research Individual Grant, requested for four years, \$1,532,438.
- Willson, R. C. and G. E. Fox. "Quantitative Polymerase Chain Reaction (Q-PCR) Technology to Measure Molecular Contamination and Validate Subsystem Cleanliness." NASA Planetary Protection Program. PI: A. Driks, Loyola University; UH subcontract, three years, \$100,000 (*not funded*).