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Genomics Investigation Of The Human Gut Microbiome In Microgravity Using Gel Microdroplets And Community Cultivation Strategies

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Long term space travel for scientific and defense related missions is inevitable. As surprising evidence has shown that commonly nascent bacterial species – isolates that are known members of the human gut and skin microbiomes – increased their mutation rates and virulence due to exposure to microgravity conditions, it is, thus, conceivable that certain members of the normal human microbiota may become pathogenic to the human host during long-term space travel. While this scenario entails an enrichment of pathogens due to microgravity exposure, we also postulate that certain key bacterial species that normally place the opportunistic pathogens in check may become depleted, thereby creating a suitable environment that permits pathogenicity. Because of challenges to study such processes in humans in space, our understanding of the mechanisms that lead to pathogenicity of such microbes in gut is completely lacking.

Herein, Los Alamos National Laboratory Bioscience Division and Rhodium Scientific seek to understand the effects of microgravity exposure to the normal human gut microbiome at the community level. By potentially preserving the native cell-to-cell interactions within the gut microbial community, our novel cultivation-to-genomic investigation offers a means to identify the enriched or depleted gut bacterial taxa during microgravity exposure and, thus, present insights into maintaining the health of the human host through the developments of both medical countermeasures against pathogens and/or probiotics. Our team utilized a novel, dual in vitro cultivation strategy involving bulk cultures and gel microdroplets (GMDs), both purposed to genomically identify changes in the human gut microbial community structure over time, when comparing flight (microgravity) to ground (control) cultures.

Extensive analyses of the bulk community revealed potential trends of bacterial growth – increasing or decreasing in relative abundance – which permitted the identification and further investigation of specific bacterial taxa exhibiting such trends. Our findings, among others, revealed a notable enrichment of bacteria associated with human gut diseases, including *Dialister*, *Enterococcus*, and *Dorea* spp. Our findings also identified trends in suggested beneficial bacteria, including *Roseburia* and *Faecalibacterium* spp. Furthermore, our bulk community analyses revealed the novel human gut bacteria at various taxonomic levels. For the GMD cultures, our team viably recovered several bacterial isolates from the flight cultures, including *Enterococcus*, *Staphylococcus*, *Bacillus*, and putative *Catelicococcus* spp. Based on 16S rRNA phylogenetic analysis, these post-flight recovered bacterial isolates represent novel Genus and species in the bacterial Domain, and particularly for the *Enterococcus*, *Bacillus*, and *Catelicococcus* isolates, have direct ties to the human gut microbiome.

Our dual cultivation approach was designed as a low-cost, rapid means to interrogate the complex human gut microbiome amenable to genomic analyses to infer how microgravity may affect the gut microbiome at the community level. Our study demonstrates the simultaneous utility of both cultivation strategies as the foundation for expanded, in-depth, 'omics-based investigations (e.g., transcriptome, proteome, metabolome) that will, undoubtedly, provide revealing insights to the dynamics of gut microbiome interactions in the human gut, and how these interactions will affect the space traveling human host.

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