

The Effect of Simulated Microgravity on Microbial Gene Expression

by George E. Fox and Duane Pierson

BACTERIA ARE CAPABLE of living in and adapting to a far larger range of environmental conditions than are normally encountered in the usual laboratory environments. Even with full knowledge of an organism's gene content, it is currently impossible to predict how expression patterns will change in different situations. Thus, usual laboratory growth conditions may not invoke key aspects of an organism's potential response. Such studies thereby may conceal behaviors that in a different environment may contribute to undesirable phenomena such as pathogenesis. One such case is the low-shear, low-turbulence environments present in utero, at the brush border microvilli of epithelial cells, and other

medically important host environments.¹ Another example is the space environment characterized by microgravity and high background radiation. In this case, the absence of gravity also produces a low shear environment, which likely results in microorganisms having difficulty in removing themselves from immediate surroundings that have been nutrient depleted and have received waste products.²

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. It is not certain, therefore, which organisms may be problematic. For example, a recent study³ 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 study⁴ revealed that a significant number of the genes are transcriptionally regulated in



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organisms may dramatically differ in their responses to medically significant low-shear and space environments. Current efforts are focused on assessing the extent to which the response changes over the long-term as a result of evolutionary adaptation. To this end, a cell line has now been cultured in the LSMMG environment for 1000 generations and is currently being studied. The project is part of a larger effort to identify, monitor, and assess the effect of exposure on microorganisms in spacecraft environments.

ABSTRACT—The effect of long-term exposure to low-shear modeled microgravity (LSMMG) on microbial gene expression and physiology is being examined using functional genomics and molecular techniques in *Escherichia coli*. In short-term studies, reproducible changes in transcription were seen but no direct response to changes in the gravity vector was 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. Comparison with earlier studies of *Salmonella enterica serovar Typhimurium* conducted under similar growth conditions revealed essentially no similarity in genes, which were significantly up or down-regulated. Given the substantial overlap in gene content between these closely related organisms, this result clearly demonstrates that different

response to LSMMG. Thus, it is essential to better understand both the long- and short-term effect of the microgravity environment on bacterial behavior.

Methodology

Wild type *E. coli* MG1655 (CGSC7740) was grown aerobically at 37°C, in rich (LB) and minimal MOPS plus glucose medium in low shear modeled microgravity (LSMMG) and a normal gravity vector control environment. They were compared with each other as well as in control experiments conducted with a normal gravity vector.

In order to conduct long-term studies, a sterilization procedure was developed in which two reactors were alternatively used. The reactor not in use was rinsed with an antibiotic solution and exposed to UV light. This protocol has allowed cultures to be maintained for more than 1000 generations. Thirteen separate minimal MOPS and 16 LB medium growth experiments were performed. Total RNA was extracted from cells and labeled following removal of ribosomal RNA, radioactively. Whole genome transcriptional assays were per-

formed using matched pairs of PCR-product DNA macroarrays (Panorama *E. coli* Gene Arrays; Sigma-Genosys, Houston, TX). The hybridized membrane phosphorimages were imported into image analysis software. Data were normalized by reporting each spot as a percentage of the sum of intensities of all spots on the array image. Significant changes in gene expression were identified based on three previously documented criteria: (1) an overall p-value of < 0.05 which implies a 95 percent probability that a change in expression between strains or media was significant; (2) a log ratio of gene expression which differed from the mean of the log ratios by > 3.0 standard deviations giving a 99.9 percent confidence in gene expression; and (3) similar gene expression in all three biological replicates.

Online databases were used for gene nomenclature, gene location and orientation, putative co-transcription, product function, and presence in *S. Typhimurium*. The Colibri WWW Server v3.1 (<http://genolist.pasteur.fr/Colibri/genome.cgi>) was used to determine individual gene locations and orientations in the *E. coli* genome as well as possible co-transcription with other expressed genes. EcoCyc (Institute for Genomic Research, University of California; San Diego, CA (<http://ecocyc.org/>)) and EcoSearch (University of Miami School of Medicine; Miami, FL (<http://bmb.med.miami.edu/search.htm>)) were used in determining gene names / synonyms and gene product function. Researchers used the coliBase website (<http://colibase.bham.ac.uk/>) to identify genes significantly expressed in this study that are present or have an orthologue in *S. Typhimurium*.

Separately, following growth in the LSMMG environment, the cultures were subjected to antibiotic sensitivity and stress resistance studies to determine if their response had been changed by the exposure to the LSMMG environment. Antibiotics employed were ampicillin, kanamycin, polymyxin E, chloramphenicol and rifampicin. Stress conditions included acidic and basic conditions, oxidative stress, osmotic stress, alcohol stress, and heat shock. Culture survival was compared to controls that had not been grown under LSMMG conditions.

Equipment/Special Technology

The project takes advantage of the High aspect rotating vessel (HARV) bioreactor which was originally developed by NASA scientists⁵ 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.

Results

Statistical analysis identified 19 up-regulated and 24 down-regulated genes in LSMMG compared to the control during the mid-log phase of growth in minimal MOPS medium. Of these, one up-regulated and 12 down-regulated genes coded for hypothetical proteins. Among the up-regulated genes of known or putative function in LSMMG are genes involved in the *E. coli* acid tolerance response: transcriptional regulator *gadE*, the putative chaperone *hdeA*, and associated genes *hdeB*, *hdeD* and *dctR*, *flg* and *fli* genes involved in cell motility, chemotaxis regulating genes *cheY*, *cheZ* and *tar*, and the phage related gene *ydfD*.

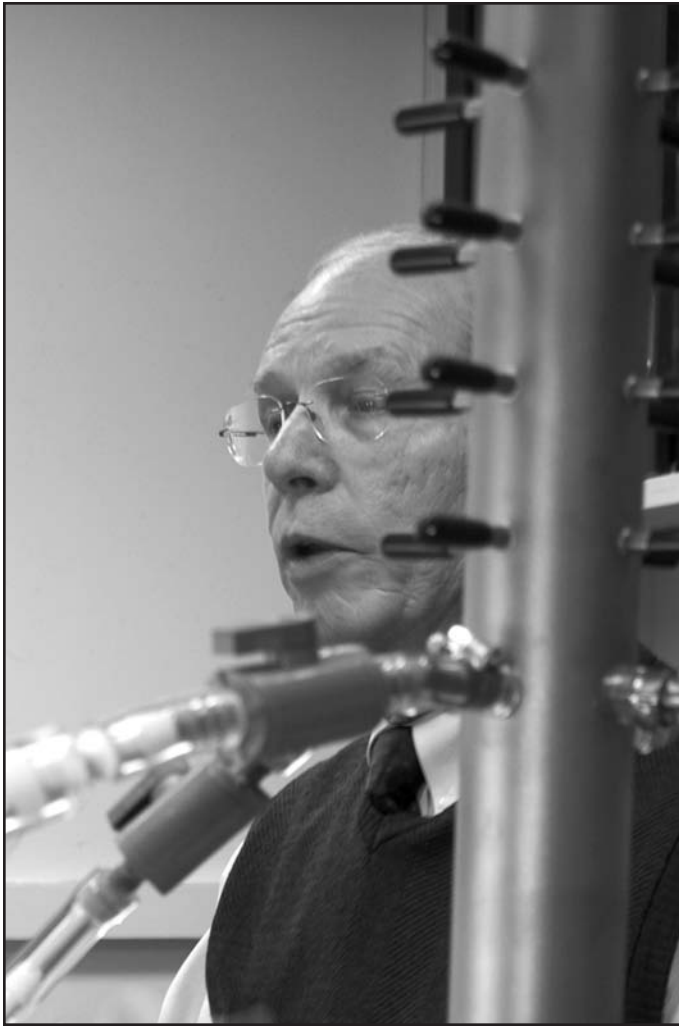
Among the MOPS LSMMG down-regulated genes were five genes involved in heavy metal efflux (*CusCFBA* and *copA*). Other LSMMG down-regulated genes included five putative bacteriophage lambda homologs, four genes involved in various stress responses, the drug resistance gene *emrE*, the acetylCoA carboxylase subunit *accB*, and the putative NAD(P) binding enzyme *ybeM*. Two of the MOPS LSMMG down-regulated genes (*cpxP* and *yfiA*) were regulators. *CpxP* serves as repressor of the *Cpx* envelope / extracytoplasmic toxicity stress response system, protects the cell from toxic, transitory stresses, is involved in adhesion and virulence of pathogenic *E. coli*, and may also act as a periplasmic chaperone. *YfiA* stabilizes 30S rRNA under cold shock conditions. Possible co-transcribed genes of putative operons were identified based on genomic location and orientation.

Physical mapping of the LSMMG MOPS regulated genes found 34 of 43 genes in four gene clusters. Similar analysis of the LB LSMMG cultures identified seven down-regulated genes in LSMMG. These genes were mostly involved in biosynthesis and energy utilization. No significant change in response relative to the controls was seen with any of the antibiotics or stresses that were tested.

Salmonella Typhimurium is an evolutionarily very close relative of *E. coli* and 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 earlier Salmonella results. 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.

Discussion

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, *E. coli* chemotactic and flagellar genes, as well as genes involved in the acid tolerance



CONTRIBUTOR—Dr. Duane L. Pierson, NASA-JSC, conducts seminars on bacteria in space focusing on the characterization of *E. coli* grown in a low-shear modeled microgravity environment.

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.⁶ 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 rapidly respond to the changes in a multifaceted way. 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 appears to be responding to LSMMG by activating genes associated with adhesion in an attempt to promote colonization in the low-shear environment. These are the same genes usually associated with pathogenicity. In contrast, *E. coli* MG1655 is a commensal that lacks many of these genes and hence adhesion in preparation for colonization is apparently not its preferred response to LSMMG.

Conclusions

Bacteria, having lived on the Earth for billions of years, have not typically encountered microgravity and hence 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, it would be anticipated that low-shear is more important in the bacterial transcriptional response than a direct effect of the randomized gravity vector. The experiments to date have confirmed this conclusion. In addition, our studies have reinforced the notion that the cell envelope is superlative in sensing changes in its local environment and able to rapidly respond to the changes in a multifaceted way. 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. However, the dramatically different response to LSMMG that is 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 the effect of exposure to low-shear or the space environment for microorganisms in general.

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