APPLICATION P97T79WR

1. Describe the scientific problem that you propose to address. What is the question you are trying to answer? What makes it significant, relevant, and interesting?

Water contamination by pathogenic bacteria has been observed a number of times in human spaceflight, particularly on the International Space Station [1,2]. Despite the health threats posed by pathogenic infection, detecting and monitoring water contamination remains a challenge. Approaches used on Earth include culturing the pathogenic bacterium and sequencing its 16S rRNA, a process that can take multiple days and requires access to specialized equipment [3]. Here, I propose a cell-free biosensor for rapid detection of the water contaminant Pseudomonas aeruginosa. This pathogen requires minimal nutrition and is able to form biofilms in a variety of environments, including freshwater sources, the lungs and bloodstreams of intensive care patients, and water appliances [4]. Moreover, this multidrug-resistant bacterium is frequently found as a pathogen in drinking water and is the cause of several waterborne diseases, the risks of which increase in microgravity, where biofilm growth is accelerated [5,6,7]. To control population density, P. aeruginosa utilizes quorum sensing, a form of cell-cell communication mediated through the exchange of metabolites [8]. This biosensor would detect a homoserine lactone involved in the QscR quorum sensing pathway to produce a fluorescent readout, informing astronauts whether their drinking water is contaminated by P. aeruginosa.

2. State your hypothesis and explain your reasoning. Based on your background research, what results do you expect to see?

My study aims to test whether a cell-free biosensor could be used to detect the presence of P. aeruginosa in drinking water aboard the ISS. Using the BioBits kit and P51 Fluorescence Viewer, this system is engineered to produce and detect a fluorescent readout in the presence of the P. aeruginosa quorum sensing molecule N-3-(oxododecanoyl)-l-homoserine lactone (OdDHL). Previous studies have reported the development of whole-cell biosensors for the detection of waterborne bacterial pathogens, and cell-free systems have also been developed to an extent [9,10,11]. The use of BioBits would streamline this process, as users would add a water sample to a cell-free biosensor and examine if a fluorescent readout is produced. Although microgravity conditions differ from conditions on Earth, I hypothesize that this biosensor will still function aboard the ISS and will only fluoresce in the presence of OdDHL. The objective of this study is to optimize a set of parameters for the use of the biosensor, including the time required for a fluorescent signal and its detection limit. The development of such technology aboard the ISS may provide the means for point-of-care detection of waterborne pathogen metabolites, prompting astronauts' further investigation into potential bacterial contamination.

3. Outline your experimental plan. How will you use the tools in the Genes in Space Toolkit to test your hypothesis? Be sure to specify the samples you will analyze, controls that you will use, and the possible experimental outcomes.

The basis of the biosensor, BioBits, will contain two modules: a reporting and a sensing module. The sensing module will constitutively express the transcription factor qscR, which can bind to OdDHL. This qscR-OdDHL complex can then bind to the inducible promoter QscR, which is contained in the sensing module, thus activating the expression of red fluorescent protein (RFP). The experiment will contain 1 negative control, 3 experimental tests, and a final prototype test, each of which will be run in triplicate both on Earth and in space. Every 0.5 hours for 3 hours, all trials will be viewed under the P51 Fluorescence Viewer to record whether a fluorescent signal is observable. In the negative control, the biosensor will be resuspended with distilled water to confirm that the system is only activated in the presence of OdDHL. In the preliminary experimental tests, distilled water samples containing 1.0E-9, 1.0E-8, and 1.0E-7 molar concentrations of purified OdDHL (commercially-available) will be used. Concentrations were selected based on previous studies in cell-free systems and will be used to gauge the detection limit [12]. Once the biosensor's optimal parameters have been identified, drinking water from Earth and the ISS will be tested.

4. Explain why you selected the tools you incorporated into your experimental plan. What makes them a good fit for your research question?

In space, cell-free systems such as those enabled by BioBits are more efficient compared to whole-cell and sequencing-based means of bacterial detection, as they require minimal equipment and, unlike cell lines, do not need to be cultured and maintained [13]. In addition, both the P51 Fluorescence Viewer and the bioengineered cell-free system are inexpensive and user-friendly, enabling astronauts to test their drinking water quality frequently and with ease. By complementing BioBits with the fluorescence viewer, we are able to selectively produce and detect a fluorescent signal in the presence of a certain signaling molecule. With regards to the experimental design, I chose to utilize the QscR quorum sensing pathway as OdDHL is specific to and secreted in large amounts by P. aeruginosa, and exhibits high binding affinity to qscR [14]. Moreover, unlike the metabolites involved in many other Pseudomonas quorum sensing pathways, less than 1% of OdDHL is contained within vesicles, allowing drinking water samples to be directly transferred into the biosensor for analysis [15]. Finally, I chose RFP as the reporting molecule as one key attribute of P. aeruginosa biofilms is their fluorescent green-blue color [16].

5. Justify your use of the International Space Station (ISS) by explaining how the unique environmental conditions found on board are essential for your experiment. Then explain what future space travelers stand to gain from your experiment. Write your answer as if you were speaking to an audience of non-scientists.

Safety and health are of utmost importance when voyaging into space. Furthermore, reducing the risk of disease, particularly the diseases caused by microbes that can contaminate water supplies, is critical on long-term space missions. I have proposed a bioengineered sensor that produces a visible, fluorescent red color when a certain dangerous microbe that otherwise can't be seen with the human eye is detected in water. This inexpensive biosensor can be used to efficiently and safely detect pathogenic bacteria. The implications of such technology on spaceflight require that it is first tested aboard the ISS, where microgravity conditions may affect its performance. The development of this biosensor in space would help to ensure human health on prolonged in-space missions. Astronauts would feel safer knowing that this technology can be used to reliably test their drinking water as frequently as they wish. Traveling miles into space won't isolate astronauts from the biomedical issues faced on Earth, and preparation is the best strategy. If this technology is proven to be operational in space, future biosensors could be developed to detect other pathogenic bacteria using similar genetic circuits, or detect for multiple species in one kit, each with its own fluorescent signal.

6. How did you hear about Genes in Space?

Student/Friend

7. Citations

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