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Chapter

Extremophiles and Limits of Life in a Cosmic Perspective

Nawab Ali, Muhammad Nughman and Syed Majid Shah

Abstract

Extremophiles are one of the most extreme entity on planet earth which can withstand many harsh conditions considered lethal for other life form of terrestrial life. From an evolutionary prospective, extremophiles are considered to be primitive cells that used to live in the early earth's harsh environment living on this planet since billions of years, it can be found in almost in any environmental conditions on our planet. There are many established valuable uses of these extremophiles and particularly their bioactive compounds. The enzymes produced by extremophiles have significant applications in different industries like detergent, food, feed, starch, textile, leather, pulp and paper, and pharmaceuticals This chapter discuss extremophile, their survival mechanism and astrobiology, discussing life in a cosmic prospective.

Keywords: extremophiles, bioactive compounds, limits of life, astrobiology

1. Introduction

Over the period of the last century, the conditions at which life is able to flourish have been pushed in every possible direction, expanding to include wider range of temperature, pH, pressure, radiation, salinity, energy, and nutrient limitation. This has allowed for the discovery of life in previously unimaginable environments. Microorganisms have the ability to thrive in different harsh conditions on earth and in space, which include microgravity, high radiation, vacuum pressure, and extremely diverse temperature [1, 2]. Extremophiles are those organisms which can withstand many harsh conditions which are considered lethal for other life form of terrestrial life. They are able to flourish in extremely higher and lower temperature, as well as acidic and alkaline environments. Many extremophiles can also thrive in different organic solvents, heavy metals and hazardous waste [3]. Space has been often called “The Final Frontier,” where most of the universe’s conditions are hazardous for human habitation [4]. Extremophiles are essential for discovering biosignatures that can be used to find habitable environments beyond the earth. Different ecological habitat of earth has similarity to that of other planets in term of nutrient composition and biogeochemistry [5]. The aim of this chapter is to discuss extremophiles, importance of their bioactive compounds. Later on, the chapter is focused on life in extreme conditions, limits of life and astrobiology, discussing life in a cosmic prospective.
2. Extremophilic microorganisms

The term extremophile was first coined by McElroy in the year 1974 [6]. Most extremophiles are classified within the archaea, bacterial, and eukaryotic kingdoms [7]. Around a decade ago extremophiles were considered exotic organisms that were only investigated by few researchers around the world. Now, it has become a promising field for enzymologists to explore and utilize these microorganisms in a variety of industrial applications [8]. Studies on extremophiles have advanced substantially over the past twenty years where first international congress on extremophiles was held in Portugal (1996) and the peer-reviewed scientific journal “Extremophiles” was launched in 1997. In addition, in 2002, the “Worldwide Society for Extremophiles” (ISE) was established as an international organization for the purpose of facilitating the sharing of knowledge and expertise in the rapidly expanding field of study on extremophiles [9].

Extremophilic organisms have the ability to grow in such environments which are considered inhospitable to other life forms. These conditions include extreme hot and cold environments, and highly alkaline and acidic environments. Several extremophiles can also thrive in different hazardous waste, organic solvents and heavy metals. It’s been discovered that extremophiles can also live in more than ten kilometers deep in the ocean and 6.7 kilometers deep in the earth’s crust; in conditions ranging from 0 to 12.8 pH, in temperatures from 122°C to 20°C; and at pressures of up to 110 megapascals (MPa) [10]. Some organisms are not only stable at harsh conditions, but they need them to survive. Extremophiles are classified into different classes based on the conditions in which they thrive, they are thermophiles, which can withstand extreme temperature, psychrophiles, which can withstand extreme low temperature, acidophiles and alkaliphiles, which can withstand extreme higher and lower pH, barophiles, which can withstand higher pressure and halophiles which can withstand higher salt concentration [11]. Extremophiles are sometimes considered as polyextremophiles which mean that they can withstand multiple harsh conditions. Some hot springs have diverse harsh conditions i.e., they are alkaline and basic at the same time and also have higher amount of heavy metals. Different hypersaline lakes are extremely alkaline, and the deep oceans have also diverse harsh condition like cold, oligotrophic and high pressure [12].

Extremophiles have attracted a lot of attention because they are able to catalyze reactions despite harsh conditions and also have significant applications in industries [13–15]. Now researchers are focusing more on genetic engineering of enzymes to improve their activity and to make them interesting candidate in industrial and biotechnological processes. This is because extremozymes are more difficult to isolate than other types of enzymes. Microbes with desirable industrial traits are genetically engineered by a large number of research organizations and companies across the world, and these microorganisms has been used in different industrial processes [16]. Industrial enzymes had a market value of more than one billion dollars in 2010, and it was projected that this value will increase to five billion dollars in 2021 at 4% growth per year [17]. This was achieved earlier, as they hit $5.5 billion in 2018, and various research associations now estimate that they will reach $7.0 billion by 2023 [18, 19]. Extremozymes are the most promising options to take into consideration to meet the ever-increasing demand on the global market [14]. Extremophiles can produce different industrially important enzymes which are stable at different harsh conditions. We have reviewed different extremophiles, there habitat, growth characteristics, bioactive compounds and their industrial importance as shown in the Table 1.
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Table 1.  
Overview of different extremophiles, their habitat and application of their bioactive compounds.
2.1 Thermophilic microorganisms and their importance

Researcher have shown a lot of interest in extremophiles specifically thermophilic bacteria, due to its ability of growing at above 50°C and producing thermostable enzymes [33]. Thermophiles are categorized into 3 classes: moderate thermophiles which have the ability to withstand 50–60°C, extreme thermophiles which can withstand 60–80°C and hyperthermophiles which have the ability to withstand 80–110°C. Thermophiles have been found in different environments, from hot springs to the deep ocean. Members of Ascomycete, Zygomycte families of fungi and archaean genera Pyrobaculum, Pyrodictium, Pyrococcus, and Melanopyrus are the organisms that are capable of growing at temperatures ranging from 103 to 110°C [34], whereas Thermotoga maritime and Aquifex pyrophilus bacterial species have the ability to grow at extremely high temperature ranging from 90 to 95°C [35, 36]. Utilizing enzymes that are both stable and active at higher temperatures is important for a number of purposes, the primary one is that these enzymes are better suited for conducting biotechnological activities at higher temperatures [8].

Many polymers-degrading enzymes (such as amylases, cellulases, chitinases, pectinases, pullulanases, and xylanases), as well as proteases, isomerases, esterases, lysases, phytases, dehydrogenases, and DNA-modifying enzymes, have been characterized from extremely thermophilic and hyperthermophilic microorganisms [37]. Because of their ability to amplify DNA in the polymerase chain reaction (PCR), thermostable DNA polymerases that were isolated from hyperthermophiles have been responsible for a significant breakthrough in the field of molecular biology. Taq polymerase, which originated from the bacterium Thermus aquaticus, is the best-known success story in this regard [38]. The enzymes that are produced by thermophiles have significant applications in the industries of detergent, food, feed, starch, textile, leather, pulp and paper, and pharmaceuticals [39, 40].

2.2 Halophilic microorganisms

Halophiles are organisms which have the ability to grow at higher salt concentration. They can survive in both moderate and extreme saline environment like salt mines and dead sea. Halophiles can thrive at 0.3–5.1 M NaCl concentrations [41]. Halophiles can also be discovered in ordinary habitats such as certain food products. For example, halophilic archaea are believed to play a key role in the fermentation process of kimchi, which is a popular dish in Korea [42]. Halophilic bacteria produce negatively charged enzymes which have promising applications in biotechnology due to their unique properties. The high concentration of halophiles and the lack of non-halophilic pollutants in hypersaline brines make them suited for a wide variety of biotechnological applications. In addition to their potential use in bioremediation and bio fermentation, halophiles may be a good source for obtaining a wide variety of other novel biomolecules, such as stable enzymes and biopolymers. Different studies are conducted on the conversion of plant and animal polymers in high salt environments, including the production of biofuels. Cellulolytic activity was detected in Haloarcula isolated from a Turkish salt mine at high salinities [43]. A strain from a Chinese salt lake showed cellulolytic activity in non-polar solvents, and it can ferment bioethanol from alkali-pretreated rice straw [44]. A species of Halolactibacillus was found able convert raw maize starch into compounds that could be then processed into bioethanol [45]. It was discovered that an extremely halophilic green alga known as Dunaliella salina produces a high lipid content that is ideal for the production of
Other halophilic lipases have also been reported, some of which have potential in biodiesel production [47, 48].

2.3 Psychrophilic microorganisms

Psychrotolerant or psychrophilic are organisms that can thrive at temperatures of 15°C or lower. These microorganisms can live in a variety of cold habitats on earth, such as the polar regions, glaciers, ocean depths, shallow underground regions, upper atmosphere and refrigerated equipment [49]. The majority of these microorganisms are classified as members of the bacterial family such as *Pseudoalteromonas*, *Vibrio*, *Pseudomonas*, *Arthrobacter*, and *Bacillus*, [50], *Methanogenium*, *Halorubrum*, *penicilium*, an *Cladosporium* are examples of archaea, fungi, and yeast [51]. Psychrophilic enzymes are those that are produced by microorganisms that are adapted to colder environments and having high catalytic efficiency at low temperatures. These characteristics offers a significant opportunity in the industries of detergent, textile, food, pharmaceutical, leather, brewing and wine, and paper and pulp. Psychrophiles and their enzymes have been suggested as an alternative in bioremediation of polluted soils and waste water [52]. The structural properties of cold-active enzymes, such as a decrease in core hydrophobicity, a decrease in ionic interactions, an increase in surface charge, and longer surface loops, contribute to the flexibility required for optimal activity at low temperatures [53].

2.4 Alkaliphilic and acidophilic microorganisms

2.4.1 Alkaliphiles

Microorganisms which can withstand extreme alkaline conditions i.e., pH value greater than 9. One of the most common examples of naturally occurring alkaline environments are the soda lakes (with a pH between 10 and 12) or extremely saline [54]. Even though these conditions are extremely harsh, they can be home to a wide variety of bacteria and archaea and are among the most metabolically active marine ecosystems due to the presence of alkaphilic cyanobacteria [55].

The alkaliphiles offer a significant amount of potential for use in biotechnology. Alkaliphiles have the potential of producing different industrially important enzymes like proteases, amylases, cellulases, lipases, xylanases, pullulanases, pectinases, and chitinase [56]. The most important uses of these enzymes are in the production of detergents, the dehairing of hides, the production of pulp and paper, the hydrolysis of starch, and the preparation of food [57].

2.4.2 Acidophiles

Acidophiles are organisms which can withstand extreme lower pH, like hot springs and mine drainage systems. Acidophiles can be found in both natural and man-made environments. Acidophiles and thermophiles are frequently grouped together because thermophilic conditions are present in the majority of acidophilic habitats [58]. Bioleaching is the main application of acidophiles through which microorganisms decompose metal ores in order to remove the metal ions into solution. These metal ions may be then harvested, which enables economical metal extraction from low-grade ores. Among the most valuable properties of acidophiles is their ability to bioleach a variety of metal ions, including heavy metals, which are generally harmful
to other organisms [59]. Acidophiles also have the ability to break down other hazardous organic molecules (such aliphatic compounds), and can be used to bioremediate acid mine drainage systems [60]. Enzymes isolated from acidophiles can be used in different industries like detergents and leather (Table 1).

2.5 Piezophilic microorganisms

Piezophiles and barophiles refer to organisms that are able to adapt to high levels of barometric pressure. Several different piezophiles have been successfully cultivated, however this process require the utilization of complex apparatus to maintain necessary pressures, such as hydraulic pumps and complex gas systems, which are able to keep up pressures up to 38 MPa [61]. Since many laboratories lack the resources and expertise to obtain and safely operate the specialized equipment required to culture these organisms, an increasing number of researches are relying on non-culturing methods, such as genomics, to learn more about them. Hydrothermal vents in the deep sea are well-studied examples of a high-pressure niche. Hydrothermal vents in the deep ocean are a prominent example of a high-pressure niche that has been extensively researched [62]. Piezophilic enzymes have the ability to withstand extreme pressure without requiring any pressure-related modifications. Enzymes extracted from piezophiles are resistant to high pressure and do not require any specialized modifications related to pressure. Enzymes isolated from piezophilic microbes have great potential in biotechnology (Table 1), especially in the food industry where high pressure is used to process and sterilize food [63]. Another enzymes chymotrypsin isolated from piezophilic microorganisms can function at high pressure and temperature and is used in many industrial processes [64].

2.6 Radiophilic microorganisms

Radiation-resistant or radioresistant extremophiles are microorganisms that can survive at extremely high levels of radiation. They have been discovered at high UVR altitudes (mountain ranges) and in broad fields. Continuous ozone depletion had a significant impact on global biosphere exposure to ultraviolet radiation. In addition, radioactive wastes have been released into the environment due to the widespread utilization radioactive compounds and elements for energy, medicine, research, and in industries [65]. Nuclear disasters like Fukushima Daiichi in 2011 and Chernobyl in 1986 have increased radionuclides and radioisotopes in the environment. X-rays and Gamma radiation are two more kinds of environmental radiation that can cause harm to humans. Different kinds of microbes have found strategies to survive in high radiation environments despite the damaging effects of radiation on humans. The bacterium *Deinococcus radiodurans* is resistant to extremely high level of radiation, both ionizing and ultraviolet (> 1000 J/m²) [66]. A number of bacteria, including *Rhodanobacter sp.* and *Desulfuromonas ferrireducens*, have been found to thrive in environments with elevated radioactive concentrations [67]. Researchers have found a correlation between the DNA repair mechanisms and the production of protective primary and secondary metabolic products of radioresistant organisms and their ability to withstand high dose of radiation [68]. Biotechnological techniques can stimulate or trigger the production of radiation-responsive metabolites, pigments, and enzymes, which can be used to create pharmaceuticals, particularly anticancer treatments, antibiotics, and commercially important agricultural products [69].
3. Limits of life and extremophiles

Searching for life beyond earth is linked to our understanding of life on earth. In order to detect possible extraterrestrial habitats, it is essential to know the conditions that can sustain earth life. This does not mean that other planets and moons cannot support life similar to that on earth. Although different types of life may have different origins and biochemistry, it is possible that studying life on earth can help us understand life elsewhere. It is also possible for life to exist in environments completely different from those on earth (for example, the bacterium Deinococcus radiodurans has been shown to withstand radiation levels far exceeding our natural environment, and Escherichia coli has been shown to be able to withstand pressures ten times higher than those found in the deepest ocean trenches) [70, 71]. Life as we know it depends on water, light source or chemical energy, nutrients like nitrogen, phosphorus, sulfur, iron, are just few of the 70 elements on earth that either need or interact with life [72]. Currently, the search for extraterrestrial life focuses on planets and moons that had liquid water, geological and geophysical factors that encourage the synthesis and polymerization of organic molecules, as well as energy sources and nutrients necessary to sustain life on these planets. Once life start on a planet, then evolution will work to fill every possible niche, even if some niches have environments substantially different from where life originated. Since our understanding of life is based on what we can observe and measure, it can only be applied to earth-based life. Based on the universal laws of chemistry and physics, we can extrapolate the conditions for life on other planets. This suggests that life requires a solvent, an energy source, and building blocks in order to survive [73].

Considering the fact that everything requires energy for their chemical reactions, redox chemistry seems to be universal. The emergence, evolution, and diversity of life has often been influenced by physicochemical gradients that create non-equilibrium redox conditions [74]. A proton gradient and redox gradient were likely the two main mechanisms involved in the origin of life, driving metabolism and growth [75]. As a result, the search for life's limits has expanded beyond temperature, pH, pressure, salinity, and radiation gradients to include energy and nutrient limits which can be considered as well [76, 77]. Temperature, pH, pressure, salinity, and radiation are all related and can affect nutrient and energy availability. There are some parameters that affect microbial diversity more than others, like temperature in geothermal waters [78], pH in soil communities [79], salinity in saline lakes [80], and water content in dry climates [11].

Some organisms can thrive under conditions that limit their growth or prove lethal to other organisms. Majority of earth's organisms are killed by extreme temperatures, pH levels, salt concentrations, toxic metals and radiation levels. However, organisms in all three domains have adapted to many extremes on earth [5]. As a result of the emergence of life on earth, microbes have colonized habitats encompassing almost every conceivable physicochemical factor. It was previously believed that terrestrial environments are not suitable for growth because of high temperatures and low water activity (desiccation). It is now known that environments with MgCl₂ above 2.3 M also inhibit life, and this is due to MgCl₂ denaturing macromolecules in biological systems [81]. These conditions aren't necessarily sterile; many organisms have adapted mechanisms to survive at temperatures above 100°C, or even in a desiccated environment. Very few environments that claim to be sterile are genuinely free of all life forms. The Atacama Desert in Chile has been found to contain a small amount of viable microbes despite it being one of the driest environments on earth and believed
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to be similar to the environment on Mars [82]. Some liquid water environments do not support life, like the high-brine liquid in sea-ice inclusions at −30°C and the water above 40°C in submarine hydrothermal vents [83]. However, microbes have been observed to be able to survive in many extreme conditions outside their normal growth range, which still indicates their survival ability [84].

The limits of life, extremophile characteristics, and astrobiology implications are discussed in different studies [7]. Many discussions about the limits of life concentrate on extremes of one physical or chemical condition, such as temperature, salinity, heavy metal concentrations, desiccation, and pH [85]. To survive in nutrient-poor conditions, organisms have evolved a variety of metabolic and physiological strategies. Researchers found that *Pelagibacter ubique* a cosmopolitan microorganism in oligotrophic oceans, grew only at in situ micromolar concentrations of organic carbon. Despite its small genome, *P. ubique* has all the genes it needs to grow independently (without help from other organisms) [86]. The lowest concentrations of organic substances that can support heterotroph growth are set by *P. ubique* and related marine oligotrophs. *P. ubique* and similar marine oligotrophs could be used as models to develop detection strategies for organisms in the Lake Vostok, the subsurface of Mars, and Europa’s ocean [87].

4. Survival mechanism of extremophiles under harsh conditions

Extremophiles have adapted a wide variety of strategies to survive in the inhospitable environments (Figure 1). Proteins are easily denatured and unfolded when exposed to temperatures over their normal range, as this breaks down intracellular bonds which is harmful to that particular organism. To prevent protein from degradation due to high temperature, thermophilic microbes produce chaperones or thermosomes, which allow them to recover their protein structure and function even in harsh environments [88]. Bacteria that thrive in high temperatures have evolved hydrogen bonds that interact with hydrophobicity to prevent protein unfolding. In contrast, the enzymes of thermophilic bacteria are structurally stable due to the abundance of salt and disulfide bridges. Additionally, structural compactness, oligomerization, glycosylation, and hydrophobic interactions between subunits all contribute to thermo-resistance and are therefore essential for stability [89]. The chaperones DnaK, GroEL, and GroES also assist protein folding in thermophiles through the role of heat shock proteins (HSPs). The DNA-repair system responds to DNA damage as well (Figure 1). Thermophiles use branched chain fatty acids and polyamines (such as spermidine) to stabilize their membranes [90]. In order to resist UV stress, ultraviolet resistant extremophiles have developed a variety of strategies (Figure 1). DNA repair, chaperone induction, and active defense against UV-induced oxidative stress (e.g., glutathione accumulation) are involved in these strategies [91]. Radiotolerance has been associated with the ability of these microorganisms to repair DNA damage because they accumulate a high level of intracellular manganese and a low level of iron, conferring UV resistance to them [92].

The cellular cold-adaptability mechanisms (Figure 1) of psychrophiles allow them to survive in extremely cold environments [93]. The presence of unsaturated fatty acids, cyclopropane-containing fatty acids, and short chain fatty acids in membranes prevents membrane fluidity loss [94]. Another mechanism is the high synthesis of cold-shock proteins (CSPs) and chaperones that protect RNA and protein synthesis [95]. The third mechanism involves the synthesis of antifreeze proteins (AFPs) that
bind to ice crystals and cause thermal hysteresis [96]. A fourth mechanism is the accumulation of mannitol as a cryo-protectant [97].

In acidophiles, the cytoplasmic pH is maintained near neutrality in order to safeguard acid-labile cellular components. Adaptation to an acidic environment involves different mechanisms (Figure 1). In the first mechanism, protons are actively pumped to maintain a pH to 1 by proton flux systems. Several bacteria have been reported to efflux protons via transport pumps in the electron transport chain, as well as influx protons via F0F1-type ATP synthases [98]. Proton flux systems include primary proton pumps (symporters) and secondary proton pumps (e.g., cation/H+ antiporters). A second mechanism suppresses the entry of protons into the cytoplasm by lowering the permeability of the cell membrane. Inside the membrane, K+ ions form a positive potential that inhibits proton influx [99]. Thirdly, acidophiles possess a more advanced protein synthesis and DNA repair system than neutrophiles. Acidophiles are induced by an external pH shift from 3.5 to 1.5 to produce proteins like chaperons involved in the heat shock response [100]. To survive in a saline environment, microorganisms have adapted different strategies (Figure 1). A first strategy involves maintaining a salt concentration within the cell equal to that in the environment. As a result, all intracellular systems have been adapted to the new environment. It is done by chloride and potassium transporters conjugated to bacteriorhodopsin and ATP synthase. Betaine and ectoine balance osmotic pressure by keeping intracellular salts low [101]. Another example of a microbial adaptation is the overexpression of a protein complex involved in DNA repair, replication, and recombination in two Halobacterium sp. NRC-1 mutants that are capable of withstanding extremely high radiation levels.
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(LD50 > 11 kGy) [102]. To deal with high pH, alkaliphile bacteria use both symporters and antipor ters (Figure 1). Symporters allow Na+ and other solutes into cells, and electrogenic antiporters produce a gradient of Na+ and H+ [103]. The respiratory system uses cytochrome C-552 to store electrons and hydrogen. By altering the distribution of ions (e.g., Na+), these systems allow protons and solutes to enter the cell, maintaining hydrosaline homeostasis and thermodynamic stability [104].

5. Relationship between extremophilic microorganism and astrobiology

Astrobiology studies how life evolved, distributed, and might continue in the universe in the future. Among other things, astrobiology brings a common biological perspective to astronomy, astrophysics, biochemistry, chemistry, extreme ecology, geology, molecular biology, microbiology, paleontology, physiology, planetary sciences, space exploration, technology, without omitting law and philosophy [105]. A major focus of astrobiology is finding evidence of life on other planets. This objective requires a clear understanding of the biophysical properties of life and the physical and chemical boundaries of earth’s life [106]. Despite the fact that there are numerous definitions of life, none of them are widely accepted. Considering the gradual transition between abiotic structures and indisputable biological forms, any boundary between them must be based on a questionable standard. There is widespread agreement among biologists that the existence of DNA or RNA is a necessary condition for life to exist [107]. Mars and Jupiter’s moon Europa are the best candidates for supporting life [108]. Several authors also proposed habitable environments on Venus and Saturn’s moon Titan [105].

Different planetary bodies have similar environments like earth, having diverse range of each parameter. In order to discover habitable environments outside the earth, it is important to study extremophiles on earth to discover novel biosignatures. As far as biogeochemistry, nutrient composition, or topological similarities are concerned, extremophile habitats on earth share many similarities with those on other planetary bodies [109]. Based on this different (poly)extremophiles may persist in different planets depending on the planetary body. Halopsychrophiles may be able to survive on Titan, Ceres, and Europa because of their salty underground oceans [110], and on Mars because of its chlorine-rich brines [111, 112]. In addition, these living forms would have to withstand intense pressure, because the hydrostatic pressure in Titan’s subterranean ocean varies between 140 and 800 MPa [113]. Despite the fact that these conditions are well outside the range of even the most extreme cultured piezophile (Thermococcus piezophilus, Pmax = 125 MPa) [114], microorganisms have successfully been exposed to pressures up to 2000 MPa and found to be metabolically active in fluid inclusions within type-IV ice [115]. Based on these observation it is possible that life may exist on other planets, such as Enceladus (Pmax = 50 MPa) and Europa (Pmax = 30 MPa) [5].

6. Conclusions

Extremophiles produce numerous extremozymes having different industrial applications, including agricultural, chemical, and medicinal. They are found in almost any habitat. Due to these advantages, extremozymes will be increasingly used in a wide range of consumer products. Extremophiles improve our understanding of
macromolecular stability and physiochemical requirements for life. According to new research adaptations that enable survival under one stress, may also enable survival under other stress conditions. Finally, the study of extreme organisms contributes greatly to astrobiology's ongoing development. Understanding how life can thrive on earth may help astrobiologist better understand and locate potential life in other planetary bodies.
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