

The likelihood of terrestrial microbes colonising Mars is...

Humans have long been fascinated by the nature and mechanics of micro-organisms. Whilst contested, it is believed the 17th century microbiologist Antoni van Leeuwenhoek first recorded their discovery in his paper 'letter on the protozoa' under the name 'animalcules'.^[1] Along with the support of Robert Hooke, they fabricated and used simple microscopes that magnified objects up to 250 times.^[2] Unfortunately, due to his mistrustful secrecy of his methods, micro-organisms were only found to be pathogenic through Robert Koch's experimentations with anthrax on cows in the late 19th century.^[3] Over the succeeding years, the significance of micro-organisms on Earth has been proven to be invaluable, and are ubiquitous, accounting for 1-3% of a human body's mass. Whilst these 'silent' beings have conquered planet Earth, there has been much debate around the question: Are micro-organisms able to survive on Mars?

Both domains of Archaea and Bacteria divide through binary fission, a form of asexual reproduction in which a parent cell reproduces by undergoing fission, forming two genetically identical daughter cells. Owing to their microscopic size (100 times smaller than an animal cell) and a circular strand of DNA which is easy to replicate, prokaryotes normally take around 4 to 20 minutes to reproduce.^[5] These expeditious rates can only be reached if they're in a suitable area such as one with high nitrogen concentration; if it's not available, the cell won't be able to respire, synthesise proteins for growth and die.

However, over the past half-century, the discovery of extremophiles has had vital implications for astrobiology,^[4] as the prevailing view until recently was that life could only exist in environments like Earth's. However, extremophiles have expanded our understanding of habitability by proving that life may exist in conditions that were previously assumed to be inhospitable, such as high pressure, radiation, and extreme temperatures, stimulating innovative ideas and models for the possibility life to exist on Mars and has prompted a re-evaluation of the criteria for habitability beyond Earth.

Due to the atmosphere of Mars being abnormally thin - coupled with the lack of a magnetic field - studies at NASA have concluded that the surface of Mars experiences about 0.7 milligrays of cosmic radiation daily, approximately 112 times higher than Earth's.^[8] One reason why Mars is deemed uninhabitable by some is that this ionising radiation (IR) causes severe oxidative damage to cells through the radiolysis of intracellular water which generates reactive oxygen species^[7] and hydroxide radicals, breaking DNA double strands, ultimately leading to necrosis, making it extremely difficult for microbes to survive.^[7]

To combat this, a multitude of bacteria have evolved to become resistant to IR, like *Deinococcus radiodurans*, a polyextremophile bacterium originally found in canned meat in 1956,^[9] which is resistant to dehydration, cold, and has been tested to have surviving cells after exposure of over 10000 Gy, 200 times higher than the lethal human dose of radiation.^{[6][7]} *D. radiodurans* is believed to survive under the surface of Mars because it has shown to develop efficient anti-oxidative systems to remove reactive oxygen species (ROS) which form through exposure to IR, reducing damage to the tertiary structure of proteins.^[9] Ensuing research by Michael Daly and his team concluded that there's an abundance of genes encoding catabolic enzymes that give rise to small antioxidant molecules.^{[10] [11]} Daly et al. demonstrated

that *D. radiodurans* accumulates abnormally high concentrations of Mn^{2+} ions, ($\sim 0.2\text{--}4\text{mM}$), which act as an oxidative scavenger, principally protecting proteins from IR-induced oxidation and inactivation.^{[7][12]}

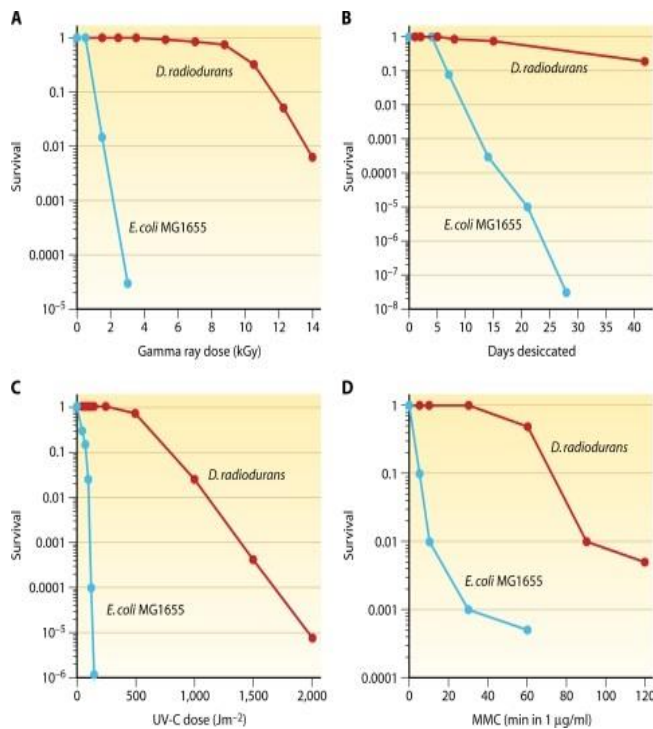


Figure 1: Extreme dose resistance of *D. radiodurans* to gamma rays (A), desiccation (B), UV-C radiation (100 to 295 nm) (C), and mitomycin C (D) in comparison with *E. coli*.^[15]

A 2011 study led by Dea Slade supports Daly's research, declaring that *D. radiodurans* has undergone additional adaptations to accommodate the high levels of Mn^{2+} , which would inhibit the growth of all prokaryotes, through utilising the ion as a cofactor for numerous proteins,^{[12][13]} ultimately reducing the oxidative damage, permitting the bacterium to replicate DNA without any complications such as DNA double strand breaks, meaning it can divide safely by binary fission to produce two genetically identical daughter cells, despite the highly toxic doses of IR smothering the surface of Mars, lethal to any other organism. It is for this reason that research and scientists indicate *D. radiodurans* would be able to withstand the conditions of Mars, making it rather likely for it to grow on Mars.

On the other hand, despite it being able to survive on radioactive surfaces, it is still unknown whether this polyextremophile will be able to survive on the surface of Mars, due to it being an obligate aerobe,^[14] meaning it requires oxygen to derive energy from organic compounds in its environment, which would be difficult on the surface of Mars; a 6 year study involving NASA's Curiosity rover discovered that the Martian atmosphere is composed of 95% carbon dioxide, and 0.16% oxygen.^[13] These findings, being supported by NASA's Perseverance rover, insinuate how difficult it would be for *D. radiodurans* to grow on the surface without significant modifications to its metabolism.

Contrarily, subsequent research by J. Frederickson and Daly found that *Deinococcus radiodurans* was able to oxidise lactate to CO_2 under anaerobic conditions, mimicking the Martian atmosphere.^[15] Whilst this result was not able to be linked to growth,^[15] the remarkable ability to withstand chronic doses of radiation coupled with the relative ease of genetic manipulation,^{[15][16]} shows *D. radiodurans* is an attractive candidate for genetic modification to adapt to the harsh Martian climate.

With the atmosphere of Mars being highly anaerobic, cyanobacteria are theorised to survive, especially the *Chroococidiopsis* genus, a photosynthetic, coccoidal bacterium containing extremophile species that

are capable to surviving extreme temperatures and high salinity; in 1982, research carried about by E. I. Friedmann in 1982 found that the cyanobacterium lived in the Arctic desert, ^[18] illustrating that *Chroococcidiopsis* would be able to survive the sub-zero temperatures of Mars.

A research team under Friedmann in 2000 also conducted multiple experiments on 10 different *Chroococcidiopsis* strains isolated from desert and hypersaline environments and discovered viable cells after 15kGy of X-ray irradiation, but not after 20kGy.^[19] Additionally, further research by a team from the University of Rome Tor Vegata studied the resistance of *Chroococcidiopsis* to ionising radiation under both hydrated and dried states; Cyprien Verseux and his team discovered that no detectable damage was induced at 11.59 kGy of gamma radiation, and when dried, it was possible for some *Chroococcidiopsis* species such as CCMEE 029 to withstand 24kGy of gamma radiation.^[20] Thus, research into the effects of IR on the growth of *Chroococcidiopsis* is still undergoing and unknown, yet these results all indicate that like *D. radiodurans*, *Chroococcidiopsis* would be able to withstand the intense and toxic IR on the Martian surface, and possibly even grow and photosynthesise there, which would create oxygen for more microbes to be able to respire and survive there.

The genus is also able to photosynthesise, converting the abundant carbon dioxide in the atmosphere to oxygen using the thermal energy from the sunlight that easily penetrates the extremely thin atmosphere. *Chroococcidiopsis* is also capable of enduring desiccation in dry surroundings, partly owing to its habitation on the lower surface of see-through stones –which are present on Mars. The condensed moisture present on the underside of these rocks is sufficient for the organism's growth, and the translucence of the rocks facilitates photosynthesis by allowing adequate light to penetrate. Hence, it is theorised that *Chroococcidiopsis* could survive on Mars and colonise it.

The discovery of extremophiles also partially validates the lithopanspermia hypothesis - a philosophical belief that life and organic compounds exist all over the Universe and can be propagated through space from one location to another, either through space dust, meteoroids, and planetoids.^[21] It is currently considered as a fringe theory among some mainstream scientists but has begun to gain popularity after studies by Allen and Wickramasinghe which claimed that a major proportion of interstellar dust was composed organically were proven to be correct.^[22] Their findings are further supported by the analysis of the Martian meteorite ALH84001, which immediately made headlines worldwide, culminating in then-U.S. president Bill Clinton addressing the world on the significance of the findings.^[23]

Moreover, subsequent research by Iowa State University probed the carbonate globules in ALH84001 to find signs of biogenicity which would solidify the theory of panspermia. Kathie Thomas-Keprta and her team characterised an abundance of magnetite (Fe_3O_4) within the globules,^[24] which she revealed were 'both chemically and physically identical to terrestrial, biogenically precipitated, intracellular magnetites produced by magnetotactic bacteria strain MV-1.'^[24] These findings are further consolidated by Koike et al., 2020 which reports discovering nitrogen bearing organics within the carbonates of ALH84001.^[25]

Koike further describes nitrogen as an 'essential element for all life on Earth' which is accurate as it is necessary for the formation of nucleic acids and proteins, thus, without it, organisms would not be able to exist. Therefore, the findings by Thomas-Keprta and Koike insinuate that living organisms could be distributed around the universe by meteoroids as vectors. Bruce Braun states that the bacteria form nodules and spores, which are extremely tolerant of low temperatures,^[25] which could further validate Thomas-Keprta's discovery of magnetite and carbonate globules in ALH84001.^[26] Through the formation of endospores, the microbes would be transported on the surface of spacecraft and withstand the intense radiation in space on their journey to Mars. However, the presence of opposition to lithopanspermia suggests that there may have been errors involving the testing of these samples, such as contamination by equipment, giving false positive results.

Directed panspermia hypothesises that micro-organisms could therefore be deliberately transported in space. Contrarily, this has been shown to be unfeasible due to the planetary protection rules formed by the Committee on Space Research (COSPAR) which outlined in 1964 that 'contamination of Mars would prevent research into extra-terrestrial life... spacecraft sterilisation is required to avoid such contamination.'^[27] This is consolidated by the 1967 Outer Space Treaty, which all current space-faring nation-states, have both signed and ratified. Consequently, all spacecrafts are sterilised vigorously. COSPAR also classifies missions into distinct groups, to categorise the importance of sterilisation. Missions to Mars are category IV, which is further split into IVa (Landers that do not search for life, with a maximum of 300,000 spores per spacecraft) and IVc (Any component that accesses a Martian special region; they must be sterilised to have 30 spores at maximum.)^[28]

There are numerous ways space probes are sterilised, such as dry heat sterilisation for sensitive missions under category IVc, this, whilst costly, is effective at reducing the probability of contamination by causing the emergence of ROS, destroying the nucleic acids of the prokaryotes. Gamma and UV radiation are also used for Martian missions,^[28] yet their efficacy is limited due to extremophiles like *D. radiodurans* being resistant to 15kGys of radiation and using enough energy to generate that would not be cost effective.

Despite these conditions, it is still possible for polyextremophiles to contaminate the probes; Madhusoodanan outlines microbes from swabs of the Curiosity rover were subjected to desiccation, UV exposure, cold and pH extremes, nearly 11% of the 377 strains survived more than one of these severe conditions.^[29] This implies that there are flaws in the sterilisation process, and also validates the panspermia hypothesis; the microbes must have formed endospores to resist the IR of space, meaning that microbes have already managed to reach Mars. Colonisation is still deemed unlikely though, as the micro-organisms would have to find an ideal habitat on Mars, which is unlikely.

Ecologist Daniel Dykhuizen proposed in 2005 that there are at least '1 billion species of bacteria alone.'^[31] Currently, the accepted target probability of contamination per Martian mission is 0.01%, though

scientists are also relying on Mars' hostile conditions to further reduce the possibility of growth. However, I believe there are micro-organisms capable of surviving this lethal atmosphere, like *Chroococcidiopsis*. *D. radiodurans* is also capable of overcoming the intense IR on Mars, but I conclude that it will not be able to survive the anaerobic conditions of Mars without major alterations to its metabolism. Whilst there are obligate anaerobes suited to this environment, there are not any that have been proven to currently withstand the radiation on the Martian surface. Further research into this field will no doubt develop this theory, but the future of humankind is bound to include a colony of Mars, and so with us, there is a huge likelihood of these 'silent beings' accompanying us there.

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