RADIATION RESISTANCE – WHAT CAN WE LEARN FROM ENDOSPORE FORMERS FROM CLEANROOM FACILITIES?

Madhan Tirumalai¹, V Stepanov¹, A. Wünsche¹, S. Montazari¹, R. O. Gonzalez¹, K. Martin¹, K. Venkateswaran², G. E. Fox^{1*},

¹Dept Biology & Biochemistry, University of Houston, 3455, Cullen Blvd, Houston, TX, 77204-5001, USA,

²California Institute of Technology, Jet Propulsion Laboratory, Biotechnology and Planetary Protection Group; M/S 89-2, Pasadena, CA 91109

(mrtirum2@central.uh.edu;*fox@uh.edu).

Introduction:

. Microbial contamination in the closed environments of spacecraft assembly and clean room facilities is a significant planetary protection concern [1]. In particular, endospore forming Bacillus species are of special concern, because many species produce radiation and peroxide resistant spores. These spores have been shown to survive cosmic levels of experimental (simulated) radiation as well as on the International Space Station (ISS) [2-3]. Two of the most dominant organisms in this specialized econiche of spacecraft cleanroom facilities are *B. safensis* FO-36 b^{T} [4] and *B.* pumilus SAFR-032 [5], Both strains produce spores that exhibit unusual levels of resistance to peroxide and UV radiation [6,7]. A third strain, B. safensis MERTA8-2, was initially isolated from the Mars Odyssey Spacecraft and associated facilities at JPL (1999-2001) and later also found on the Mars Explorer Rover (MER) before its launch in 2004. This strain was found to grow significantly better on the International Space Station than on Earth [8].

Methods:

To further our understanding of the basis for enhanced spore resistance, the whole genome sequence of *Bacillus safensis* strain FO-36b was determined and annotated. The resulting sequence was compared to draft genomes of *B. safensis* MERTA8-2 (MER) and *B. pumilus* ATCC7061 as well as the complete genome of *B. pumilus* SAFR-032 (SAFR),

Results:

The FO-36b gene order is essentially the same as seen in SAFR-032 and other *B. pumilus* strains. The FO-36b genome has 3875 open reading frames including at least 98 non-coding RNAs and riboswitches. 65 of these are not shared by the SAFR ATCC, or MER strains. This includes genes coding for a dUTPase (with roles in viability, DNA replication, transcription and uracil base-excision repair), a spore coat protein and others involved in DNA recombination, and, cell signalling, all of which that may have potential roles in the strain's resistance properties. Furthermore, 25 genes are part of phage element(s), 52 are hypothetical protein coding ORFs.

Thirteen of the 65 locally unique genes are in fact totally unique to FO-36b with no homologs in the entire NR/NT databases. Eight of these are part of two different phage elements homologous to *Bacillus* phage SPP1 (NC_004166.2) and *Brevibacillus* phage Davies (NC_022980.1), respectively; thus they are likely transferred / acquired between species.

We propose that both *B. safensis* FO-36b and *B. safensis* MERTA8-2 strains are candidate strains likely to survive the effects of radiation levels encountered in cosmic space (as in the ISS).

References:

- [1] Rummel J.D *et al.* (2012) *Astrobiology 12*(11):1017-1023.
- [2] Moissl-Eichinger C et al. (2015) Sci Rep, 5:9156.
- [3] Venkateswaran K et al. (2014) Microbes Environ, 29(3):243-249.
- [4] Satomi M, et al. (2006) Int J Syst Evol Microbiol, 56(Pt 8):1735-1740
- [5] Link L, et al. (2004) Microb Ecol 2004, 47(2):159-163.
- [6] Stepanov V.G. *et al.* (2016) *PLoS One*. Jun 28;11(6):e0157331
- [7] Tirumalai M.R. *et al.* (2013) *PLoS One. 2013* Jun 14;8(6):e66012.
- [8] Coil D.A et al. (2015). *Genome Announc*. Nov 19;3(6).

Acknowledgement: This work is supported by NASA Grant NNX14AK36G to GEF.