***GEODERMATOPHILUS* SP. STRAIN MN04-01 SURVIVES HIGH DOSES OF SIMULATED PRESENT-DAY MARTIAN UV RADIATION.** I. G. Paulino-Lima[1], G. G. Araujo[2], F. Rodrigues[3], E. P. Silva[4], L. J. Rothschild[5] and D. Galante[6]. [1]NPP Fellow at NASA Ames Research Center, Moffett Field, CA, USA, 94035-0001, [2]Interunities Biotechnology Graduate Program, University of São Paulo, Brazil, 05508-900, [3]Institute of Chemistry, University of São Paulo, Brazil, 05508-000, [4]Institute of Chemistry, University of São Paulo, Brazil, 05508-000, [5]NASA Ames Research Center, Moffett Field, CA, USA, 94035-0001, [6]Brazilian Synchrotron Light Laboratory, Campinas, Brazil, 13083-100.

Introduction: The ultraviolet radiation present during daytime on the surface of Mars is highly damaging for most forms of life if unprotected and totally exposed [1]. Even *Deinococcus radiodurans*, a microbial model for radiation-resistance studies, would be effectively inactivated if totally exposed to Martian UV [2], [3]. However, there might be other organisms yet to be discovered, that would not be inactivated so easily by Martian UV radiation if deposited in a clean surface such as a spacecraft. *Geodermatophilus* sp. strain MN04-01, was recently isolated from a manganese deposit in the Sonoran Desert after a screening method developed for selecting highly UV-resistant microorganisms. This isolate is 3 times more resistant to UV-C radiation than *D. radiodurans*, as measured through colony counts on agar plates. Here we report its survival to a simulated Martian UV irradiation experiment performed at NAP-Astrobio, University of São Paulo, Brazil.

Material and Methods:

Microbial cultures: Cells of *Geodermatophilus* sp. strain MN04-01 were grown in GOM Medium (1.5% Malt Extract, 1% starch, 1% sucrose, 0.5% yeast extract , 0.2% $CaCO_3$) for 7 days at 30 °C, 200 rpm. Cells of *Deinococcus radiodurans* were grown in TGY medium (1% tryptone, 0.6% yeast extract, 0.2% glucose) for 15h at 30 °C, 200 rpm. 0.5 ml aliquots of each organism were centrifuged at 8,000 rpm for 3 min and the cell pellet was washed twice through centrifugation in the same conditions using saline solution (0.9% NaCl). Finally, the cells were resuspended in 0.5 ml of saline solution.

Sample loading: Four replicates of 2 µl aliquots of the cell suspension were loaded in four 5 mm x 5 mm silicon support (Si 111). After 10 min of dehydration in a laminar flow hood at room temperature the silicon supports were fixed on a metallic mount using a carbon tape. The mount containing the samples was then fixed inside the Mars Simulation Chamber.

Experimental conditions: The Martian uv flux was simulated using a non-ozone free Oriel Solar Simulator containing a xenon-arc lamp emmiting a broad spectrum of UV, visible and infrared radiation. To minimize the amount of infrared radiation delivered, an air mass 0 (AM0) filter was used to correct the lamp spectrum and a water filter was placed between the solar simulator and the vacuum chamber (Fig. 1). The uv flux was measured usin a Vilber Lourmat radiometer as 87 W/m^2 for UV-A, 118 W/m^2 for UV-B and 23 W/m^2 for UV-C.

The samples were irradiated at room temperature under an atmosphere of 8 mbar containing 95% CO_2 and 5% N_2, to the following Martian full uv doses, in kJ/m^2: 3, 6, 10, 30, 60 and 100.

Analytical techniques: After the irradiation, individual silicon supports containing the samples were placed in microfuge tubes containing 100 µl of appropriate culture media and vortexed for at least 20 seconds. Cell suspensions were serial diluted 10^{-1} to 10^{-4} and 10 µl aliquots were inoculated on agar plates, which were incubated at 30 °C for up to 10 days. Colonies of irradiated samples (N) and the non-irradiated control (N_0) were counted and the results (N/N_0) were plotted in a graph showing survival curves. The remaining volumes of the 10^{-1} dilutions were also stained with propidium iodide and SYTOX® green for live/dead quantification through fluorescence microscopic analysis.

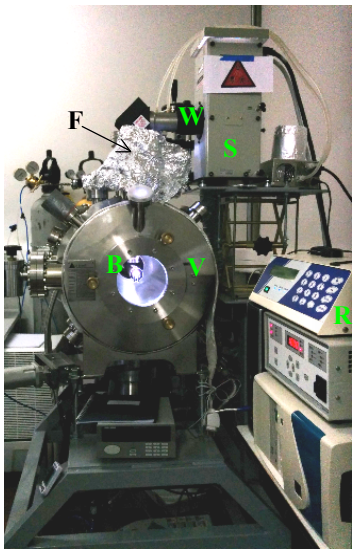

Fig. 1. Experimental setup showing: solar simulator (S), water filter (W), AM0 filter (F), radiometer (R), vaccum chamber (V), and biological specimens being irradiated (B).

Results:

Colony counts: LD_{10} (dose that kills 90% of the population) was 3.27 ± 0.25 kJ/m^2 for *D. radiodurans* and >100 kJ/m^2 (maximum dose tested in our experiments) for *Geodermatophilus* sp. strain MN04-01 (Fig. 2).

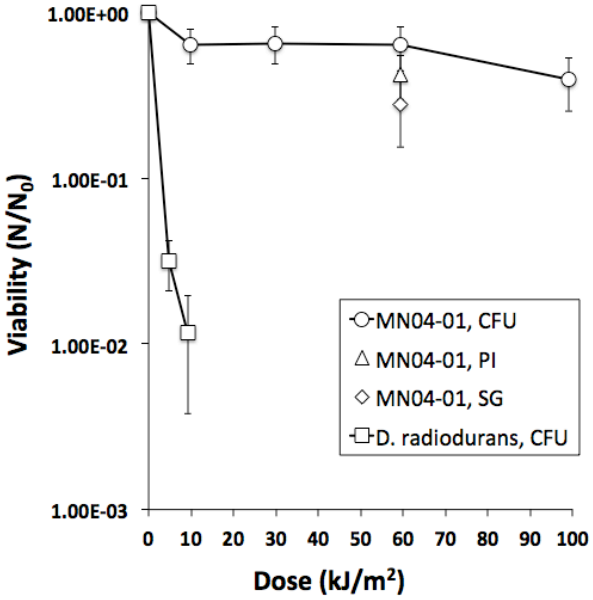

Fig. 2. Survival of *Geodermatophilus* sp. strain MN04-01 to full uv (200-400 nm) under martian atmosphere, and comparison with *D. radiodurans*. Fluorescence microscopy data is also shown on the graph. CFU, colony forming-units. PI, propidium iodide. SG, SYTOX® green.

Fluorescence: After analyzing 13 micrographs for the non-irradiated control and 14 for samples irradiated with 60 kJ/m^2 of Martian UV irradiation (Fig. 3), both staining methods resulted in similar numbers (Fig. 4).

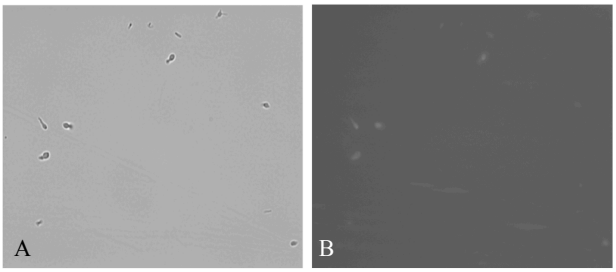

Fig. 3. A, Phase contrast micrograph showing cells of *Geodermatophilus* sp. strain MN04-01. B, Fluorescence micrograph (SYTOX® green) showing dead cells of the same organism after 60 kJ/m^2 of Martian full uv irradiation.

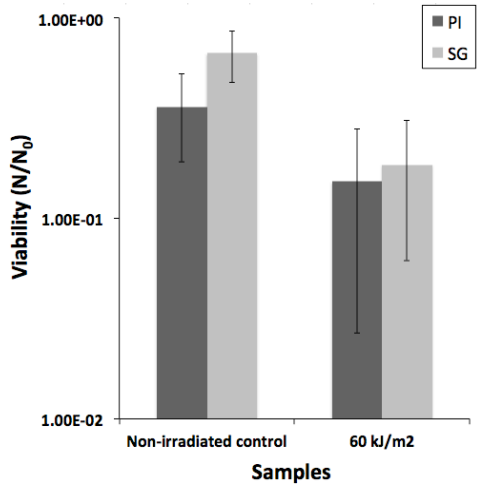

Fig. 4. Viability of strain MN04-01 to 60 kJ/m^2 of Martian full uv irradiation as determined by fluorescence microscopy using propidium iodide (PI) and SYTOX® green (SG).

Raman spectroscopy of colonies (Fig. 5) indicates the production of a melanin-like pigment that strongly absorbs UV-C radiation.

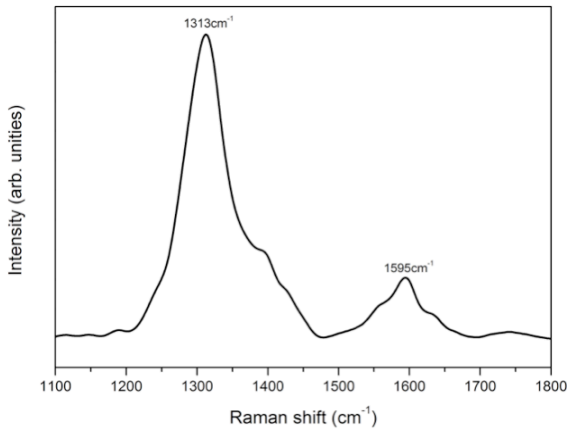

Fig. 5. Raman spectrum showing two shifts, at 1313 cm^{-1} and 1595 cm^{-1}, consistent with melanin.

Conclusions: The results obtained in this research have implications for planetary protection and space exploration using biological systems. This microbial isolate represents an excellent biological model for photobiology studies including DNA damage and repair analysis.

References: [1] Horneck G. et al. (2010) Microbiol Mol Biol Rev, 74(1) 121-156. [2] Paulino-Lima I. G. et al. (2010) Planet Space Sci, 58(10), 1180-1187. [3] Abrevaya X. C. et al. (2011) Astrobiology 11(10) 1034-1040.

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