IMPLICATIONS OF COSMIC RADIATION ON THE MARTIAN SURFACE FOR MICROBIAL SURVIVAL AND DETECTION OF FLUORESCENT BIOSIGNATURES.

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Introduction: The martian surface experiences an ionising radiation environment significantly greater than that of the Earth due to the penetration of cosmic rays through the minimal shield of the Mars atmosphere. Here we report experimental results concerning the likely survival time of microbial life bathed in this radiation field at different depths in the martian near-subsurface [1], which builds on our recent results from computational modelling of the cosmic radiation environment on Mars [2][3]. These results are discussed with respect to their significance to both the chances of finding surviving martian microbial life, and the persistence of terrestrial contaminants in the interests of planetary protection. Novel bacterial isolates have been cultured from the Antarctic Dry Valleys region, and found to be very similar to strains discovered contaminating assembly clean rooms in both Kennedy and Johnson Space Centers [4]. On-going work is concerned with the alteration and degradation rate of detectable fluorescent biosignatures exposed to this cosmic radiation over geological timescales [5].

Cosmic radiation Beyond the protection of the atmosphere and magnetic field of the Earth, the solar system is pervaded by an ionizing radiation field known as cosmic rays. Solar Energetic Particles (SEP) are accelerated by flares and coronal mass ejections from the Sun at high fluxes and moderate energies, whereas Galactic Cosmic Rays (GCR) are accelerated by supernovae to very high energies, but with a peak flux much lower than that of SEP [6]. When they strike shielding material such as the casing of a space probe, or atmosphere or unprotected surface of a planetary body (e.g. the Earth or Mars, respectively), these energetic particles produce extensive showers of deeply-penetrating secondary radiation [7]. Ionising radiation is a major threat to life, the persistence of detectable biosignatures, and the operation of spacecraft instruments [8]. On Mars these particle cascades penetrate far deeper into the subsurface than ultraviolet radiation, and so cosmic rays represent one of the primary hazards of the martian near-surface [2][3][9]. This cosmic ray ionising radiation field is complex and dynamic, and we have reported the results of computational modelling of the radiation environment at increasing depths beneath the martian surface [2][3].

Microbial survival We experimentally determined the survival responses of several bacterial strains to ionising radiation exposure whilst frozen at a low temperature characteristic of the martian near-subsurface [1]. Novel psychrotolerant bacterial strains were isolated from the Antarctic Dry Valleys, an environmental analogue of the martian surface, and identified by 16S rRNA gene sequencing as representatives of *Brevundimonas*, *Rhodococcus* and *Pseudomonas* genera. These isolates and the known radioresistant extremophile *Deinococcus radiodurans* were exposed to gamma-rays whilst frozen on dry ice (-79°C). We found *D. radiodurans* to exhibit far greater radiation resistance when irradiated at -79°C than similar studies performed at higher temperatures, which has important implications for the estimation of potential survival times of microorganisms near the martian surface. Furthermore, the most radiation resistant of these Dry Valley isolates, *Brevundimonas* sp. MV.7, was found to show 99% 16S rRNA gene similarity to contaminant bacteria discovered in clean rooms at both Kennedy and Johnson Space Centers [4], and so presents a concern to efforts for the planetary protection of Mars from our lander probes. Mission failure during the landing sequence could emplace contaminant cells sufficiently far beneath the surface for protection from rapid inactivation by solar UV or SEP, and even a successfully-landed probe could shield bacteria on its underside or deposit them in a shaded enclave for them to persist for significant periods or be widely redistributed and buried by dust storms. Results from this experimental irradiation, combined with the previous radiation modelling, indicate that *Brevundimonas* sp. MV.7 emplaced only 30 cm deep in martian dust could survive the cosmic radiation for up to around 100,000 years before suffering 10^6 population reduction. A population of contaminant Deinococcus cells, or native martian microbes of comparable radiation resistance, at 30 cm depth in the subsurface could persist for 1.2 million years before experiencing 10^6 inactivation [1].

Fluorescent biosignatures On-going work is focussed on the characteristics and degradation rate of remnant biosignatures of past life within this ionising radiation field – to determine the window of opportunity for detection. One method proposed for surveying for both organics and organisms on the martian surface

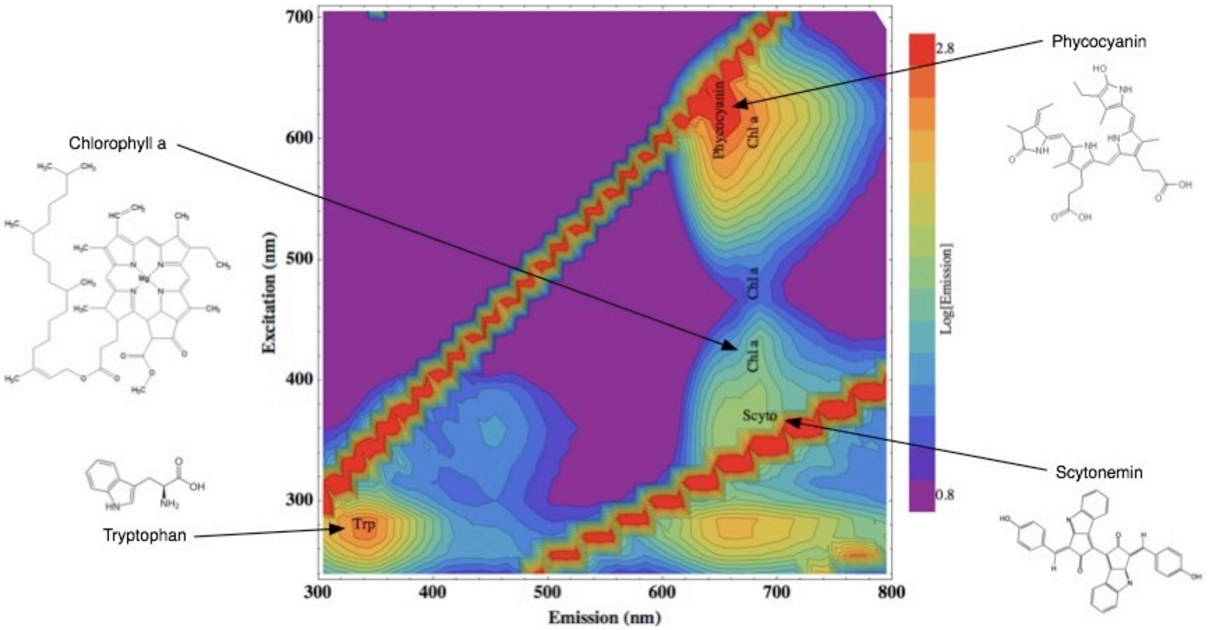

Fig. 1 Excitation-Emission Matrix (EEM) of fluorescence from the cyanobacterium *Synechocystis* sp. PCC 6803, exhibiting emission from small aromatic compounds such as the amino acid tryptophan, larger light-harvesting molecules phycocyanin and chlorophyll, and the UV-screening compound scytonemin.

is using laser-induced fluorescence [10][11][12][13][5]. After absorption of short-wavelength light, many organic molecules de-excite by emission of fluorescence. The excitation and emission spectra are characteristic of different molecules, and so fluorescence can be used to both localise and identify organics. All terrestrial microorganisms fluoresce due to cellular components such as protein-bound tryptophan, and photoautotrophic cells such as cyanobacteria are particularly conspicuous through fluorescence of photosynthetic pigments including chlorophyll and phycocyanin, as well as UV-screening compounds [5]. Even in the absence of microbial life, prebiotic molecules such as polycyclic aromatic hydrocarbons (PAHs) or aromatic amino acids may be revealed by fluorescence. Fluorescent signatures of different molecules can be clearly seen in Excitation-Emission Matrices, as shown in Fig.1. Fluorescence-based detection systems have proved themselves to be sensitive and discriminatory, and have been used for assessing the organic content or pollution of aquatic and terrestrial samples [14][15][16], identifying potentially pathogenic or toxic microorganisms in environmental water or food preparation [17][18][19][20], and detecting trace biomolecules or life in glacial [21] and Antarctic ice [11], and the Atacama desert [22]. It is largely unknown, however, how rapidly fluorescent biosignatures are destroyed by radiation under martian conditions. The change in fluorescence response after radiolytic degradation is being characterized for samples of both microorganisms, including *D. radiodurans* and the cyanobacterium *Synechocystis* sp. PCC 6803, and non-biological organics such as PAHs, as a function of radiation exposure, and thus time at different depths in the martian subsurface.

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