HYPOTHESIZING POSSIBLE LIFE FORMS IN VENUSIAN CLOUDS. Romi Rishit George[1] and Ayush Bagchi[2], [1]Department of Microbiology, St. Xavier's College (Autonomous), Kolkata, India (81A, Dr. S C. Banerjee Road. Beleghata, Kolkata - 700010, India and romi_george@yahoo.com), [2]Department of Microbiology, St. Xavier's College (Autonomous), Kolkata, India (8, Ekdalia Road Santosh Garden Kolkata 700019, India and ayush.bagchi10@gmail.com).

Background: When we think of extraterrestrial life the first planet that comes to mind is Mars. Recent signs of biological life in Venus' atmosphere have, however, gripped the attention of the scientific community and made Earth's sister planet one of the most probable planets to potentially harbor life.

Venus is situated near the edge of the sun's habitable zone. The cloud layers hovering roughly 48-60 kilometres above the surface have temperature around 60°C and pressure around 0.1 MPa. These conditions make the lower and middle cloud regions habitable for microbial life, although we do need to factor in hyperacidity and low water activity. [1]

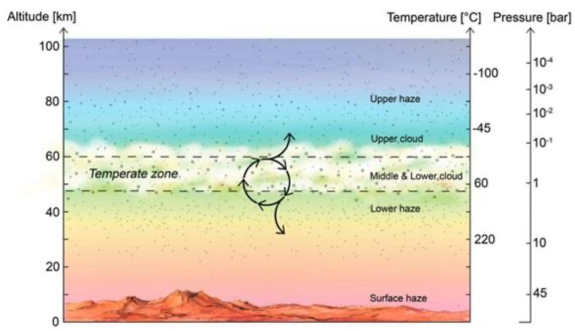

Figure 1: Venusian cloud layers. Temperate zone is thought to be at around an altitude of 50-60km above the surface [4]

Evidence from NASA's Goddard Institute for Space Studies (GISS) suggests that Venus was once part of the Circumstellar Habitable Zone and could have had surface temperatures low enough to sustain water for upto 2 billion years [2]. Moreover, the JCMT and ALMA telescopes have recently made possible detections of phosphine (PH_3) gas in Venus's atmosphere [3]. The presence of Phosphine could be due to biological activity similar to what is seen on Earth and therefore is hypothesized as a possible biosignature [4]. However, more research is needed to definitively predict the presence of phosphine. [5]

Keeping this in mind and using our current knowledge on the Origin of Life on Earth and different hyper-extremophilic survival mechanisms, here we hypothesise possible cloud habitating polyextremophilic microbial populations and how they might have originated and found ideal habitable conditions in the clouds of Venus.

Acidophilic Extremophiles on Earth: Acidophiles like *Sulfolobus acidocaldarius* maintain a near neutral pH in highly acidic conditions by multiple methods including proton efflux via primary transport pumps in the electron transport chain. Another method to maintain pH homeostasis is by maintaining a positive membrane potential that repels the influx of protons. This positive membrane potential is formed of K+ ions. In addition, *Leptospirllum ferriphilium* has several secondary proton pumps such as cation/H+ anitporters. Another effective way to maintain pH homeostasis is by consuming the H+ in chemical reactions such as those using amino acid decarboxyalses. [6]

Extremozymes or extremophilic enzymes are very resistant to extreme conditions because of their great solidity. The endo-β-glucanase from *Sulfolobus solfataricus* demonstrated stability at optimum pH of 1.8. This is because it can resist protonation in its catalytic domain and when compared to other mesophilic counterparts, glutamic acid residues were found to be responsible for this high resistance[7]. Similarly, the α-glucosidase from Ferroplasma acidiphilum has demonstrated stability at low pH. This enzyme is not only stable at such a low pH but also has a preference for pH of 3 in place of 5.6, which is the internal pH of *Ferroplasma acidiphilum*.[6]. This stability is due to the presence of catalytic triad-like mechanisms involving Histidine, Arginine or Lysine in the active site of the enzyme. Another possibility is the complete lack of ionic amino acids in the active site so the external pH does not matter.

Enzymes from acidophilic microorganisms are of particular interest since they show low pH resistant properties on Earth and hence there is a possibility of such acid tolerant enzymes to withstand denaturation in the highly acidic environment of Venus.

Thermophilic Extremophiles on Earth: Thermophilic microorganisms like *Methanopyrus kandleri* have the capability to develop at great temperatures between 41°C and 122°C. This temperature is quite similar to the proposed "habitable zone" in Venus' clouds. A wide number of enzymes from thermophilic microorganisms have been characterized, such as cellulases, amylases, pullulanases, xylanases, mannanases, pectinases, chitinases, proteases, lipases, esterases, and phytases [8]. Enzymes from thermophilic enzymes or thermozymes of extremophilic microorganisms are capable of staying intact and resisting denaturation under extreme conditions like the presence of denaturing agents and organic solvents and high salinity.

The thermozymes possess certain physical properties and electrostatic interactions to keep activity at high temperatures. They possess different adaptations to maintain their conformations and functions in extremes of temperatures. They also have an increased number of hydrophobic deposits, forming bisulfide liaisons between two ions with opposite charges [8]. Di-sulfide linkages are of particular importance since they make proteins stable and heat resistant for instance, Taq Polymerase isolated from *Thermus aquaticus* which is routinely used in PCR technologies.

The aforementioned enzymes when aligned with their mesophilic counterparts were found to have little to no similarity in identity. BLAST search results returned no results. So these enzymes have completely unique structures while having similar functions which would mean different proteins over the time developed similar functions.

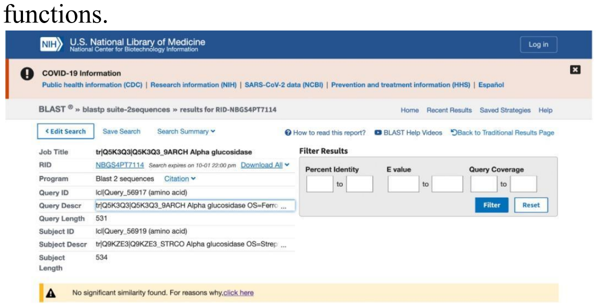

Figure 2: No similarity in sequence found between UniprotKB Q5K3Q3 alpha glucosidase of *Ferroplasma acidiphilum* and UniprotKB Q9KZE3 alpha glucosidase of *Streptomyces coelicolor*.

Extrapolating Data from Extremophiles on Earth: These examples show us that if two different enzymes, one acid resistant and one acid labile, with the same function, having completely different sequences can exist on earth then there is a possibility of such independent enzymes existing on the clouds of Venus where the pH is even lower. We hypothesize the existence of hyperacid-resistant enzymes and thermozymes similar to the ones found on earth but on a more extreme scale. Thus, we hypothesize microorganisms that might have a combination of highly efficient proton efflux pumps on their membranes, along with highly resistant enzymes to both heat and acid, and any other environmental stresses that they could face in the harsh Venusian atmosphere. Organisms on Earth, over the years, have adapted and in some cases completely changed their genetic composition in favor of a suitable phenotype to adapt to their environments which in some cases are extremely harsh and unforgiving. We propose a similar hypothesis where organisms might have evolved completely independent of the evolution on earth, where they utilised the energy available on venus like lightning, volcanic eruptions and miniscule amounts UV light that can penetrate the atmosphere as well as the chemicals like Nitric oxide [9], carbon dioxide that is available on Venus. However, in order to adapt to a harsher environment they ended up with highly resistant macromolecules very different from the ones seen on Earth. Our normal DNA and proteins would not be able to resist the harsh environment of Venus. So, it is likely that the macromolecules formed over the course of evolution in Venus are much more resistant, with stronger interactions to adapt to the environment. One such possibility could be disulphide bridges in nucleic acids or in the Venusian life counterpart of genetic information storing macromolecules. Nitrogenous bases and sugar-phosphate backbone may just be a characteristic of nucleic acids on Earth.

Based on our limited knowledge of extremophiles on Earth, it is very difficult to define the boundaries of life. Life can be said to be defined by parameters like capability to store information, replicate, grow and capability of performing metabolism. However, there are many exceptions, for instance computers can store information and fires can metabolize by using oxygen. So, life itself cannot be defined by just a few parameters and based on just a few examples on a larger scale.

A New Origin of Life Theory: Here, we also propose a modification to Oparin-Haldane's Model of origin of life, where life originated in the once shallow oceans of Venus (as hypothesized by NASA research [2]) and in order to adapt to the drastic climate changes found refuge in the clouds where some selective life forms found habitable conditions just like how life gradually shifted to land on Earth. Those organisms that could survive the climate changes and had suitable mechanisms to survive high in the clouds under the acidic and hot conditions managed to do so. Life may or may not be flourishing if present, but based on our knowledge of extremophiles, they probably are not flourishing under such extreme conditions but rather are able to survive. However, microbes in Venus' clouds could very well have adapted to the extreme conditions and now flourish, floating in the skies. The only way to find out would be through more exploration for signs of life. Presence of phosphine does give us hints where we can predict that microbes present could be metabolically active, so it wouldn't be surprising if they are able to flourish.

Other Hypotheses: Another hypothesis is that life could exist inside the cloud droplets to avoid fatal net loss of water and relative high water activity and less acidity. Desiccated spores are present at the lower haze layer which are carried upwards to the lower cloud layer by upward diffusion and gravity waves. In this conducive environment inside cloud droplets, they germinate to become metabolically active. As the size increases and water activity decreases, the life forms divide and sporulate. These spores settle at the lower haze layer and the cycle continues. [4]

Another speculation is that elemental sulfur present in the clouds of Venus, which is not wetted by sulfuric acid, provides chemical protection from the concentrated sulfuric acid environment. But this does not fit in the life cycle theory.

Conclusion: The possibility of life in Venus has been hypothesized due to recent detection of phosphine [3] in the Venusian atmosphere. However, this evidence is not conclusive enough and is highly disputed [5] in the scientific community. Henceforth, here we went for a different, a rather bottom-up approach, where we predict the possibility of existence of biomolecules like proteins in such hostile conditions that are crucial to life itself. We used current knowledge of extremophiles on earth and the proteins that they need to survive and achieve such levels of endurance to predict possible life forms that can be present on the clouds of venus. The existence of extremophilic versions of important enzymes in extremophiles tell us that similar hyper-extremophilic versions of enzymes could exist in the microorganisms in Venusian atmosphere if life is found. This enables us to construct a possible life form.

So, with our rather loose definition of life we can predict or hypothesise life on other planets like Venus. An anaerobic, polyextremophilic sulfate-reducing chemoautotroph with hyper-extemophilic enzymes and stable biomolecules is quite possibly the best model considering the available data since it matches with the cloud chemistry with respect to CHNOPS and energy availability. However, we have to keep in mind that life on other planets might be completely different from our own and this wouldn't be surprising considering it is far more likely that they had an independent origin of life and completely different timelines of evolution.

Since all this is hypothetical, we believe further investigations should be done which might show very promising results. Moreover, taking a closer look at our own atmosphere and the life it harbors like clouds of regions with high frequency of acid rain could also provide us some clues regarding possible life forms on Venus. Signs of life, if found on Venus could also reignite the Theory of Panspermia suggesting that life might have found a way to escape to Earth during drastic climatic changes on Venus adding to the diversity of life found on our home planet.

Acknowledgment: We would like to thank our Father Principle Dr. Rev. Dominic Savio SJ, Head of Microbiology Department, Dr. Sudeshna Shyam Chowdhruy and Controller of Examinations, Dr. Arup Kumar Mitra and all the other professors of the Department of Microbiology, St. Xavier's College (Autonomous), Kolkata, India for their continued support.

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