

Report on “Thiol levels in *Bacillus* species exposed to ultraviolet radiation”

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As mandated by the Committee of Space Research, space-faring nations must take precautions in preventing contamination of extraterrestrial bodies by limiting the amount of microbes present to the greatest possible extent. Surveys of spacecraft assembly clean rooms for microbes have revealed the existence of strains of bacteria resistant to high levels of ultraviolet (UV) radiation and vaporous hydrogen peroxide, which are used to sterilize clean rooms. In order to develop effective ways to eradicate these bacteria prior to the spacecraft leaving Earth, we must understand how these bacteria are able to survive these extremophilic conditions.

We are particularly concerned about spore forming bacteria, such as *Bacillus pumilus* SAFR-032 and *Bacillus horneckaie*, which are highly resistant to UV and oxidative stress. First, we have demonstrated that these species like other *Bacilli* contains a novel low molecular weight thiol (LMW), bacillithiol. LMW thiols like bacillithiol play a critical role in maintaining a reducing environment and are involved in protection of organisms against a variety of stresses. Bacillithiol has been shown to protect against hypochlorite stress by S-bacillithiolation of cysteines in critical proteins such as glyceraldehyde-3-phosphate dehydrogenase. We have examined samples of *B. pumilus* SAFR-032 spores exposed to four different extreme conditions at the International Space Center: (1) deep space, (2) Martian atmosphere, (3) deep space with UV radiation, and (4) Martian atmosphere with UV radiation. Thiol analysis of the surviving spores indicates that levels of bacillithiol are ten times higher in UV radiation treated samples exposed to both deep space and Martian atmosphere conditions. Spores that survived exposure in a parallel experiment conducted on Earth had ten-fold higher bacillithiol levels, also. Thus, we hypothesized that LMW thiols, such as bacillithiol, protect *B. pumilus* SAFR-032 against UV radiation by (1) protecting the cell against oxidative stress that results from UV radiation and (2) protecting the integrity of proteins such as SP lyase, a DNA repair enzyme, by bacillithiolation of critical cysteine residues, thereby preventing enzyme inactivation.

Aim 1: to determine whether mutants in spore forming and non spore forming bacteria that lack bacillithiol are more sensitive to UV radiation

To determine if bacillithiol plays a role in protection against ultraviolet radiation, we exposed *Bacillus subtilis* and *Staphylococcus aureus* mutants lacking bacillithiol to UV radiation. Mutants lacking bacillithiol are more sensitive to UV irradiation as compared to the wild-type strain. However, the sensitivity is not in the orders of magnitude as expected.

We are continuing to project determine the bacillithiol levels in *B. pumilus* SAFR-032, *B. horneckaie* and *Bacillus subtilis* that have been exposed to UV radiation. We are examining whether there is a change in thiol levels during sporulation and germination in *B. pumilus* SAFR-032, *B. horneckaie* and the laboratory strain, *Bacillus subtilis*, since it has been demonstrated that *B. subtilis* mutants lacking bacillithiol are hundred fold less likely to sporulate.

Aim 2: to determine whether *Mycobacterium smegmatis* mutants lacking mycothiol are more sensitive to UV radiation

We have determined that *Mycobacterium smegmatis* mutants lacking mycothiol, another LMW thiol found in actinobacteria, and *Synechococcus* PCC7942 mutants lacking glutathione, are more sensitive

to ultraviolet radiation. However like bacillithiol lacking mutants, the sensitivity is not in the orders of magnitude as expected.

Results of Aim 1 and 2 were presented at 2012 by Andrew Strankman in an oral presentation at the Astrobiology Science Conference in Atlanta and a poster at Annual California State University Biotechnology Symposium in Santa Clara in 2012.

Aim 3: to ascertain if bacillithiolation or mycothiolation increases in UV stressed cells

We have validated a protocol to measure protein thiolation using monobromobimane to label thiols instead of the avidin/biotin system proposed and are examining the levels of protein thiolation in *B. pumilus* SAFR-032, *B. horneckaie* and *Bacillus subtilis* that have been exposed to UV radiation. This work will be presented by Samantha Haranto at the Central California Research Symposium in 2013.

Research presented at Conferences

1. A Strankman#, CB Subsilla#, **M Rawat**. Thiol levels in *Bacillus* species exposed to ultraviolet radiation (24th Annual CSU Biotechnology Symposium, January 2012)
2. A Strankman#, C Subsilla#, **M Rawat**. Thiol Content in *Bacillus* exposed to ultraviolet radiation. (Astrobiology Conference, April 2012)
3. A Strankman# and **M Rawat**. Thiol Content in *Bacillus* exposed to ultraviolet radiation. (Graduate Research and Activities Symposium, May 2012)

graduate student

Students trained:

1. Calvin Subsilla- left Master's program; performed ultraviolet sensitivity assays on different bacteria lacking thiols such as *Bacillus subtilis* and *Staphylococcus aureus* mutants lacking bacillithiol and *Mycobacterium smegmatis* mutants lacking mycothiol.
2. Andrew Strankman- Master's student; total thiol analysis of *Bacillus pumilus* SAF032 and recovered spores from spores sent to International Space Station
3. Samantha Haranto- Freshman Undergraduate student; protein thiol analysis of *Bacillus pumilus* SAF032 and recovered spores from spores sent to International Space Station