The Effect of Modeled Microgravity on Microbial Gene Expression

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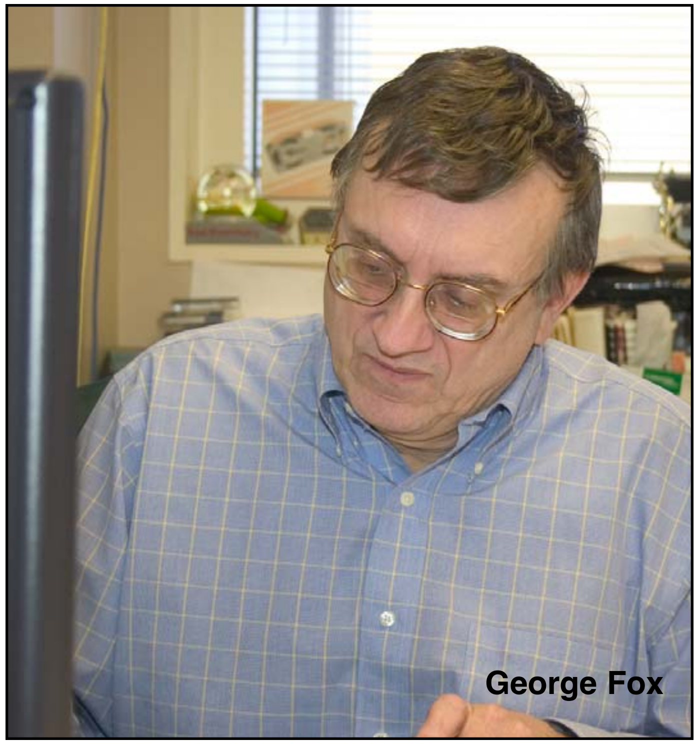
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Abstract—The space environment is characterized by microgravity and high background radiation. The absence of gravity produces low shear environments, which likely result in microorganisms having difficulty in removing themselves from immediate surroundings that have been nutrient depleted and which have received waste products. In order to adapt to life in a low shear world, bacteria likely express different combinations of genes than they do in more usual laboratory environments and may ultimately make evolutionary adaptations as well. Thus, a particular bacterium may exhibit properties such as antibiotic resistance, biofilm formation, or virulence that are not generally associated with it. For example, recent evidence indicates increased *Salmonella* virulence in response to modeled microgravity. Changes in physiology, gene transcription and regulation are being examined in *Escherichia coli* cells grown in the low-shear modeled microgravity environment (LSMMG) found in a high aspect rotating vessel (HARV).

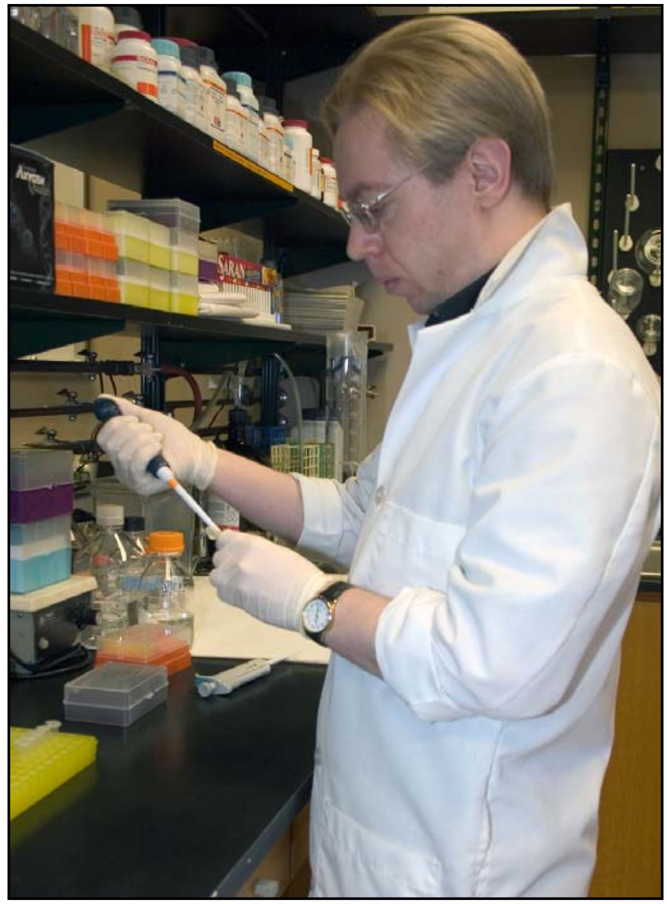
Aerobic LSMMG cultures were grown in rich (LB) and minimal (MOPS + glucose) medium with a normal gravity vector HARV control. Reproducible changes in transcription were seen but no direct response to changes in the gravity vector identified. Instead, absence of shear and a randomized gravity vector appeared to cause local extra-cellular environmental changes, which elicited reproducible cellular responses. In minimal media, the majority of the significantly up- or down-regulated genes of known function were associated with the cell envelope. Conversely, in rich medium the majorities of genes were LSMMG down-regulated and involved in translation. Comparison with earlier studies of *Salmonella enterica* serovar Typhimurium conducted under similar growth conditions revealed essentially no similarity in genes that were significantly up- or down-regulated. Given the substantial overlap in gene content between these closely related organisms, this result clearly demonstrates that different organisms may dramatically differ in their responses to medically significant low-shear and space environments.

THE ABILITY TO ADAPT TO ENVIRONMENTAL CHANGE IS extremely important to bacteria. As a result most free-living organisms can adapt to survive in a variety of environments. Such adaptation is facilitated in the short-term by changes in the types of genes expressed and the relative levels of expression. For example, *Escherichia coli* can use nitrite as a terminal electron acceptor and thrive under anaerobic conditions. In the long-term, evolutionary changes may occur as well. In principle, complete knowledge of the genes carried in an organism's genome and how they are regulated would be sufficient to anticipate the full range of possible responses that can be elicited from any organism. Although genomics has largely identified the genes, it is not always clear what the gene products actually do. The regulatory information is to a significant extent unknown. Thus, even an intensively studied organism may respond to novel environments in unanticipated ways. One such environment is the low shear-high radiation background environment encountered in 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.[1] 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.[2] For example, a recent study[3] showed that *Salmonella enterica* serovar Typhimurium grown under low-shear modeled microgravity (LSMMG) appeared to have increased virulence potential in a murine model system. A follow-up study[4] revealed that a significant number of the genes are transcriptionally regulated in response to LSMMG. Increased resistance to antibiotics and low pH was also identified.[4] 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 HARV's rotation 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 and 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.

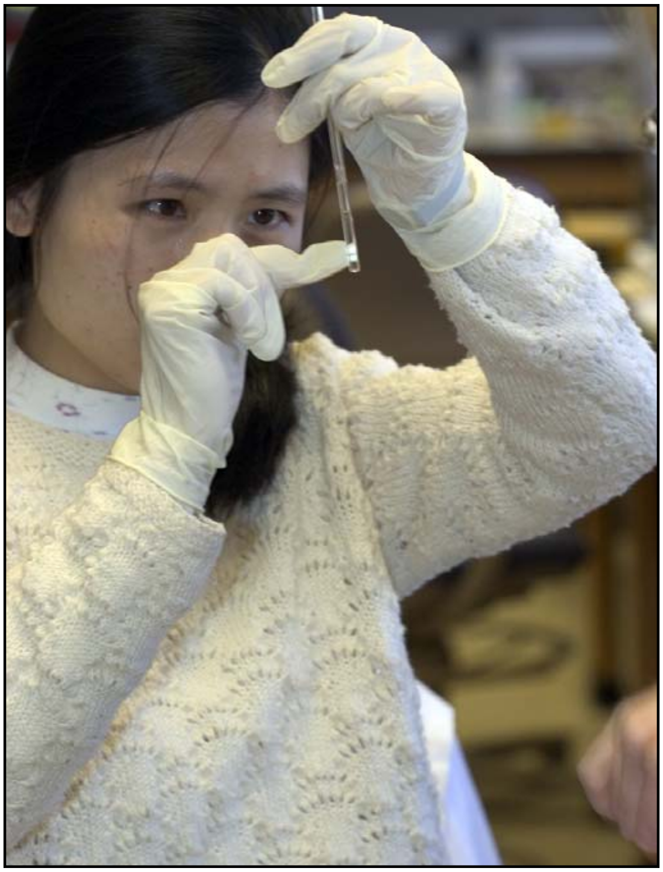


MICROGRAVITY—Viktor Stepanov, post-doctoral Aeronautical Fellow (PDAF), specializes in molecular and structural biology. A native of Russia, he earned his Ph.D. at the Aarhus University in Denmark. He is a principal in studies of physiology.

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 identically 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.

These methods of analysis are being repeated in cultures grown in complete (LB) media and under anaerobic conditions. Future work will look at long term adaptation as well.



BIOCHEMISTRY—Qin Zhao, a graduate student in biochemistry, conducts research in Dr. George Fox's laboratory. Originally from Anhui, P.R.China, she earned her B.S. degree in biochemistry at Wuhan University.

Results

The primary differences between the LSMMG environment and the control are the randomized gravity vector and low shear present in LSMMG. In attempting to interpret the differences seen, one must consider that they might be due to either or both of these factors or as an indirect effect of one or both. In minimal MOPS medium, chemotactic and flagellar genes as well as genes involved in the acid tolerance response were up-regulated in LSMMG.

It is attractive to theorize that the LSMMG up-regulation of flagellar and chemotactic genes in minimal medium is related to a cellular requirement for relocation away from zones of local nutrient depletion and excreted waste hypothesized to occur in the low mixing environment of space.[5] Although an enticing hypothesis, the presence of these zones in HARV-produced LSMMG and the determination whether or not *E. coli* MG1655 is responding to these zones by up-regulating flagellar and the chemotactic genes requires further study.

The majority of minimal medium LSMMG down-regulated genes are involved in metal or drug transport, cell lysis, or in regulating cellular stress responses, which alludes to the importance of the cell envelope in regulating the LSMMG response in minimal medium grown *E. coli* MG1655. More generally, all of the LSMMG up-regulated genes and a majority of the down-regulated genes of known function are present in or involved with regulation of the cellular envelope. This suggests that the cell envelope is superlative in sensing changes in its local environment and able to respond rapidly to the changes in multifaceted ways. Future time course studies of the LSMMG response to minimal media in cells preadapted to the HARV control environment may allow detailed study of how the genes involved are coordinated.

S. Typhimurium is an evolutionarily very close relative of *E. coli*. Its response to LSMMG had been studied previously.[4] In fact, the majority of the *E. coli* MG1655 LSMMG up- and down-regulated genes have homologues or orthologues in S. Typhimurium. We therefore reduced the statistical stringency of our analysis so that a direct comparison could be made with the *Salmonella* results of these earlier studies. A greater number of genes were down-regulated rather than up-regulated in both organisms under a rich medium condition. In addition, many of the genes that responded to LSMMG conditions were clustered in known or likely operons. However, when individual genes were intercompared, it was abundantly clear that the vast majority of genes affected by LSMMG in *E. coli* MG1655 and S. Typhimurium were not affected in the same manner in the other organism. S. Typhimurium may be responding to LSMMG by activating genes involved in pathogenicity and adhesion in an attempt to promote colonization in the low-shear environment. *E. coli* MG1655 is a commensal that lacks many of the genes associated with pathogenesis in S. Typhimurium; hence, adhesion in preparation for colonization is apparently not its preferred response to LSMMG. Thus, the dramatically different response to LSMMG observed between *E. coli* MG1655 and S. Typhimurium emphasizes that different species can respond to LSMMG in very different ways. This is a frustrating conclusion for those seeking to ascertain what the effect of exposure to low-shear or the space environment will be for microorganisms in general.

Bacteria, having lived on the Earth for billions of years, have not typically encountered microgravity; therefore, it would seem unlikely that genes governing a direct response to variations in gravity would have evolved. With specific reference to the LSMMG environment then, researchers would anticipate that low-shear is more important in the bacterial transcriptional response than as a direct effect of the randomized gravity vector. If there were a specific response to changes in the gravity vector, these changes would likely be seen regardless of growth condition. Based on the comparison of LSMMG regulated genes in rich and minimal media, and on preliminary studies under anaerobic growth conditions, there does not appear to be such a generalized LSMMG response system or gene in *E. coli* MG1655. This conclusion is further supported by the absence of a strong correlation with the responses seen in S. Typhimurium. Thus, the response to LSMMG seen here is more likely a response to conditions created by the loss of the gravity vector, e.g., low shear, than to gravity itself.

Acknowledgments

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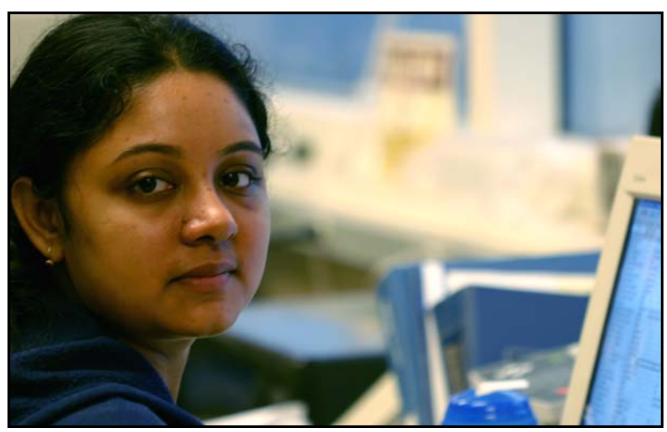

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