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TRANSCRIPTIONAL AND PHYSIOLOGICAL CHARACTERIZATION OF ESCHERICHIA COLI K12 MG1655 GROWN UNDER LOW SHEAR SIMULATED MICROGRAVITY FOR 1000 GENERATION

Abstract

Microbes inhabit and survive in a wide range of ecological niches and adapt to changing environmental conditions. Low-shear and low turbulence are unique conditions encountered in space that can negatively impact human health and living conditions. Low shear stress has been shown to induce increased virulence and biofilm formation in several Gram-negative bacteria. Understanding this microgravity stress response is at a nascent stage. Little is known about the effects of long-term exposure to such environments. The initial response is clearly the result of changes in gene expression patterns, but will this be followed by genetic changes and hence a permanent response? The lac+ E. coli MG1655 was grown for 1000 generations in low shear modeled microgravity, (LSMMG). Transcriptional analysis revealed that expression of 357 genes differed from the immediate response. Initial genomic re-sequencing studies revealed genomic changes in some of these genes. Fimbriae encoding genes were significantly up-regulated, whereas genes encoding the flagellar motor complex were down-regulated. 81 genes whose expression patterns changed are implicated in and/or associated with biofilm formation. The remaining up-regulated genes may be triggering expression of genes associated with the type II secretion complex. This complex has been associated with virulence factors and members of the multidrug efflux system, which confer resistance

to antimicrobial agents and antibiotics. Biofilm formation and the aggregation of cells were evaluated by scanning electron microscopy. The analysis revealed that extracellular matrix and complex cellular networking were present among cells that were exposed to LSMMG for the extended period. Thus, the immediate response to LSMMG is apparently replaced by a permanent one that includes genomic adaptation. In order to facilitate long-term response experiments, a competition assay was developed using E. coli strain NCM520, which is a lac- variant of MG1655. When grown on MacConkey agar, MG1655 produces red colonies while NCM520 produces white colonies. When grown together neither strain has a selective advantage. The lac+ strain was exposed to LSMMG for one 24 hour cycle under LSMMG, while the lac- strain was grown under control 1g conditions. The two strains were then combined and grown under LSMMG for a second hour period. With just one cycle of exposure to microgravity conditions, the lac+ strain had a definite selective advantage over the unadapted lac- strain. By competing these isogenic strains from generation X and generation X+N, this assay will allow us to determine how many generations are required before adaptation has largely stopped.