Quantitative Proteomic Analysis of *Bacillus pumilus* from Spores that Survived in Outer Space Conditions

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Introduction

The hardy spores of *Bacillus* species are well known for their resistance to unfavorable conditions such as UV and gamma radiation, hydrogen peroxide desiccation, chemical disinfection, or starvation. In particular, *Bacillus pumilus* strain SAFR-032 that was originally recovered from the Jet Propulsion Lab Spacecraft Assembly Facility has shown to exhibit unusually high resistance to UV radiation and peroxide treatment compared to other *Bacillus* species. To further understand the resistance of bacterial endospores to relevant outer space environments, spores of *B. pumilus* SAFR-032 were exposed for 1.5 years to selected parameters of space on board of the International Space Station (ISS). Here we are using a quantitative proteomics approach to gain insights into the resistance mechanism of *B. pumilus*.

Methods

Spores of *B. pumilus* SAFR-032 were exposed to different extraterrestrial conditions during the EXPOSE-E mission on the ISS: UV-Space, UV-Mars, Dark-Space, and Dark-Mars. The surviving spores were retrieved and cultured under aerobic conditions in trypticase soy broth medium for 48 hours. Proteins were extracted from bacterial cell pellets using a bead beater, and then TCA precipitated, reduced, alkylated, and digested with trypsin/LysC. The digested peptides were labeled with isobaric tandem mass tags (TMTsixplex) and fractionated with a High pH Reversed-phase Peptide Fractionation kit (Thermo). The eight resulting fractions were analyzed in triplicate by LC/MS on an Orbitrap Fusion Tribrid mass spectrometer equipped with an Easy nano HPLC (Thermo). The experiment was repeated with an additional biological replicate.

Preliminary Data

2146 and 2225 proteins were identified in each bioreplicate, respectively; accounting for approximately 60% of all proteins (3601) encoded in the *B. pumilus* SAFR-032 genome. Only proteins that were identified and quantified in all biological replicates were used for the analysis (1611 proteins). The protein expression levels of four space-surviving strains were further normalized to those of unexposed *B. pumilus* SAFR-032 (ground control). Previous studies showed enhanced UVC resistance and sporulation of space-surviving strains of *B. pumillus* SAFR-032. Our results showed that several DNA repair and peroxide resistance proteins were dysregulated in the space-surviving strains. Most of these proteins are conserved in *Bacillus* and are known to contribute to the genus' resistance to UV radiation and H_2O_2 treatment. Proteins essential for the initiation of sporulation were also significantly upregulated in all space-surviving strains compared to the ground control. Furthermore, to identify candidate proteins that might be responsible for the enhanced resistance of *B. pumilus*, the whole genome of *B. pumilus* SAFR-032 and *B. subtiltis* 168 were compared using Artemis Comparison Tool. 424 proteins were identified and considered as unique/characteristic of *B. pumilus* SAFR-032. Gene ontology analysis of these proteins that were either up- or down- regulated compared to the control included involvement in carbohydrate metabolic process [GO:0005975], cell redox homeostasis [GO:0045454], regulation of transcription [GO:0006355], oxidoreductase activity [GO:0016491], and catalytic activity [GO:0003824]. While more in-depth proteomic analyses are required, the preliminary results revealed the candidate biological pathways and proteins that may be responsible for the unique characteristics of *B. pumilus* SAFR-032. The results from this study will contribute to a better understanding of microorganisms that selectively adapted and resisted the extreme environment of interplanetary outer space.

Novel Aspect

Exploring mechanisms of enhanced resistance to outer space conditions of *Bacillus pumilus* SAFR-032 by a quantitative proteomics