

Microorganisms in extreme environments with a view to astrobiology in the outer Solar System

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ABSTRACT

We review the various manifestations of the evolution of life in extreme environments. We review those aspects of extremophiles that are most relevant for astrobiology. We are aware that geothermal energy triggering sources of heat in oceanic environments are not unique to our planet, a fact that was exposed by the Voyager mission images of volcanic activity on Io, the Jovian moon. Such activity exceeded by far what was known from terrestrial geology. The science of astrobiology has considered the possible presence of several moon oceans in the vicinity of both giant gas and icy planets. These watery environments include, not only Europa (strongly suggested by data from the Galileo mission), but the Voyager flybys exposed, not only the unusual geothermal activity on Io, but also the possible presence of subsurface oceans and some geothermal activity on the Neptune's moon Triton. More recently, calculations of Hussmann and coworkers with available data do not exclude that even Uranus moons may be candidates for bearing subsurface oceans. These possibilities invite a challenge that we gladly welcome, of preliminary discussions of habitability of extremophiles in so far novel environments for the science of astrobiology. Nevertheless, such exploration is currently believed to be feasible with the new generations of missions suggested for the time window of 2030 - 2040, or even earlier. We are envisaging, not only the current exploration of the moons of Saturn, but in the coming years we expect to go beyond to Uranus and Neptune to include dwarf planets and trans-neptunian worlds. Consequently, it is necessary to begin questioning whether the Europa-like conditions for the evolution of microorganisms are repeatable elsewhere. At present three new missions are in the process of being formulated, including the selection of payloads that will be necessary for the exploration of the various so far unexplored moons.

1. INTRODUCTION

We attempt to demonstrate in this paper that our knowledge of terrestrial microbiology based on Darwinian evolution, considered by others to be the backbone of universal biology¹, provides multiple examples not only in the polar regions, but elsewhere as well. Hence, we feel justified to extend the range of testing potential habitability of moon oceans beyond our previous considerations that were focused on the Jovian moons, especially Europa². To implement these ideas we dwell on the outer Solar System and keep in mind mainly life in extreme environments according to the range suggested by McKay³, namely temperatures between $-15\text{ }^{\circ}\text{C}$ and $122\text{ }^{\circ}\text{C}$, and a total pressure high enough to keep liquid water stable ($P > \sim 0.01$ atmospheres). In that region we find a large number of satellites: 63 around Jupiter, 60 in the Saturn System, 27 and 13 around Uranus and Neptune, respectively⁴. Out of these we have at least three orbiting around different planets that are potentially significant for astrobiology, namely, the Jovian moon, Europa, the Saturn moon Enceladus and Triton, the satellite in retrograde motion around Neptune.

In some of these icy worlds we have discovered plumes of internal liquid from its interior that are jetting out of their surfaces, emission of liquid has been observed first in the most distant one, Triton, due to the Voyager observations in 1989.

Enceladus, was next, due to the Cassini discovery with the sophisticated instrumentation on board (the ion and neutral mass spectrometer and the ultraviolet imaging spectrograph). A cloud of water vapor was found over the moon's

south pole. High-resolution Cassini images show icy jets and towering plumes ejecting large quantities of particles at high speed. Last but not least, Europa's plumes were discovered with the Hubble Space telescope ⁵.

The surface age of Triton is approximated between 10 and 100 Myr old ⁶, suggesting recent geologic activity. The formation of Triton would have been in the solar nebula, unlike the case of Titan, which may have been formed in the disk of material that surrounded Saturn.

2. MICROBIOLOGY IN MOON OCEANS OF GIANT GAS AND ICY PLANETS

Since we are aware that geothermal energy as sources of heat in oceanic environments are not unique to our planet, we look in more detail to the outer Solar System. The science of astrobiology has considered the possible presence of several moon oceans in the vicinity of giant gas and icy planets. These watery environments include not only the Jovian moon Europa (from data of the Galileo missions), but even Voyager flybys exposed that the Neptune's moon Triton, the possible presence of subsurface ocean with geothermal energy resources.

The surface expression of this energy has manifested itself in the Solar System after the case known on Earth, in volcanoes (the Galilean moon Io), and several cases of steam geysers discovered since the 1980s, firstly, Neptune's moon Triton, Enceladus the Saturn moon and most recently Europa the Galilean moon. We now focus on those aspects of microbiology that are most relevant for astrobiology:

I. The Canadian Arctic at Ellesmere Island, where sulfur patches, as on the surface of Europa are accumulating on glacial ice lying over saline springs that are rich in sulfide and sulfate ⁷. Besides the biogenic sulfur-related traffic through the icy surfaces of the lakes that is well understood in the dry valley lakes lying on the western shore of McMurdo Sound ⁸, there is another major European analog in North America. Once again, we are dealing with a valley that has been given the name of "Borup Fiord Pass", where minerals accumulate on glacial ice. This site is a geologically relevant feature that lies on Ellesmere Island in northern Canada. The valley lies over saline springs that are rich in sulfide and sulfate that make their way all the way to the icy patchy surface. This additional traffic of surficial sulfur provides a terrestrial laboratory to test the instrumentation that may be used later on a future mission to Europa ⁹. The biosignatures that have been studied in this environment are not related to the sulfur isotopes, as we have done in our above-mentioned work of 2006. Rather, what has been followed up in Ellesmere Island is the process of biomineralization ¹⁰. This interesting biogenic process takes place when biominerals are generated as a result of interactions of microbial life and its environment. In chemosynthesis this is a well-understood process ¹¹.

II. A second ecosystem has microbially produced icy patches at Blood Falls in the McMurdo Dry Valleys ¹². An intriguing feature—Blood Falls—suggests the presence of microbial mats underneath the Taylor glacier. The name is due to the resemblance with a blood-red color waterfall at the glacier's extreme end. Isotopic measurements of sulfate, water, carbonate, ferrous iron and gene analyses imply that a microbial consortium facilitates a catalytic sulfur cycle analogous to the metabolic events that may sustain life elsewhere in the Solar System. This is especially relevant to the icy satellites of the outer Solar System, including Europa, where the Galileo Mission discovered sulfur patches (1995-2003). These stains on the icy surface of the Jovian satellite are suggestive of chemosynthetic products of metabolism ¹³. On Earth there are extremophilic environments.

III. The ice-covered lakes of Antarctica's McMurdo Dry Valleys have long been of interest to astrobiology ¹⁴. These environments harbor unique microbial ecosystems that could orient us how to plan our experiments on Europa. Lake Joyce is of special interest to NASA, as it is ice covered year-round: Its icy surface is 6 meters deep. Yet, even the few percent of light that penetrates through the ice is enough to support an algal ecosystem in the lake. Many of the structures on the lake bottom look like what we see in the Archean rock record from about 3 Gyr before the present (BP), because its waters harbor carbonate structures known as microbialites. These unique structures are formed with layers of cyanobacteria. The research team is interested in how these organisms are able to grow in the dark, cold waters of Lake Joyce: In these environments the extremophiles that are trapped in microbial mats may also be living under the Taylor Glacier in the Taylor Valley. These microbes probably lived in the ocean at one time, but when the floor of the Dry Valleys rose more than a million years ago, the glacier covered seawater when it advanced and trapped the microorganisms in pockets of water.

IV. We should finally consider a lake called Vostok, which is the largest of about 80 subglacial lakes in Antarctica ¹⁵. Its surface is of approximately 14,000 km² and its volume is 1,800 km³. Indeed this Ontario-sized lake in Eastern Antarctica is also deep, with a maximum depth of 670 m. On the other hand, from the point of view of microbiology, the habitat

provided by Lake Vostok presents us an analogue for the Europa environment. The ice above the lake has been cored to a depth of over 3,600 m, stopping just over 100 m over the surface of the lake itself. This work has revealed great diversity of single-celled organisms: yeast, actinomycetes, mycelian fungi (which remain viable for almost 40,000 years), the alga *Crucigenia tetrapodia*, diatoms, and most interestingly, 200,000 year old bacteria. Besides it appears that in the lake water temperatures do not drop too far below zero centigrade, with the possibility of geothermal heating raising the temperatures above this level. Extrapolation of data retrieved from work deep in the ice core to the lake itself, implies that Lake Vostok may support a microbial population, in spite of the fact that that large volume of water has been isolated from the atmosphere for over one million years.

Bacterial density is found to be two to seven-fold higher in accretion ice than in the overlying glacial ice. This implies that Lake Vostok is a source of bacterial carbon beneath the ice sheath. Phylogenetic analysis of the amplified small subunit ribosomal ribonucleic acid (rRNA) gene sequences in this accretion ice has revealed the presence of several microbes: *alphaproteobacteria*, *betaproteobacteria* and *gammaproteobacteria*¹⁶. It has been estimated that the youngest water is at least 400,000 years old. It is a window into life forms and climates of primordial eras. The zone of ice layer up to 3,309 m (referred to as I), and the layer between 3,310 to 3,509 m (zone II) provide detailed information about the paleoclimate record spanning during the last 420,000 years. The basal portion of the ice core from 3,539 to 3,623 m has many features differing from overlying glacial ice and its geochemical composition indicates that it represents actual lake water that has accreted (i.e., frozen) to the underneath of ice sheet. In spite of extremely cold air temperatures above the ice (an average of -55 °C), liquid water is stable in the lake owing to the combined effect of background geothermal heating, together with the insulating properties of the overlying icy sheet¹⁷.

Lake Vostok appears to be harboring hydrothermal vents beneath the water surface. This is suggestive of what may be occurring on Europa. The circulation of pure water in Lake Vostok will be driven by the differences between the density of melt water and lake water. Geothermal heating will warm the bottom water to a temperature higher than that of the upper layers. The water density will decrease with increasing temperature resulting in an unstable water column. This leads to vertical convective circulation in the lake, in which cold melt water sinks down the water column and water warmed by geothermal heat ascends up the water column¹⁸. Similarly, Europa may also have geothermally-heated warm water under its ice-crust. Processes of the type that occur in Lake Vostok may be taking place on Europa, where sulfate-reducing microbes may be processing sulfur originating from hydrothermal vents. Such isotopically modified sulfur may be reaching the surface. In principle, discoveries of extremophilic multicellular animals may be relevant as the "extreme shrimps"¹⁹.

3. ARE THERE HABITABLE OCEANS IN THE OUTER SOLAR SYSTEM?

Already, for a considerable time, we have enquired about the habitability of the Jovian moon Europa, having realized from the Galileo early data that conditions of a likely ocean are compelling for habitability by extremophiles²⁰, such as some of the ones we have discussed in this work in Secs. 1 - 2. We are envisaging not only continuing the exploration of the moons of Saturn, but in the coming years to go beyond to Uranus and Neptune²¹. Consequently, it is necessary to begin questioning in those moons of the Outer Solar System (OSS) whether the Europa-like conditions are repeatable. In other words, whether conditions in likely oceans are compelling for habitability by extremophiles. We begin by considering what is known so far, having Galileo data for the Jovian satellites, especially for the putative oceans on Europa and Ganymede, Cassini data for Titan and Enceladus, as well as Voyager data for Uranus and Neptune. These observations have allowed raising the question: *Where are possible ecosystems in the Solar System?* Calculations by Hussmann and collaborators have suggested the following²²: Starting with the smallest and ordered by size, the satellites and dwarf planets that may have internal oceans are Rhea (Saturn), Oberon and Titania (Uranus), Pluto, Triton (Neptune) and Europa (Jupiter) with estimated ocean thickness ranging from 16.4 km (Rhea) to 192.5 km (Triton). If life were possible in these liquid layers then, in view of the planned missions for the icy planets in the next two decades, a second question that will eventually require an answer is: *How can we identify biomarkers with feasible instrumentation?* The forthcoming approved ESA JUICE Mission can in principle bear instrumentation adequate for detection of biomarkers²³. Even though relevant missions are planned for measurements within two decades, in the meantime we should keep in mind the exploration of two dwarf planets: Firstly, the *New Horizons Mission* will have close flybys in the Pluto System including the moon Charon, and the *Dawn Mission* performance systematic measurements around Ceres. Even though Ceres has not been included amongst the suggested ocean-bearing bodies in the Hussmann et al calculations, they may be similar to Triton, where the first liquid jets were detected in the 1980s. Triton, due to its anomalous rotation, has been

suggested to be a captured body, possibly not unlike some of the dwarf planets that are about to shed some of its secrets²⁴.

4. OTHER POSSIBLE OCEANS IN THE SOLAR SYSTEM

Possible tests of biogenicity are feasible in Europa with the instrumentation that has been approved by the forthcoming mission to the Jovian system, the European Space Agency JUUpiter ICy Moons Explorer (JUICE) mission. Its Jupiter Ganymede Orbiter (JGO) will include orbits around Europa and Ganymede. For example, the sulfates known to be present in the low albedo regions should produce a quantity of sulfur atoms in the thin Europa atmosphere by micrometeorite bombardment.

The sulfur-contaminated thin exosphere of Europa remains within the range of what can be measured with current developments in miniaturised instrumentation. Indeed, there is a wide selection of miniaturized instruments. They employ heritage from earlier instrumentation prepared for a series of missions, such as the Ptolemy and COSAC GC/MS instruments²⁵⁻²⁷ on-board the ROSETTA lander package, and Beagle-2 Gas Analysis Package²⁸, and could cover a large mass range of species including some isotopic differentiation. In addition, there is a new generation of instruments that have been developed at the University of Bern: A NGMS is a TOF-MS using a grid-less ion mirror (a 'reflectron') for performance optimization²⁹. One of the science goals of a component of the JUICE/PEP instrument is FOR the isotopic analysis of the Galilean satellites' atmospheres, when the signal levels are sufficiently high. The heritage of instruments goes back to those developed or measuring the chemical composition of the terrestrial stratosphere³⁰. Results from the Cassini mission have shown that the source region of the plume of Enceladus could in fact provide a habitable zone for terrestrial microorganisms with internal liquid from its interior is jetting out of its surface: we need look no farther than our own planet to find examples of the types of exotic ecosystems that could make life possible on Saturn's geyser moon^{31,32}. In view of the relatively accessible distance of Saturn's satellites, it is conceivable to think in terms of a sample return mission³³. Enceladus retains an atmosphere suggesting it to be sufficiently active geologically to reintroduce the water vapor, as some of it may be escaping into interplanetary space, due to its low gravity. The answer appears that it could be possible. In recent years, several extremophiles have been found on Earth that thrive in places where the Sun does not shine and oxygen is not present, because no photosynthesis takes place. Microbes have been discovered that survive on the energy from the chemical interaction between different kinds of minerals, and others that live off the energy from the radioactive decay in rocks.

Amongst the 13 Neptune satellites, the icy moon Triton is can in principle be explored³⁴. Its mass is 40% greater than that of Pluto. Triton is one of the few moons in the Solar System known to be geologically active with a relatively young, with a complex geological history demonstrated by its complex cryovolcanic and tectonic terrains³⁵. As such it is suggestive to translate the proposals for the search for biomarkers to this large moon that was first visited by the Voyager Mission. Even though specific missions to the outer Solar System have not been endorsed at present by any space agency, the biogeochemical tests will eventually be relevant.

In particular, a mission concept is referred to with the acronym ODINUS that is derived from the main areas of proposed scientific research: "Origins, Dynamics and Interiors of Neptunian and Uranian Systems". As the proposers of this mission concept suggest²¹, the ODINUS mission is based on the use of two twin spacecraft to perform the exploration of the ice giants and their regular and irregular satellites with the same set of instruments. This will allow performing a comparative study of the outermost giant planets of the Solar System, with the aim of revealing their histories in the context of the evolution of the Solar System.

5. DISCUSSION

We shall now dwell in a little more detail on the biogeochemical arguments that may apply to all the three icy moons highlighted above — Europa, Enceladus and Triton — but our arguments will refer specifically to Europa (and inevitably to Ganymede, its Galilean neighbor). Our choice is motivated by the experimental feasibility of searching for biomarkers, outlined recently (Chela-Flores et al., 2014) that could, in principle, discriminate between the possible presence, or absence, of life in these two worlds^{36,37}. The low expected abundance of organic compounds on Europa is based on terrestrial systems such as the oceans, hydrothermal vents and the Vostok Lake. This suggests that measuring organics on Europa will require high sensitivity, such as the one provided by the ESA PEP technology³⁸. Surface icy

materials on Europa include sulfur that is present at less than 1 wt % to even 50 of weight %, depending on the species and on the surficial location.

It is likely that an eventual test of biogenicity proposed earlier should be feasible. Large 70 ‰ $\delta^{34}\text{S}$ variations suggested for microbial sulfate reduction was discovered recently in pure culture experiments³⁹ and is only observed in a small handful of natural environments. This result does not exclude an European biota composed of sulfate reducers and disproportionators that over geologic time may lead to a euxinic ocean that can be tested with instruments on board of JUICE.

The Galileo orbital mission discovered surficial patches of non-ice elements on Europa that were widespread and, in some cases, possibly endogenous. Instrumental capability is available both with the above-mentioned approved ESA technologies, as well as with the more recently proposed (and approved) NASA instrumentation. Indeed, we are suggesting that it will be possible to measure biogenic stable S-isotope fractionation δS^{34} of the order of up to 70 ‰. Such sensibility of the instruments is not beyond the efforts of a proposed NASA Europa Clipper spacecraft with nine scientific instruments that will assess the icy moon's ability to support life. The instruments on this second Europa probe will study the satellite's surface composition and the nature of its salty subsurface sea⁴⁰. Firstly, the MAAss SPectrometer for Planetary EXploration/Europa (MASPEX) will determine the composition of the surface and subsurface ocean by measuring Europa's extremely tenuous atmosphere and any surface material ejected into space. Secondly, the SURface Dust Mass Analyzer (SUDA) will measure the composition of small solid particles ejected from Europa, providing the opportunity to directly sample the surface and potential plumes on low-altitude flybys. The instrument is capable of reliably identifying traces of organic and inorganic materials in the ice matrix of ejecta⁴¹.

In addition, we have also to rely on ESA's mission PEP technology for careful consideration of the exosphere of Ganymede. Fortunately, even though a lander is not under consideration, JUICE is expected to have a broad mission profile, including several orbits around the largest moon of the Solar System that will begin in September 2032, well before the Mission nominal end a year or so later, when the orbiter will be disposed of on Ganymede itself^{42,43}, yet not posing any significant planetary protection risk. In addition, JUICE is also expected to include 2 close flybys around Europa. With these planned flybys the possibility of performing measurements is feasible with the required accuracy of ‰. To sum up, we expect both JUICE and Ice Clipper technologies to give us robust fingerprints of life in the moons of the Jovian System.

At a later date it is possible to discuss, along the same lines of the biogeochemical arguments that apply to Europa, what would be required to analyze the isotopic sulphur content of the Ganymede exosphere (faithfully reflecting the surface contaminants), as well as in the exoatmospheres in other outer Solar System moons. Such studies would go a long way to complete the test of biogenicity of the Jovian moon oceans, but analogous arguments could be extended to the Saturn and Neptune icy moons. With the instrumentation pointed out in this review, we can attempt to answer questions such as whether a second genesis of life⁴⁴ has taken place in our Solar System (cf., additional references⁴⁵). The most urgent worlds are Europa, Enceladus, or Triton.

Before the ODINUS proposal two additional missions that have also been proposed for the outer Solar System: The Uranus Pathfinder (UP)⁴⁶, as well as the Outer Solar System (OSS) for Neptune, Triton and the Kuiper Belt⁴⁷. Potentially, with ODINUS, OSS and UP forthcoming data by 2060 or hopefully earlier, we will be able to begin appreciating most extraordinary insights on microorganisms' in unexplored habitats. Such science can be inferred from our earlier work on the necessary payloads²³, originally intended for the similar environments on Europa.

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