COMPARISON OF THE WHOLE GENOMES OF TWO CLOSELY RELATED AND EXTREME RADIATION AND PEROXIDE RESISTANT SPORE PRODUCING STRAINS, BACILLUS SAFENSIS F036B AND BACILLUS PUMILUS SAFR-032 FROM SPACECRAFT CLEANROOM FACILITIES.

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Introduction: Planetary protection policies are integral to space missions and exobiology. Protocols under these policies require the construction and assembly of spacecraft under optimal clean conditions. More than 30 years of extreme radiation, peroxide treatment, and desiccation of spacecraft cleanroom facilities have created a special habitat for multi-resistant bacteria. As part of NASA's planetary protection program, monitoring of microbial diversity in the NASA Jet Propulsion Laboratory Spacecraft Assembly Facility (JPL-SAF) resulted in the isolation of a number of microbial species inhabiting various parts of the facility.

Radiation and peroxide-resistant spores producing *Bacillus sp*. are the predominant organisms amongst the microbial diversity found in the NASA Jet Propulsion Laboratory Spacecraft Assembly Facility (JPL-SAF). Two of these strains, namely, *B. safensis* FO36b and *B. pumilus* SAFR-032, are closely related species that produce spores that can survive and persist in the extreme environment of the JPL-SAF (characterized by very low nutrient levels, strictly controlled humidity, and periodic disinfection), and also exhibit elevated resistance to UV and H_2O_2. In terms of the spore resistances, SAFR-032 is more resistant to UV radiation than FO36b, while FO36b is more resistant to peroxide than SAFR-032.

In our previous reports [1, 2], our comparison of the genome of SAFR-032 with its closely related genomes included an earlier version of FO36b genome sequence which was available only as 408 small individual contigs, and thus only qualitative estimations of the presence/absence of a gene could be made. We have now resequenced the genome of FO36b towards obtaining a better quality of the sequence, which was further annotated using the Rapid Annotations using Subsystems Technology [3]. The Open Reading Frame (ORFs) sequences thus obtained were blasted against the gene sequences of SAFR-032. The genomes of these two genomes were further compared for potential rearrangement of gene segments.

Result: The draft genome of *Bacillus safensis* F036b was assembled into five super contigs with a total length of 3735438 bases, a GC % value of 41.7 and the RAST annotation gave a total of 3882 ORFs. Our preliminary comparative analysis of the two genomes showed extensive re-arrangement of the segments of the *Bacillus safensis* F036b genome. 196 genes are possibly unique to F036b, 31 genes are possibly uniquely shared by F036b and SAFR-032, and 303 F036b characteristic genes (shared possibly by other Bacillus/non-Bacillus genomes, but not by SAFR-032). Interestingly, of the 130 genes we had reported to be characteristic to SAFR-032 [1], and thus not shared by either the type strain *B. pumilus* ATCC7061^T or the older version of the FO36b genome (spread over 408 contigs), only four are shared by the current resequenced version of the FO36b genome. The 40 SAFR-032 unique genes are still not shared by the type strain ATCC7061^T or the current version of the FO36b genome. We had also reported that 105 genes while being shared by SAFR-032 and ATCC7061^T, are absent in FO36b, most likely because the earlier FO36b genome was incomplete. Examining the resequenced genome of FO36b shows that 6 of these genes are indeed shared by FO36b as well. The operon *oppABCDF* is known to play important roles in sporulation initiation, competence as well as resistance to aminoglycoside antibiotics [4]; we observe that while there is an overlap of *oppA-oppB* as well as *oppD-oppF* in SAFR-032, indicating translational coupling, the homologs in FO36b, ATCC7061^T, *B. subilis* and *B. licheniformis* do not overlap, further suggesting efficient gene expression regulation of this operon in SAFR-032, perhaps impacting sporulation initiation positively.

The potential roles of the genes not shared by the two genomes as well as the genome rearrangement, in the spore resistances to radiation and peroxide, despite the close phylogenetic relationship of the strains will be discussed.

References: [1]Tirumalai M. R. et al. (2013) *PLoS One* Jun 14; 8(6):e66012. [2]Tirumalai M. R. and Fox. G. E. (2013) *Extremophiles*, Sep; 17(5):767-74. [3]Overbeek R. et al. (2014) *Nucleic Acids Res*. 2014 Jan; 42(Database issue):D206-14. [4]Perego M. et al. (1991) *Mol Microbiol*. Jan; 5(1):173-85