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LONG-TERM EVOLUTION STUDIES OF E. COLI MG1655 UNDER THE COMBINED STRESS OF LOW SHEAR MODELED MICROGRAVITY (LSMMG) AND THE BROAD SPECTRUM ANTIBIOTIC CHLORAMPHENICOL

Abstract

Introduction: Organisms exposed to the space environment for extended periods of time may evolve in unanticipated ways thereby negatively impacting long duration space missions. We report here, an experimental study of microbial evolution in which the effect of long-term exposure to LSMMG on microbial gene expression and physiology in *Escherichia coli* (*E. coli*) was examined using functional genomics, and molecular techniques with and with-out simultaneous exposure to broad spectrum antibiotic chloramphenicol. *E. coli* MG1655 was grown under simulated microgravity for 1000 generations in High Aspect Ratio Vessels (HARVs) that were either heat-sterilized (115 deg C, 15 min) or by using/rinsing the HARVs with a saturated solution of the broad-spectrum antibiotic chloramphenicol. Gene expression patterns and cellular physiology were analyzed in comparison with short-term expo-sure.

Results: In the case of the cells evolved using the antibiotic sterilized HARVs, the expression levels of 357 genes were significantly changed. In particular, fimbriae encoding genes were significantly up-regulated whereas genes encoding the flagellar motor complex were down-regulated. Resequencing of the genome revealed that a number of the flagellar genes were actually deleted. The antibiotic resistance levels of the evolved strains were analyzed using VITEK analyzer. The evolved strain was consistently resistant to the antibiotics used, even after 11 cycles of ‘erasure’ of the ‘adaptation memory’ – this ‘erasure’ was accomplished by re-growing the evolved cells under shaker flask conditions and 1 cycle equals 10 generations.

In the case of the cells evolved using heat sterilized HARVs, no resistance was observed to any of the antibiotics used, even after 1000 generations of growth under LSMMG.

Competition experiments using an isogenic pair revealed that the adaptive advantage of the 1000G strain (in both cases) over an unexposed strain was rapidly eliminated.

While this obviously implies that the adaptation was both environmental and genomic, the levels of antibiotic resistance observed to be consistently maintained, raises the concern of persistent resistance conferred to bacterial communities through exposure to antibiotics on space missions.