

# A Review on Astrobiology and Astromicrobiology

Disti Vaghela<sup>\*</sup>, Bhumika Yadu<sup>\*\*</sup>, Rakhi Bajpai<sup>\*\*\*</sup>

<sup>\*</sup>Student of School of Life & Allied Sciences, ITM University, Raipur, Chhattisgarh

<sup>\*\*</sup>Assistant Professor, School of Life & Allied sciences, ITM University, Raipur, Chhattisgarh

<sup>\*\*\*</sup>Associate Professor, School of Life & Allied Sciences, ITM University, Raipur, Chhattisgarh

Corresponding author: Rakhi Bajpai,

Email: [rakhib@itmuniversity.org](mailto:rakhib@itmuniversity.org)

\*\*\*\*\*

## Abstract:

Astrobiology is an interdisciplinary field that investigates the big bang theory, evolution, the origin of life, and the existence of extraterrestrial life. Geology, astrophysics, chemistry, biology, mythology, and other disciplines are all included. "Astromicrobiology" or "Exomicrobiology," which focuses on the existence of microbial life in an extraterrestrial environment, their growth, metabolic activity, and pathogenicity in space-like conditions, is a much more focused subfield of astrobiology for those already working in the fields of microbiology or biotechnology. The subject, terminology, microbiota (especially extremophiles), and subdivisions of the microbiota are covered in the first half of this paper. The second half explores exoplanets that support life, such as Mars, Saturn, as well as their natural satellites, Titan, Enceladus, and Europa.

**Keywords:** Astrobiology, Exomicrobiology, Extraterrestrial life, Exoplanets, Microbiota, Extremophiles.

\*\*\*\*\*

## I. INTRODUCTION

Astrobiology is an interdisciplinary field of science that investigates life throughout the universe by studying its origin, development, and ongoing distribution in connection with wide-ranging discipline areas including geology, astronomy, astrophysics, chemistry, biology, biotechnology, and often mythology as well as within narrower focus areas such as those of microbiologists and/or biotechnologists via a sub-field named exobiology or astromicrobiology. Astromicrobiology concerns itself with issues related to the study of microorganisms and their capability to survive and pathogenicity as related to the potential existence of microbial life in outer space or in environmental conditions similar to those present in outer space. The word "astrobiology" was first used by Russian astronomer Gavriil Adrianovich Tikhov in 1953 (Tikhov 1953; Thombre *et al.*, 2020). The space age began shortly thereafter with the launch of the first manmade satellite—the Soviet Union's Sputnik in 1957 and accelerated research into

astrobiology. In 1960, Joshua Lederberg conducted an investigation into exobiology and searched for clues of extraterrestrial life (Lederberg 1960). Lederberg along with Carl Sagan established exobiology as an area of scientific research for NASA (Emlik and Marakli, 2023).

Many model microorganisms exist in astrobiology & most of these type(s) of microorganisms have been created specifically for the purpose of observing the different stages of the life cycle of those organisms. While most of these model organisms fall under the category of marine microorganisms (exemplified by bacteria), many of the new model organisms created are classified as 'extremophiles.' This is due to the overwhelming number of extremophilic species that have adapted to & are thriving within virtually every domain & extreme environment (e.g., deserts, acidic mine drainage, thermal springs, glaciers, extreme salinity).

Hydrothermal vents and the environment around them could be analogs for early Earth and icy moons, which have great potential as living

environments and targets of astrobiology (Klenner *et al.*, 2024). For this reason, much research is devoted to studying Jupiter's moons (Europa and Ganymede) and Saturn's moons (Enceladus and Titan), including their surfaces and chemistry and whether or not they can support life. Mars has received more attention from an astrobiology perspective than the icy moons of the outer solar system. It was Italian astronomer Giovanni Schiaparelli who first observed Mars using a telescope with limited resolution and drew a map of what he saw. He claimed that he saw dark, straight lines across the surface of Mars, which he referred to as "canali", the Italian word for "channels", but did not make any comment about their natural or artificial origins (Schiaparelli, 1878). Because of Schiaparelli's descriptions of what he saw, and subsequent confirmation of his findings by other astronomers, the misinterpretation of "canali" as "canals" in English led to worldwide speculation regarding the existence of water on Mars (Dick, 1996).

## II. ISS- A MICROBIAL OBSERVATORY

The International Space Station (ISS) is a closed system and has been a microbial observatory for research into microbes' adaptation and survivability in outer space conditions over the last 10 years. Two types of stress applied to these microbes are: microgravity and radiation, both of which affect microbial growth in space, allowing scientists to comprehend the nature and growth characteristics of the model microbes under these two conditions, thus enabling scientists to look for specific microbes, or exoplanets that will allow these microbes to grow and contribute to proving the existence of extraterrestrial life. The ISS is in a stable orbit within the thermosphere, which is located in the upper atmosphere of the Earth, and travels around the Earth at an approximate height of 400 km (Castro *et al.*, 2013). The microbes that were in outer space (our low earth orbit), are the only ones on Earth that have been exposed to multiple types of stress. These stresses include: altered gravity (gravity between  $10^{-3}$  to  $10^{-6}$  g), high temperature (from 153 to 393 K) in a vacuum

(low pressure  $10^{-7}$  to  $10^{-4}$  Pa), and high levels of CO<sub>2</sub> (partial pressure of 0.2 to 0.5 kPa) (Sielaff *et al.*, 2019; Horneck *et al.*, 2010; Huang *et al.*, 2018).

### I. Radiations

There are 2 different kinds of cosmic radiations in LEO. Galactic Cosmic Radiation (GCR), these are the radiations, accelerated mostly by the effect of supernova, which comprise high energy particles such as, high energy protons, heavy particles, and alpha particles. Solar Cosmic Radiation (SCR), these radiations are produced due to intense solar activity such as Coronal Mass Ejection (CMEs) and comprise mostly of protons, alpha particles, electrons, and heavy particles.

In a study, it was observed that DNA is more susceptible to radiation damage by the formation of double-stranded breaks that ultimately lead to mutations, as noticed in *E. coli*, *B. subtilis*, and *Deinococcus radiodurans* (Micke *et al.*, 1994; Schafer *et al.*, 1994; Zimmermann *et al.*, 1994).

### II. Microgravity

Microgravity is defined as being in a state of weightlessness and is a result of gravity's force being reduced or zero'd out. In this case microgravity is acting on a body between  $10^{-3}$  and  $10^{-6}$ g (Herranz *et al.*, 2013; Huang *et al.*, 2018; Nickerson *et al.*, 2004).

Studies conducted in microgravity have resulted in new hypotheses regarding how microgravity affects the biological cells growing in it. The lack of motility exhibited by non-motile cells is proposed as the basis for an increase in cell density. Since the fluid surrounding non-motile cells is quiescent, mass transfer between the cell and the surrounding environment is limited (nutrient uptake and metabolic waste removal). This creates a different chemical composition of the medium on the outside of the cell which may elicit a physiological response of the cell. On the other hand, during the flagellar movement of motile cells, the motile cell will mix with the surrounding fluid on a continuous basis creating improved mass transfer between the two entities and thus countering the effect of microgravity i.e.,

countering a low-shear environment) (Thevenet *et al.*, 1996).

### III. EXTREMOPHILES- THE NOTEWORTHY MICROORGANISMS

Extremophiles (“extrem” = “extreme”, “philes” = “loving”) are those organisms that can thrive in extreme environments such as temperature, pH, alkalinity, and so on. These were first described by MacElroy as “organisms able to populate environments hostile to mesophiles, or organisms which grow only in intermediate environments.”

Certain types of extremophiles are found to be of diverse nature and thus are taken into account and are considered model organisms for research in astrobiology or exomicrobiology. The different types of divisions or categories of extremophiles include thermophiles (grow above 55°C) (Atalah *et al.*, 2019), psychrophiles (grow below 20°C) (Kirkinci *et al.*, 2021), oligophiles (can survive at low nutrient concentration) (Stan-Lotter, 2019), alkaliphiles (grow at pH above 9.0) (Merino *et al.*, 2019), acidophiles (grow at pH below 3-4) (Tripathi *et al.*, 2021), halophiles (grow above 15% NaCl) (Stan-Lotter, 2019), halotolerant, Examples of extremophiles include, *Chroococcidiopsis* sp., which is a cyanobacterium that can survive in extreme desiccation (Billi and Potts, 2002; Li *et al.*, 2022), *Colwellia psychrerythraea*, which belongs to the Colwelliaceae family is a marine heterotrophic bacterium (Liu *et al.*, 2020). This bacterium has become important for studying adaptive strategies against cold and salinity habitats and may be for extraterrestrial conditions (Mudge *et al.*, 2021; Casillo *et al.*, 2022), *Planococcus halocryophilus*, which is an ideal model organism for Martian environments (Heinz *et al.*, 2018), *Haloarubrum lacusprofundi*, an archae, a halophilic species with variation in its pigmentation and *Halobacterium*, a halophilic archae that is also referred to as a polyextremophile due to their ability to live in multiple extreme conditions. Among the above-listed extremophiles, halophiles and halotolerants have been the most studied organisms because they thrive in the same

extreme environments as those that are scientifically observed on planetary environments such as Mars, Europa, and Enceladus.

#### I. Halophiles and Halotolerants

Halophiles are salt-loving organisms, so they require salt to survive. There are three types of halophiles, classified according to the amount of salt they need for optimal growth. Moderate halophiles have a salt concentration of approximately 29 to 146 g L<sup>-1</sup>; borderline extreme halophiles approximately 88 to 234 g L<sup>-1</sup>; and extreme halophiles approximately 146 to 304 g L<sup>-1</sup> (Vieira & Tótola, 2025). On the other hand, halotolerants can survive in both a saline and non-saline environment. They are often selected as model organisms and are primarily studied because of their ability to live in salty oceans on moons such as Europa and Enceladus and in the presence of perchlorate on Mars.

Halophiles are able to endure extreme salt content; thus, they do not become dehydrated due to their ability to utilize a salt-in and salt-out mechanism with the help of halophilic enzymes that have a high concentration of amino acids (e.g. aspartate, glutamate) that when combined with salt form hydrated salts and therefore do not allow for protein aggregation. Halophilic enzymes also contain a high concentration of small hydrophobic residues (e.g. glycine, alanine, valine) and due to the presence of more ionic bonds in specific locations, share the same characteristics of thermophilic enzymes, which can also explain the polyextremophile properties of halophilic enzymes as described by Vieira and Tótola (2025).

In addition to those mentioned, haloarchaea are also being studied because they can produce various pigments that can be detected using remote sensing techniques. For this reason, haloarchaea can be detected on habitable exoplanets and their moons and because they are an important model organism in astrobiology. Another example of an astrobiological model organism under study is *Deinococcus radiodurans*, a bacterium capable of remaining metabolically active at high UV radiation levels. Let us further explore the concept of biosignatures, technosignatures, exoplanets,

their moons and their atmospheric conditions to appreciate the significance of studying extremophiles.

#### **IV. THE CONCEPT OF BIOSIGNATURES AND TECHNOSIGNATURES**

Biosignatures are biological markers that indicate potential extraterrestrial existence. Fossil fuels something that could provide evidence, as do the existence of microbial mats or stromatolites in layers, signs of life providing evidence of lipids, amino-acids or specific isotopes related to certain cellular/genetic characteristics (life-forming) and likely associated with life-processes. The presence of potential life-giving gases (O<sub>2</sub>, CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>, etc.) may also be taken to be biosignatures.

Technosignatures are markers indicating the evolution of technology by extraterrestrial entities. Examples include spacecraft or physical probes, electromagnetic radiosignal emissions, or the detection of pollutant materials such as CFCs emitted into the atmosphere. SETI (Search for Extraterrestrial Intelligence) is a discipline that investigates the search for evidence of both biosignatures and technosignatures.

#### **V. MARS- THE PARAMOUNT EXOPLANET FOR ASTROBIOLOGY**

Of all other planets other than our own planet Earth, the planet Mars is considered the best candidate for searching/exploring other types of life outside of our own planet. Previous studies show that Mars was subject to abundant liquid water (rivers, lakes, oceans) during the Noachian (~4.1 and 3.7 billion years ago), but that liquid water bodies ultimately became extinct in the Hesperian (~3.7 to 3.0 billion years ago) (Vieira and Tótolá 2025). Martian caves are important geological features that have great potential for astrobiological research as they are possible habitats for possible microbes, (i.e., life), and most notably can serve as sheltered archives of biosignatures (the chemical, biological and/or physical evidence of the existence of any past or current life), including past known ecosystems

(Biagioli *et al.* 2025). Recent advances in high-resolution orbital imaging and new applications using artificial intelligence to image and evaluate Martian lava tube caves have confirmed that there are extensive cave systems on Mars and they represent one of the highest priorities for future robotic and human exploration (Léveillé and Datta 2010; Blank *et al.* 2018; Watson and Baldini 2024; Domínguez *et al.* 2025).

Earth's unique and extreme formations of cave ecosystems allow researchers to view cave ecosystems as valuable analogue sites for exploring life in extraterrestrial subsurface environments. Caves typically possess many extreme abiotic features (e.g., oligotrophs, complete darkness, high concentrations of minerals, unusually high levels of carbon dioxide (CO<sub>2</sub>), and variable pH levels) but are also statistically constant regarding microclimatic conditions (Léveillé *et al.*, 2010; Colak and Güngör, 2022). Caves therefore provide habitats that are substantially more stable than those of the surface due to their reduced fluctuations in surface environmental conditions (environmental variability). The aforementioned characteristics make terrestrial caves an ideal model for gaining insight into habitability, biosignature preservation, and microbial survival mechanisms in extreme habitats (Blank *et al.*, 2018). Microbial communities residing within caves on Earth have evolved and developed specialized metabolic pathways, such as chemolithoautotrophy. Chemolithotrophs are microbes that utilize reduced chemical compounds (i.e., nitrogen, sulphur, iron, manganese, and trace amounts of gases in the atmosphere) as a source of energy in order for them to grow and exist on Earth. Such metabolic pathways enable chemolithotrophs to survive in extremely oligotrophic (nutrient-limited) environments. These metabolic pathways may provide important insights into how Martian microbes would survive (Bay *et al.*, 2024; Jurado *et al.*, 2024).

The major geological features found on Mars have been identified as karst-related landforms and lava tubes. Lava tubes form when the surface of a lava flow solidifies and the interior remains fluid. Once

flow completion occurs, only the hollow space of the lava tube is left behind (Léveillé *et al.*, 2010; Sauro *et al.*, 2020; Qiu *et al.*, 2023). Lava tubes and karst-forming surface features can create environments that provide stable microclimates with protection against both cosmic (radiation from space) and solar (radiation from the sun) and may also serve as reservoirs for ice accumulation. This characteristic makes these landforms attractive for astrobiological investigation. From the perspective of astrobiological modeling, terrestrial karst caves and lava tubes represent two different paradigms for understanding what Martian habitats could have existed. Karst caves are developed through chemical (chemical weathering) processes that occur when acidic water (acidified by CO<sub>2</sub> originating from the atmosphere or formed from soil respiration) interacts with carbonate rocks (limestone, dolostone, evaporites). The chemical and physical structures of karst caves create conditions that are conducive to developing high microbial diversity and mineral deposits, as well as the formation of redox gradients, all of which make them excellent environments for astrobiological biosignature (Biagioli *et al.*, 2025).

Several caves on Earth act as astrobiological field study locations (Lechuguilla Cave, USA; Cueva de Villa Luz, Mexico; Río Tinto caves, Spain) while many ice caves, like those in Antarctica, also represent an important terrestrial analogue for potential Martian ecological settings. These caves have a very cold climate (sub-zero temperatures) that supports microorganisms living in ice and rock substrates. They demonstrate that microorganisms capable of growing at low temperatures (psychrophilic organisms) can live in extreme cold conditions where there are few nutrients. The lava tube caves of Mt. Erebus (Antarctica) have a solid basis for being compared to Martian cave habitats because they combine both volcanic and cryogenic conditions. Microorganisms living in lava tube caves of Mt. Erebus have adapted to changes from thermal to geochemical gradients, some of which (microorganisms) are using volcanic gases as an energy source, further demonstrating the astrobiological significance of lava tube caves as

analogs for suspected Martian caves with deposits of ice (Martínez *et al.*, 2014; Stibal *et al.*, 2012). These caves' environment and microbial life have provided researchers with knowledge that can help them develop technologies to detect signs of life on Mars. This information about these caves can also provide scientists with information on what types of biosignatures will exist on Mars, what metabolic processes they used to survive and thrive in, and whether or not they would have positive or negative impacts on their ecosystem and other organisms living there. In astrobiology, all of this information is critical in understanding potential for life elsewhere. This makes the identification of similarities in lava tubes and/or karst-like landscapes on Earth and Mars another major breakthrough in astrobiology.

## **I. Adaptation And Survival Strategies Of Cave Microbiota**

Previous research has indicated that Karst and Lava Tubes are two primary geological entities found on Mars; with the VT known as a developing area for higher microbe to host relationships. These geological entities and their subsurface environments have demonstrated that the factors influencing microbiota include temperature, moisture, mineral composition and the availability of nutrients. There are however, two principal strategies by which micro-organisms collectively or cooperatively grow or exist via; 1- Metabolically Complementary: cooperation between different functioning organisms or cells through the exchange of key metabolites. 2- Co-operative Stress Response: co-operative responses of micro-organisms to survive and combat/mitigate the various stressors present such as antibiotics, radiation, desiccation etc. (Biagioli *et al.*, 2023, Léveillé and Datta, 2010, Bay *et al.*, 2024) In addition to these factors, different environmental pressures placed on micro-organisms within the two geologic structures are based on the use of environmental filters (e.g., water and nutrients; temperature; pH; Redox conditions) which results in different microbiota growing or developing in each geologic structure. The existing microbiota in

each of these structures is known to be extremely adaptable to the conditions of each geologic structure based on the diverse forms of microbial adaptation and survival strategies; such as biofilm formation, chemolithotrophy or psychrophilic or halophilic or endolithic colonization activities. The details about the strategies are-

#### **A. Chemolithotrophy**

Chemolithotrophy is one of the most important metabolic processes used by cave-dwelling microbes, which helps them to derive energy from the oxidation of inorganic substances rather than from sunlight or organic matter (Biagioli *et al.*, 2025). In the case of sulphide, iron, or manganese caves, the microbes get their nutrients from the oxidation or reduction of sulphur, iron, or manganese respectively. For instance- in sulphur caves like Cueva de Villa Luz, which are rich in sulphur, the microbes (Beggiatoa and Desulfovibrio) will be able to metabolize H<sub>2</sub>S or any form of sulphur present (Northup *et al.*, 2001; Hedrich *et al.*, 2021), in the Rio Tinto caves in Spain, which are rich in iron or manganese or have an acidic environment, the microbes (Acidithiobacillus ferrooxidans and Leptospirillum spp.) will be able to produce energy through the oxidation of iron (Jones *et al.*, 2023; 2014; 2016; Martin Pozas *et al.*, 2023; Kelly *et al.*, 2023). There are also epigenic caves in the upper Tennessee River Basin (USA), which harbor Mn (II)-oxidizing bacterial biofilms, and finally, but not least, there is nitrogen-based chemolithotrophy, which is an important component of cave ecosystems. Methanotrophy is another important metabolic process in subterranean environments. Methanotrophs living in caves, such as those found in limestone caves and tropical karst environments, are involved in methane consumption and atmospheric methane oxidation (Waring *et al.*, 2017; Nguyễn-Thùy *et al.*, 2017; Cheng *et al.*, 2021).

However, these processes and their research today play an extremely important role in recognizing the existence of life on Mars as there is abundance of

iron oxide and sulphate, along with traces of nitrogen, in its atmosphere, as research has found.

#### **B. Biofilm Production or Formation**

This is one of the most crucial adaptation strategies observed in the cave microbiome. The development of biofilms (assists in retaining moisture, nutrients, and protection against harmful radiation, desiccation, and temperature changes) and microbial mats enables the microbial community to attach to nutrient-rich materials and resist environmental stress and pressure. In terrestrial caves, biofilms assist in rock surface weathering, which often results in the precipitation of minerals that can act as biosignatures on a geological timescale (Pfendler *et al.*, 2018). The presence of such biomineralized features in Martian lava tubes could be an indication of past or present microbial activity, making biofilms a prime target for future astrobiological missions.

#### **C. Psychrophilic and Halophilic Adaptation-**

It refers to the ability of microbes to survive in a permanent cold and hypersaline environment, it is one of the most important types of adaptation and is of crucial interest as these conditions are synonymous with those found in Martian environment.

Psychrophiles are those microbes that can survive in extreme cold temperatures, this ability is because of their use of antifreeze proteins and cryoprotectants that help to protect the cell from damage caused by the formation of ice crystals. Halophiles, on the other hand, are those microbes that can survive in a hypersaline environment, they can do so by the accumulation of compatible solutes that help to protect the cell.

The research on psychrophiles and halophiles and their adaptation mechanisms is critical as subsurface brines and caves that may contain pockets of liquid water that can act as an intermediate in the microbial metabolic pathway are found in the Martian surface (Ojha *et al.*, 2015).

#### **D. Endolithic Colonization-**

Endolithic organisms, as the name implies, are organisms that live inside rocks. They possess the ability to thrive in harsh conditions such as the

hyper-arid Atacama Desert and the McMurdo Dry Valleys in Antarctica, and thus they are model organisms in astrobiological research and studies (Biagioli *et al.*, 2025).

Endolithic organisms are a broad and diverse range of life forms such as bacteria, fungi, algae, lichens, and viruses (Friedmann, 1982; Archer *et al.*, 2017; Ettinger *et al.*, 2023; Coleine *et al.*, 2021, 2024). The habitation of these organisms is another survival strategy used for their survival and development in caves with harsh environments such as deep karst systems, lava tubes, and Antarctic ice caves. Their survival in the mentioned environment is because of their ability to access the trace amount of water and nutrients available in the matrix of the rock.

The melanized rock-dwelling fungi of the genera *Exophiala*, *Devriesia*, and *Cladosporium* were often mentioned in subterranean environments in a variety of substrates such as sediments, walls, and speleothems, these have been found to demonstrate slow but persistent growth in harsh environmental conditions.

#### **E. Aversion to Radiation, Desiccation and Low Nutrient Availability**

The Earthen caves are well protected from ionizing radiations and thus the survival mechanisms of microbes in such ionizing radiations cannot be analyzed in the microbes present in the Earthen caves but, the experiments conducted on the ISS had shown and proved that many microbes possess DNA repair mechanisms and protective cellular structures that help them withstand radiation and desiccation due to dryness. The model organism that is present on Earth and has shown resistance to the above-mentioned conditions is *Deinococcus radiodurans*. Besides this, certain cryptoendolithic fungi like *Cryomyces antarcticus* and *C. minteri* that are observed to be present in Antarctic deserts also show high resistance to UV radiation and desiccation (Pacelli *et al.*, 2019; Gomez-Gutierrez *et al.*, 2024).

The microbes resist the stress caused due to the limited availability of nutrients by oligotrophic resistance and this is a crucial microbial adaptation for the survival of microbes in such conditions. The

microbiome is able to survive in the nutrient-scarce environment due to the flexibility in their metabolic pathways that include a slower growth rate and efficient mechanisms to recycle the nutrients so as to survive without any reduction in the population. The study has only concentrated on the caves that exist on both Earth and Mars and their relation as, Karst and Lava tubes are proven to exist on both the planets and so are many atmospheric and environmental conditions, thus their study helps to relate about what organisms might be present, the reason of them being in that particular area, their metabolic process and the most significant- the proof that alien life can be or is present on the Martian surface, because, if a microbe can survive on Earth in the conditions that are similar to the red planet, they can and must be able to survive on the Exoplanet. Additionally, caves are excellent and long-lasting preservers of biosignatures, thus acting as natural time capsules that safeguard microbial fossils from any form of external degradation (Biagioli *et al.*, 2025).

## **VI. THE ICY MOONS OF SATURN**

The research on the Saturnian moons, Enceladus and Titan is further explored in the paper. The reason why these two moons are in the limelight for this review is that there are solid and more valuable proofs of life and life-supporting atmosphere as compared to other moons like Europa or Ganymede. Below is the discussion on two of the Saturn's satellites in brief.

### **I. Enceladus**

Enceladus, a moon of Saturn, is one of the Ocean worlds, thus meaning, a planetary body that has liquid water in the current state and its exploration or study, therefore, provides a huge assistance to the study of astrobiology and also in its development. The exploration plan for Enceladus primarily means the validation of the Organic Chemical Evolution theory (OCE).

The identification of Enceladus as an exo satellite that supports life occurred in the year 2005 when the Cassini-Huygens space exploration mission performed compositional analysis, both in situ using its mass spectrometers—the Cosmic Dust

Analyzer (CDA) and the Ion and Neutral Mass Spectrometer (INMS)—and also using the Ultraviolet Imaging Spectrograph, which obtained compositional information from plume observations that was revealed to be coming from four giant fractures on the South Polar Terrain of Enceladus (Porco *et al.*, 2006) indicating an oceanic origin of this material (Davila *et al.*, 2023; Khawaja *et al.*, 2019). The plume is suspected to be coming from the subsurface liquid water and has an irregular sheet or curtain of water vapor, gas, and ice grains of approx. 0.1-10  $\mu\text{m}$  in size (Porco *et al.*, 2014; Spitale *et al.*, 2015).

In the plume, three kinds of ice grains were identified by mass spectral analysis by CDA, and these were named as Type I (pure water ice grains), Type II (organic rich grains), and Type III (salt rich grains), 10% of these grains settle over the E ring for a time period of over days to decades at distances of 2.5-20  $R_S$  (Saturn radii  $R_S = 60,330\text{ km}$ ) after their ejection from the moon's interior. In recent findings, phosphate has been identified in the material ejected from the moon, also known as plume, this finding included the identification of five out of six bioessential elements, i.e., CHNOPS (Davila *et al.*, 2023, Khawaja *et al.*, 2024). Apart from this, there has been identification of volatile, low mass organic species with N- and O- along with some single ringed aromatic compounds in Type II plume. In a sub type of type II plume, (Postberg *et al.*, 2018) identified complex macromolecular fragments of refractory insoluble organic compounds with masses above 200 u, along with multiple aryl moieties linked to chains of saturated and unsaturated hydrocarbons, alongside N- and O-bearing groups.

The results obtained from the analysis of the plume gas and ice by the Cassini mission suggested that the Enceladus ocean satisfies the conditions to support life and the presence of bioessential elements as mentioned above (Cable *et al.*, 2020; Waite *et al.*, 2017) and geochemical parameters that either support or fall within the tolerance limits of Earth's animals such as temperature, salinity, pH, and the redox couple ( $\text{H}_2$  and  $\text{CO}_2$ ) that can be

utilized as a energy source by chemoautotrophs (Hoehler, 2022; Waite *et al.*, 2017).

The theory of OCE that forms the foundation for the research on Enceladus is that life is the outcome of a sequence of prebiotic chemical reactions, whereby complex chemical compounds and molecular assemblages are formed from the interaction of simpler molecules, and eventually give rise to the first cell. Naturally, OCE is a cyclical process that occurs in massive mixtures of components, with myriads of reactants and products over a wide chemical space of molecular composition and structure (Guttenberg *et al.*, 2017). As per the theory, the foremost research objective would be the search for compounds that are specific to the early stages of OCE, specifically in the models of hydrothermal vents for the origin of life, inorganic compounds such as  $\text{H}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{CO}_2$  that have been identified by Cassini, precursors of small organic molecules such as  $\text{CH}_4$ ,  $\text{NH}_3$ ,  $\text{HCN}$ , and  $\text{CH}_3$  (Rimmer & Shorttle, 2019). Notably,  $\text{CH}_4\text{OH}$  was also detected by the Cassini INMS (Waite *et al.*, 2009, 2017), and  $\text{HCN}$  has been provisionally detected on the basis of statistical models of INMS spectra (Peter *et al.*, 2023). Apart from these, the presence of relevant biochemical building blocks such as oligopeptides, amino acids, carbohydrates, nucleosides, and so on in the ocean samples would assist in the testing of various life theories and hypotheses. In conclusion, it can be said that according to the OCE, the atoms are transformed into molecules, which are then catalyzed or combined to form larger molecules, which in turn can lead to the formation of cell-supporting structures, thus giving rise to a cell, thereby leading to the formation of an organism as a whole.

However, the current discovery of polyelectrolytes, catalysis, or cells in or on the surface of Enceladus would be a revolution in astrobiology on a large scale, but the low energy flux of this ocean world is a challenge (Chyba & Phillips, 2001; Ray *et al.*, 2021), and the low energy is also a reason for the technical constraints that can be overcome only by the use of invention or assistance from high-volume filtration or highly sensitive sensors.

Finally, the study of this satellite is not only restricted to the discovery of life but also to the discovery of information regarding abiotic and prebiotic cursors that can further lead to the development of mutations of the living or life my mutations or as it is.

## **II. Titan**

Titan is the largest moon of the planet Saturn and is actually very similar to the planet Earth on the basis of many different principles. The reason for discussing about Titan is that it is the only world, except for the Earth, that has liquid on its surface. It also has a thick atmosphere that is made up of nitrogen and methane, and along with this, there are lakes, rain, and clouds that are made up of methane and ethane in its overall composition (McKay, 2016).

All the findings regarding the presence of liquid and organic and inorganic material on Titan is discussed because of the mission of orbiting around Saturn by the Cassini. The mission began in 2004 and was in operation till 2017. During the time of the mission, the spacecraft carried out several flybys of its moons, out of which 124 were exclusively devoted to the Titan, which is the largest moon of the planet and 2nd largest in our solar system after Ganymede. It was the findings of the Huygens lander that revealed the presence of global water on the surface. Along with the availability of water, there are many other factors that are responsible for the habitability of life on a planet, such as- sources of chemical or light energy, availability of nutrients, liquid habitats, and transport cycles or mechanisms of liquid moving nutrients and wastes. The question that arises here is that are these requirements met by the atmosphere of Titan or its surface.

### **A. Sources of Chemical or Light Energy**

The methane, ethane, and other organic compounds in Titan's atmosphere can be subjected to several redox reactions and yield energy, for example, the photochemically produced organic compounds in Titan's atmosphere would yield energy if reacted with  $H_2$  in the atmosphere, hence would act as a source of biological energy (Schulze-Makuch and

Grinspoon, 2005), the reaction of  $O_2$  with  $CH_4$  yields energy of  $\sim 900 \text{ kJ mole}^{-1}$ , etc.

In the case of light energy, the results obtained from the Huygens probe indicate that due to the distance of Saturn from the sun, and the presence of haze in the atmosphere, the maximum intensity of sunlight on the surface of Titan is about 0.1% of the overhead sun on the surface of the Earth (McKay, 2016). Despite the presence of haze, there is an even distribution of solar flux, with the maximum flux at about  $0.6 \text{ um}$ , this flux is more than sufficient for the process of photosynthesis as it is on Earth. Hence, this proves that both chemical and light energy is sufficient on Titan for the support of life and its processes.

### **B. Nutrient Availability**

The principal organic elements present in Titan are C, H, and N, in which N is most abundantly present. The issue here is the scarcity of O, and it has been observed that O is present in the form of ice only in  $H_2O$  and is not present in any other form or compound/molecule. This will cause less availability of nutrients on the moon compared to Earth. Another significant problem for life on this satellite will be the availability of inorganic elements, like- Fe, Cu, Mn, Zn, Ni, S, Ca, Na, and K, etc. These elements can be easily accessed by the life on Earth due to their solubility in water and the indirect intake of the same through the intake of water. Although these elements are needed in smaller quantities, they are still needed for the process of life. Christopher P. McKay proposes that two approaches may be regarded as applicable for nutrients to sustain life on Titan: (1) the conservative utilization of elements that are difficult to access; and (2) the utilization of  $H_2O$  to fulfil some of the roles of the inorganic elements.

There is another alternative for the 1st approach, it states that there can be a removal of the requirement for these inorganic substances altogether, i.e, the process of photosynthesis is not required, hence conserving the energy required for the fixation of  $N_2$  and the atom N itself. Furthermore, it is possible that the  $H_2O$  molecules in Titan could be utilized in a manner similar to

how metals are utilized in enzymes by living organisms on Earth, this is also possible given the fact that the low temperature in Titan leads to very strong hydrogen bonding, which can hence lead to the formation of useful structures or substances (McKay, 2016).

### C. *Liquid Habitat*

It is a reality that the presence of water is a very essential factor for the existence of life on any planet or natural satellites. From the observations of the Huygens probe, it was clear that there are large lakes in the northern hemisphere of Titan and at least one large lake in the southern hemisphere of Titan, apart from this, it was observed that the land is wet with methane and ethane in the equatorial landing site of the probe. The lakes that are visible in the northern hemisphere constitute as 97% of the total lakes that are present on the surface of Titan and these are present in only 2% of the total surface area of Titan (in the range of 900-1800 kms). There are large lakes on one hand, which are deep, have fractal shorelines, and fluvial channels (Stofan *et al.*, 2007) and small lacustrine depressions on the other hand, which are shallow and have rounded shorelines (Wasiak *et al.*, 2013). The geological processes that cause the formation of lakes on the moon are still not clear. However, there was a discussion on the status of knowledge of the lakes and the proposal of karst-like formation processes due to the dissolution of solid organics by the liquid methane and ethane (Cornet *et al.*, 2015). Orbital spectral observations had led to the classification of Titan's surface into five terrains: 1. Bright terrain; 2. Dark equatorial dune fields, or dark brown units; 3. Blue units; 4. 5  $\mu\text{m}$  bright units and the 5. Dark lakes.

The  $\text{CH}_4$  and  $\text{C}_2\text{H}_6$  liquids are abundant on Titan in lakes and the wet surface. If life can thrive in these liquids, then life is abundant on Titan without many exceptions and/or difficulties (McKay, 2016).

### D. *Transport Cycles of Nutrient and Waste Movement with Water*

However, unlike the transport cycle on Earth, which includes water and salts as non-volatile

solids, the transport cycle on Titan includes methane, ethane, as well as  $\text{N}_2$  that is soluble in the two. Nitrogen is left behind as the ethane, which is non-volatile relative to methane, evaporates. However, nitrogen and methane can readily be detected in or introduced into the surface liquids, and therefore, the rain on Titan is expected to consist of a mixture of the two gases, the presence of which is determined by either methane or ethane, depending on the hemisphere in which the cycle is taking place, as the northern hemisphere is seen to be dominated by methane, while the southern is dominated by ethane. Moreover, unlike Earth, the density of the fluids on Titan varies depending on the temperature, and the higher the temperature, the higher the density, and vice versa, which greatly complicates the transport of the liquids on the moon relative to the processes taking place on Earth.

### Conclusion

Astrobiology is an emerging and interdisciplinary field that seeks to understand the origin, evolution, and distribution of life in the universe. This review highlights astro/exomicrobiology, with a focus on extremophiles, as key models for exploring the potential existence of microbial life beyond Earth. The ability of extremophiles to survive under harsh conditions analogous to those on planets and moons such as Mars, Europa, Enceladus, and Titan strengthens the possibility of extraterrestrial life. The presence of water, along with essential organic and inorganic compounds, makes these celestial bodies prime targets for astrobiological research. With rapid technological advancements, astrobiology continues to expand its scope, encouraging future studies to explore metabolic pathways adapted to extraterrestrial environments rather than limiting the search to Earth-like life forms.

### Acknowledgements

The authors are thankful to ITM University, Raipur, Chhattisgarh, India, for providing institutional support and access to academic resources necessary for the completion of this review article.

## References

1. Archer, S.D.J., de los Ríos, A., Lee, K.C. *et al.* (2017). Endolithic microbial diversity in sandstone and granite from the McMurdo Dry Valleys, Antarctica. *Polar Biol* 40: 997–1006.
2. Atalah, J., Cáceres-Moreno, P., Espina, G., Blamey, J.M. (2019). Thermophiles and the applications of their enzymes as new biocatalysts. *Bioresour Technol.* 280:478-488.
3. Bijlani, S., Stephens, E., Singh, N.K., Venkateswaran, K., Clay, C.C. Wang (2021). Advances in space microbiology, *iScience*.24(5): 1-19.
4. Huang, B., Li, D.G., Huang, Y., Liu, C.T. (2018) Effects of spaceflight and simulated microgravity on microbial growth and secondary metabolism. *Mil Med Res.* 5(1):18.
5. Bay, S.K., Ni, G., Lappan, R., Leun, P.M., Wong, W.W., Holland, S., and Greening, C. (2024) Microbial aerotrophy enables continuous primary production in diverse cave ecosystems. *bioRxiv*.
6. Biagoli, F., Bay, S. *et al.* (2025). Caves on Earth as proxies for Martian subsurface environments. *International Journal of Astrobiology.* 24(29):1–21
7. Blank, J. G., Roush, T.L., Stoker, C.L., Colaprete, A., Datta, S., Wong, U & Wynne, J.J. (2018) Planetary caves as astrobiology targets. A white paper submitted to the Space Studies Board of the National Academy of Sciences. 5.
8. Nickerson, CA., Ott, C. & Wilson, James & Ramamurthy, Rajee & Pierson, Duane. (2004). Microbial Responses to Microgravity and Other Low-Shear Environments. *Microbiology and molecular biology reviews.* 68(2): 61-345.
9. Castro, V., Thrasher, A., Healy, M. *et al.* (2004). Microbial Characterization during the Early Habitation of the International Space Station. *Microbial Ecology.* 47(2):119–126.
10. Cheng, X-Yet *al.* (2021) USC  $\gamma$  dominated community composition and cooccurrence network of methanotrophs and bacteria in subterranean Karst Caves. *Microbiology Spectrum.* 9(10).
11. Chyba, C. F., & Phillips, C. B. (2001). Possible ecosystems and the search for life on Europa. *Proceedings of the National Academy of Science USA.* 98(3):801-804.
12. Çolak, B. and Güngör, N.D. (2022) The astrobiological significance of caves on Earth and on Mars. *International Journal of Environment and Geoinformatics.*9:57-64.
13. Coleine, C., Albanese, D., Ray, A.E., Delgado-Baquerizo, M., Stajich, J.E., Williams, T.J., and Selbmann, L. (2024). Metagenomics untangles potential adaptations of Antarctic endolithic bacteria at the fringe of habitability. *Science of the Total Environment.*917.
14. Coleine, C., Biagioli, F., de Vera, J.P., Onofri, S., and Selbmann, L. (2021). Endolithic microbial composition in Helliwell Hills, a newly investigated Mars-like area in Antarctica. *Environmental Microbiology.* 23(7):4002-4016.
15. Cornet, T.; Cordier, D.; Le Bahers, T.; Bourgeois, O.; Fleurant, C.; Le Mouélic, S.; Altobelli, N. (2015). Dissolution on Titan and on Earth: Towards the age of Titan's karstic landscapes. *Journal of Geophysical Research: Planets.* 1-2.
16. Davila, A. F., & McKay, C. P. (2014). Chance and necessity in biochemistry: Implications for the search for extraterrestrial biomarkers in Earth like environments. *Astrobiology.*14(6).
17. Davila, A. F., & Eigenbrode, J. L. (2024). Enceladus: Astrobiology revisited. *Journal of Geophysical Research: Biogeosciences.*129:1-11.
18. Dick, Steven J. 1996. *The Biological Universe: The Twentieth Century Extraterrestrial Life Debate and the Limits of Science.* Cambridge University Press.
19. Domínguez, R., Pérez-del-Pulgar, C., Paz-Delgado, G.J., Polisanò, F., Babel, J., Germa, T., Dragomir, I., Carlietti, V., Berthet, A.C., Danté, L.C. and Kirchner, F. (2025). Cooperative robotic exploration of a planetary skylight surface and lava cave. *Science Robotics.* 10(89).
20. Emlik, S., Marakli, S. (2023). Important extremophilic model microorganisms in

- astrobiology. *Frontiers in Life Sciences and Related Technology*. 4(2):105–110.
21. Ettinger, C.L., Saunders, M., Selbmann, L., Delgado-Baquerizo, M., Donati, C., Albanese and Coleine, C. (2023). Highly diverse and unknown viruses may enhance Antarctic endoliths' adaptability. *Microbiome*.11(103).
22. Gomez-Gutierrez, S.V., Sic-Hernandez, W.R., Haridas, S., LaButti, K., Eichenberger, J., Kaur, N., and Grigoriev, I.V. (2024). Comparative genomics of the extremophile *Cryomyces antarcticus* and other psychrophilic Dothideomycetes. *Frontiers in Fungal Biology*.5:1-22.
23. Guttenberg, N., Virgo, N., Chandru, K., Scharf, C., & Mamajanov, I. (2017). Bulk measurements of messy chemistries are needed for a theory of the origins of life. *Philosophical Transactions of the Royal Society A: Mathematical, Physical & Engineering Sciences*. 375(2109).
24. Hedrich, S., and Schippers, A. (2021). Distribution of acidophilic microorganisms in natural and man-made acidic environments. *Current issues in molecular biology*.40(1):1-23.
25. Hoehler, T. M. (2022). Implications of H<sub>2</sub>/CO<sub>2</sub> disequilibrium for life on Enceladus. *Nature Astronomy*.6(3).
26. Herranz, R., Anken, R., Boonstra, J., Braun, M., Christianen, P.C., de Geest, M., Hauslage, J., Hilbig, R., Hill, R.J., Lebert, M. *et al.* (2013).Ground-based facilities for simulation of microgravity: organism-specific recommendations for their use, and recommended terminology *Astrobiology*.13(1):1-17.
27. Jones, D. S., Schaperdoth, I., Northup, D.E., Gómez-Cruz, R., and Macalady, J.L. (2023). Convergent community assembly among globally separated acidic cave biofilms. *Applied and Environmental Microbiology*.89(1):1-17.
28. Jurado, V., Northup, DE. and Saiz-Jimenez, C. (2024). Microbial roles in caves. *Frontiers in Microbiology*.15:1-3.
29. Kelly, L.C., Rivett, D.W., Pakostova, E., Creer, S., Cotterell, T., and Johnson, D.B. (2023). Mineralogy affects prokaryotic community composition in an acidic metal mine. *Microbiological Research*.266:1-11.
30. Khawaja, N., Postberg, F., Hillier, J., Klenner, F., Kempf, S., Nölle, L., et al. (2019). Low-mass nitrogen-oxygen-bearing, and aromatic compounds in Enceladean ice grains. *Monthly Notices of the Royal Astronomical Society*.498(4):5231-5243.
31. Kirkinci, S. F., Edbeib, M. F., Aksoy, H. M., Marakli, S., & Kaya, Y. (2021). Identification of Dalapon degrading bacterial strain, *Psychrobacter* sp. TaeBurcu001 isolated from Antarctica. *Polar Science*.
32. Klenner, F., Baqué, M., Beblo-Vranesevic, K., Bönigk, J., Boxberg, M.S., Dachwald, B., Digel, I., Elsaesser, A., Espe, C., Funke, O., Hauber, E., Heinen, D., Hofmann, F., Hortal Sánchez, L., Khawaja, N., Napoleoni, M., Plesa, A.C., Postberg, F., Purser, A., Rückriemen-Bez, T., Schröder, S., Schulze-Makuch, D., Ulamec, S. and de Vera, J.P.P. (2024). Icy ocean worlds astrobiology research in Germany. *Frontiers in Astronomy and Space Sciences*. 11:1-18.
33. Lévillé, R.J. and Datta, S. (2010). Lava tubes and basaltic caves as astrobiological targets on Earth and Mars: a review. *Planetary and Space Science*. 58:592–598.
34. Martínez, G., and Renno, N.O., (2013). Water and brines on Mars: current evidence and implications for MSL. *Space Science Reviews*. 175: 29–51.
35. Martin-Pozas, T., Cuezva, S., Fernandez-Cortes, A., Cañaveras, J.C., Benavente, D., Jurado, V., and Sanchez-Moral, S., (2022). Role of subterranean microbiota in the carbon cycle and greenhouse gas dynamics. *Science of the Total Environment*. 20:831-154921.
36. McKay, C.P, (2016). Titan as the Abode of Life.6(8):1-15.
37. Merino, N., Aronson, H. S., Bojanova, D. P., Feyhl-Buska, J., Wong, M. L.,Zhang, S., & Giovannelli, D. (2019). Living at the extremes:extremophiles and the limits of life in

- a planetary context. *Frontiers in Microbiology*.10:1-25.
38. Micke, U., Horneck, G., Kozubek, S. (1994). Double strand breaks in the DNA of *Bacillus subtilis* cells irradiated by heavy ions. *Advances in Space Research*.14(10):207-211.
39. Nguyễn-Thuỳ, D. et al (2017). Subterranean microbial oxidation of atmospheric methane in cavernous tropical karst. *Chemical Geology*.466:229-238.
40. Northup, D.E. & Lavoie, K.H. (2001). Geomicrobiology of caves: a review. *Geomicrobiology Journal*.18:199-222.
41. Ojha, L., Wilhelm, M.B., Murchie, S.L., McEwen, A.S., Wray, J.J., Hanley, J., and Chojnacki, M. (2015). Spectral evidence for hydrated salts in recurring slope lineae on Mars. *Nature Geoscience*. 8:829–832.
42. Pacelli, C., Selbmann, L., Zucconi, L., Coleine, C., de Vera, J.P., Rabbow, E., and Onofri, S., (2019). Responses of the black fungus *Cryomyces antarcticus* to simulated mars and space conditions on rock analogs. *Astrobiology*.19(2):209-220.
43. Peter, J. S., Nordheim, T. A., & Hand, K. P. (2023). Detection of HCN and diverse redox chemistry in the plume of Enceladus. *Nature Astronomy*. 8:164-173.
44. Pfendler, S., Karimi, B., Maron, P.A., Ciadamidaro, L., Valot, B., Boust, F., and Aleya, L., (2018). Biofilm biodiversity in French and Swiss show caves using the metabarcoding approach: First data. *Science of the Total Environment*. 615:1207-1217.
45. Porco, C. C., Helfenstein, P., Thomas, P. C., Ingersoll, A. P., Wisdom, J., Wes, R., et al. (2006). Cassini observes the active south pole of Enceladus. *Science*. 311(5766):1393-401.
46. Postberg, F., Clark, R. N., Hansen, C. J., Coates, A. J., Dalle Ore, C. M., Scipioni, F., et al. (2018). Plume and surface composition of Enceladus. In *Enceladus and the icy moons of Saturn*. The University of Arizona Press.475:129-162.
47. Qiu, X. and Ding, C. (2023). Radar observation of the lava tubes on the Moon and Mars. *Remote Sensing*.15:1-28.
48. Ray, C., Glein, C. R., Waite, J. H., Teolis, B., Hoehler, T., Huber, J. A., et al. (2021). Oxidation processes diversify the metabolic menu on Enceladus. *Icarus*.364.
49. Rimmer, P., & Shorttle, O. (2019). Origin of life's building blocks in carbon- and nitrogen-rich surface hydrothermal vents. *Life*. 9(1):12.
50. Sauro, F., Pozzobon, R., Massironi, M., De Berardinis, P., Santagata Tand De Waele, J., (2020). Lava tubes on Earth, Moon and Mars: a review on their size and morphology revealed by comparative planetology. *Earth-Science Reviews*.209.
51. Schafer, M., Schmitz, C., Buckner, H. (1994). Heavy ion induced DNA double strand breaks in cells of *E. coli*. *Advances in Space Research*. 14(10):6-203.
52. Schiaparelli, Giovanni Virginio. 1878. Osservazioni astronomiche e fisiche sull'asse di rotazione e sulla topografia del pianeta Marte: fatte nella Reale Specola di Brera in Milano coll'equatoriale di Merz durante l'opposizione del 1877. Salviucci.978.
53. Schulze-Makuch, D., Grinspoon, D.H. (2005). Biologically enhanced energy and carbon cycling on Titan? *Astrobiology*. 5(4):7-560.
54. Senatore, G., Mastroleo, F., Leys, N., Mauriello, G. (2018). Effect of microgravity & space radiation on microbes. *Future Microbiology*. 13.
55. Sielaff, C. A., Urbaniak, C., Mohan, G.B.M., Tran, Q., Wood, J.M., Minich, J., McDonald, D., Mayer, T., Knight R., et al. (2019). Characterization of the total and viable bacterial and fungal communities associated with the International Space Station surfaces. *Microbiome* 7(50):1-21.
56. Spitale, J. N., Hurford, T. A., Rhoden, A. R., Berkson, E. E., & Platts, S. S. (2015). Curtain eruptions from Enceladus' south-polar terrain. *Nature*. 521:57–60.

57. Stan-Lotter, H. (2019). Survival of subsurface microbial communities over geological times and the implications for astrobiology. In: Seckbach J., Rampelotto P. (eds) Model Ecosystems in extreme Environments. Academic Press.169-187.
58. Stibal, M., Wadham, J.L., Lis, G.P., Telling, J., Pancost, R.D., Dubnick, A., and Anesio, A.M., (2012). Methanogenic potential of Arctic and Antarctic subglacial environments with contrasting organic carbon sources. *Global Change Biology*.18(11): 3332-3345.
59. Stofan, E.R.; Elachi, C.; Lunine, J.I.; Lorenz, R.D.; Stiles, B.; Mitchell, K.L.; Ostro, S.; Soderblom, L.; Wood, C.; Zebker, H.; et al. (2007). The lakes of Titan. *Nature*. 445:61–64.
60. Thevenet, D., D'Ari, R., Boulloc, P. (1996). The SIGNAL experiment in BIORACK: *Escherichia coli* in microgravity. *Journal of Biotechnology*. 47:89-97.
61. Vieira, C. D. S., Tótola, M. R. (2025). Halophiles and halotolerants: From Industry to astrobiology. *Current Microbiology*. 82(482).
62. Waite, J. H., Lewis, W. S., Magee, B. A., Lunine, J. I., McKinnon, W. B., Glein, C. R., et al. (2009). Liquid water on Enceladus from observations of ammonia and <sup>40</sup>Ar in the plume. *Nature*.460:487-490.
63. Waite, J. H., Glein, C. R., Perryman, R. S., Teolis, B. D., Magee, B. A., Miller, G., et al. (2017). Cassini finds molecular hydrogen in the Enceladus plume: Evidence for hydrothermal processes. *Science*.356(6334):155-159.
64. Waring, C.L., et al. (2017). Seasonal total methane depletion in limestone caves. *Scientific Reports*.7.
65. Wasiak, F.C.; Androes, D.; Blackburn, D.G.; Tullis, J.A.; Dixon, J.; Chevrier, V.F. (2013). A geological characterization of Ligeia Mare in the northern polar region of Titan. *Planet. Space Science*. 84:141-147.
66. Watson, T.H. and Baldini, J.U. (2024). Martian cave detection via machine learning coupled with visible light imagery. *Icarus*.411.
67. Zimmermann, H., Schafer, M., Schmitz, C., Bucker, H. (1994). Effects of heavy ions on inactivation and DNA double strand breaks in *Deinococcus radiodurans* R1. *Advances in Space Research*.14(10):203-206.