International space station plays host to innovative infectious disease research

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Performing sensitive biological experiments is always a delicate affair. Few researchers, however, contend with the challenges faced by Cheryl Nickerson, whose working laboratory aboard the International Space Station (ISS) is located hundreds of miles above the Earth, traveling at some 17,000 miles per hour.

Nickerson, a microbiologist at Arizona State University's Biodesign Institute, is using the ISS platform to pursue new research into the effects of microgravity on disease-causing organisms.

Nickerson presented her research findings and charted the course for future investigations aboard the ISS at the 2013 annual meeting for the American Association for the Advancement of Science in Boston, Mass. Her talk, titled "Microgravity: A Novel Tool for Advances in Biomedical Research," is part of a special session devoted to ISS science.

"One important focus of my research is to use the microgravity environment of spaceflight as an innovative biomedical research platform. We seek to unveil novel cellular and molecular mechanisms related to infectious disease progression that cannot be observed here on Earth, and to translate our findings to novel strategies for treatment and prevention," Nickerson said.

During an earlier series of NASA space shuttle and ground-based experiments, Nickerson and her team made a startling discovery. Spaceflight culture increased the disease-causing potential (virulence) of the foodborne pathogen Salmonella, yet many of the genes known to be important for its virulence were not turned on and off as expected when this organism is grown on Earth. Understanding how this switching is regulated may be useful for designing targeted strategies to prevent infection.

For NASA, Nickerson's findings were revelatory, given their implications for the health of astronauts on extended spaceflight missions. Already faced with the potential for compromised immunity induced by the rigors of space travel, astronauts may have to further contend with the threat of disease-causing microbes with amped-up infectious abilities. A more thorough understanding of infectious processes and host responses under these conditions is therefore vital for the design of therapeutics and other methods of limiting vulnerability for those on space missions.

The story however, doesn't end there. Further research by Nickerson's team pointed to important implications for the understanding of health and disease on Earth. Her team, including NASA scientists, showed that one of the central factors affecting the behavior of pathogenic cells is the physical force produced by the

movement of fluid over a bacterial cell's sensitive surface. This property, known as fluid shear, helps modulate a broad range of cell behaviors, provoking changes in cell morphology, virulence, and global alterations in gene expression, in pathogens like Salmonella.

"There are conditions that are encountered by pathogens during the infection process in the human body that are relevant to conditions that these same organisms experience when cultured in spaceflight. By studying the effect of spaceflight on the disease-causing potential of major pathogens like Salmonella, we may be able to provide insight into infectious disease mechanisms that cannot be attained using traditional experimental approaches on Earth, where gravity can mask key cellular responses," said Nickerson.

Nickerson's spaceflight studies also pinpointed an evolutionarily conserved protein– called Hfq– which appears to act as a global regulator of gene responses to spaceflight conditions. Further research by her team established that Hfq is a central mediator in the spaceflight-induced responses of other bacterial pathogens, including Pseudomonas aeruginosa, thus representing the first spaceflight-induced regulator acting across bacterial species.

Nickerson's examination of the post-spaceflight alterations in bacterial behavior made use of microarray technology, which allows analysis of gene expression for the entire 4.8 million base pairs found in Salmonella's circular chromosome. Data revealed that 167 distinct genes and 73 proteins had been altered during growth under microgravity conditions, including (but not limited to) virulence-associated genes. Of the 167 genes undergoing up- or down-regulation in response to spaceflight, one third were under the control of the Hfq master regulator protein.

These microgravity studies open a new window into the infectious disease mechanisms of Salmonella, an aggressive pathogen responsible for infecting an estimated 94 million people globally and causing 155,000 deaths annually. In the U.S. alone, more than 40,000 cases of Salmonellosis are reported annually, resulting in at least 500 deaths, and health care costs in excess of $50 million. However, only a small percentage of infections with Salmonella are reported, and the estimated two to four million cases of Salmonella-induced gastroenteritis which occur in the United States each year constitute a significant economic loss of productive work time, reported to exceed $2 billion annually.

While Salmonella has been a pathogen of choice for a broad range of spaceflight investigations, Nickerson stresses that her findings have spaceflight and Earth-based implications. Her confidence is based on her team's work showing that microgravity culture also uniquely alters gene expression and pathogenesis-related responses in other microorganisms.

Nickerson emphasizes that the ISS provides an unprecedented opportunity to study the infection process under microgravity conditions, enabling advances in our understanding of microbial gene expression and accompanying host responses during infection in fine-grained detail. This novel approach holds the potential to identify new classes of genes and proteins associated with infection and disease not

possible using traditional experimental conditions on Earth, where the force of gravity can mask certain cellular responses. Further, experiments aboard the ISS will permit the study of microbial transitions and cellular responses to infection over a prolonged time frame- an important advance not available during shuttle-based experiments.

Microgravity research may provide an opportunity to identify novel targets for vaccine development and the Nickerson team, in collaboration with Roy Curtiss, director of the Biodesign Institute's Center for Infectious Diseases and Vaccinology has been working toward this goal. Based on previous findings, the scientists hypothesized that results from microgravity experiments might be used to facilitate vaccine development on Earth.

In a recent spaceflight experiment aboard space shuttle mission STS-135, the team flew a genetically modified Salmonella-based anti-pneumoccal vaccine that was developed in the Curtiss lab. By understanding the effect of microgravity culture on the gene expression and immunogenicity of the vaccine strain, their goal is to genetically modify the strain back on Earth to enhance its ability to confer a protective immune response against pneumococcal pneumonia.

"Recognizing that the spaceflight environment imparts a unique signal capable of modifying Salmonella virulence, we will use this same principle in an effort to enhance the protective immune response of the recombinant attenuated Salmonella vaccine strain," Nickerson said.

Nickerson's space-based microgravity experiments are carried out in conjunction with simultaneous Earth-based controls housed in the same hardware as those in orbit, to compare the behavior of bacterial cells under normal Earth gravity. Additional information is also provided using Earth-based cell cultures which are subjected to a kind of simulated microgravity, produced by culturing cells in a rotating wall vessel bioreactor (RWV), a device designed by NASA engineers to replicate aspects of cell culture in the spaceflight environment.

Back at ASU, RWV reactor experiments were conducted by Nickerson and her team to help confirm that Hfq plays a central regulatory role in the Salmonella response to spaceflight conditions. Nickerson has also used this RWV technology to grow three dimensional (3D) cell culture models that mimic key aspects of the structure and function of tissues in the body. These 3D models are being used in the Nickerson lab as human surrogates to provide novel insight into the infectious disease process not obtainable by conventional approaches and for drug/therapeutic testing and development for treatment and prevention.

Nickerson also focuses research efforts on determining the entire repertoire of environmental factors that may influence bacterial response to spaceflight culture. For example, she found that the ion concentration in the cell culture media played a key role in the resulting effect of spaceflight on Salmonella virulence. Using the RWV, she was able to identify specific salts that may be responsible for this effect.

Nickerson's long list of firsts (first study to examine the effect of spaceflight on the

virulence of a pathogen, first to obtain the entire gene expression response of a bacterium to spaceflight, first to profile the infection process in human cells in spaceflight, first identification of a spaceflight-responsive global gene regulator acting across bacterial species), will soon be augmented with a new experiment, that will be flown on SpaceX Dragon slated for the ISS later this year. Nicknamed PHOENIX, the project will mark the first time a whole, living organism– in this case a nematode– will be infected with a pathogen and simultaneously monitored in real time during the infection process under microgravity conditions.

This and future studies aboard ISS will almost certainly deepen science's understanding of the molecular and cellular cues underlying pathogenic virulence and open a new chapter in the understanding of health and disease to benefit the general public.

"It is exciting to me that our work to discover how to keep astronauts healthy during spaceflight may translate into novel ways to prevent infectious diseases here on Earth," Nickerson said