Is Alternate Sigma Factor H (SigH) Essential for the Mycobacterium marinum Response to Low-Shear Modeled Microgravity (LSMMG)?

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Many bacteria have been observed to become more infectious in spaceflight, and astronauts are more susceptible to infection in space. Alternate sigma factor H (SigH) has been identified as a possible protein involved in the microgravity-related stress response of Mycobacterium marinum, a member of the biofilm-forming Mycobacteria genus and an excellent model for M. tuberculosis. The purpose of this experiment is to determine if SigH is essential for the Mycobacterium marinum survival response to microgravity. It is hypothesized that if sigH is disabled, M. marinum will have decreased growth and survival when grown in simulated microgravity. CRISPRi vectors were generated and transformed into M. marinum to decrease transcription of the sigH gene. PCR was used to determine the successful ligation of the three sgRNA and one control DNA sequence into the CRISPRi vector and successful integration of the CRISPRi vectors into M. marinum. Cultures were grown, and RNA was made from strains with and without the activated CRISPRi complex. Quantitative real-time PCR from cDNA indicates that the CRISPRi complex can reduce SigH expression by 90-95%. After four days and forty hours of growth in LSMMG, SigH-disabled M. marinum show increased growth compared to NG. This suggests that decreased growth in LSMMG is part of the M. marinum survival response induced by SigH.

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