THE VIABILITY OF USING MICORBIALLY-DERIVED PLASTIC AS A BIOMARKER FOR ASTROBIOLOGICAL DETECTION.

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Introduction: The search for life within the solar system is an ongoing endeavor and a key science priority of multiple space agencies. Biomarkers are a vital technique used in this search, as they provide unequivocal evidence of a biological process. Microbially-derived plastic polyesters, or 'bioplastics', are polymers that resemble consumer plastics but are derived biologically [1]. Bioplastics are chemically known as polyhydroxyalkanoates (PHA), and polyhydroxybutyrate (PHB) is a type of PHA that has been extensively researched due to it being the only bioplastic that is truly 100% biodegradable [2]. This class of molecule's potential as a biomarker is poorly understood; nevertheless, it has the potential to be stable in extraterrestrial environments for long durations. Thus, its integration into existing astrobiology detection experiments should be considered.

Bioplastics & Biomarker Potential: Research into bioplastics has mainly explored their utility in manufacturing a more environmentally safe alternative to petroleum-derived plastics [1-2]. Microbially-derived plastics have been hypothesized to be created and utilized as energy and carbon stores in times of stress and starvation in microorganisms [1].

Bioplastics are synthesized and degraded in integrated carbon metabolism pathways by bacteria in aerobic and anaerobic environments [3]. They often branch off from carbon cycles that are well understood such as glycolysis, the citric acid (TCA) cycle, fatty acid biosynthesis, and β-oxidation (Fig. 1), the glyoxylate cycle, the ethylmalonyl-CoA pathway, and from the Entner-Doudoroff pathway [1, 4-5].

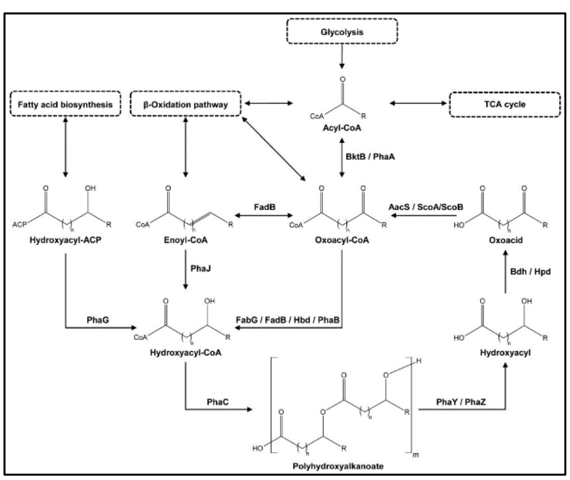

Fig. 1. Overview of the polyhydroxyalkanoate (PHA) cycle, from [1].

As bioplastics are not abiotically derived, they can serve as unequivocal biomarkers if they are found extraterrestrially. They have long half-lives and can be stable in an environment outside of cells for an extended time if not degraded by living organisms [1, 6]. They are also water insoluble and sink in aqueous environments [3]. PHAs have exhibited melting points between 50 – 180°C, crystallinity between 30 – 70%, and size ranging between ~100 nm to several µm [6-7].

PHAs are easily attacked by acids and alkalis and dissolve in chlorinated solvents [8] and thus are not chemically stable in some conditions. PHAs also have good UV resistance [8], making them an attractive candidate when looking for biomarkers on Mars and Europa where high radiation can degrade molecules [9-10].

Detection & Analytical Methods: Identification of polyhydroxyalkanoates in manufacturing has been explored and tested using infrared (IR) spectroscopy and gas chromatography-mass spectroscopy (GC-MS) in addition to many other methods [11-12]. In the laboratory, differential scanning calorimetry, wide-angle X-ray diffraction, and Raman spectroscopy have also been applied to analyzing PHAs [13-14].

Additionally, polyhydroxyalkanoate granule-associated proteins (phasins) are a group of amphipathic proteins that bind specifically and hydrophobically with the PHA core [6]. The phasin functional role has been studied in detail, and phasin-PHA binding has been exploited to replace beads for specific and sensitive antibody detection using fluorescence activated cell sorting (FACS) technology [6].

Instrument Integration: There are many potential ways in which the search for bioplastics can potentially be implemented into the search for extraterrestrial life. On Mars, the Scanning Habitable Environments with Raman & Luminescence for Organics & Chemicals (SHERLOC) instrument on the *Perseverance* rover utilizes Raman spectroscopy to search for biosignatures [15], and the Sample Analysis at Mars (SAM) tool on *Curiosity* utilizes a GC-MS to search for organics [16]. The upcoming *ExoMars* rover will be capable of doing IR spectroscopy, Raman spectroscopy, and GC-MS [17].

In addition to searching for bioplastics with the existing suite of rover instruments, PHAs could potentially be detected using the 'Signs of Life Detector' third generation (SOLID3) instrument described in [18]. SOLID3 utilizes the LDChip300, a sandwich microarray immunoassay detection tool to analyze hundreds of different biomarkers from a small sample [18]. It employs fluorescent-based immunoassay antibodies to microscopically detect and report the presence of biomarkers, and we propose that phasins could be added as a probe to target for PHAs.

Discussion: Detection of bioplastics *in situ* in natural environments is exceedingly rare in the scientific literature. A pushback to using bioplastics as a biosignature is that they are not found in all life on Earth and thus would be more difficult to find extraterrestrially than other, more universal biomarkers (which still haven't been found outside of Earth). Nevertheless, we argue that bioplastics could be a useful tool to search for extraterrestrial life.

Considering that many solar system environments that are proposed to have been habitable are extreme and potentially hostile to terrestrial life (*e.g.,* environments exhibiting extreme aridity, irradiation, pH, heat, and/or cold), a stable carbon and energy store such as PHA would be considerably beneficial. In the crater lake of the Poás Volcano in Costa Rica, which exhibits a pH of ~0 and is identified as an extreme analog to acid-sulfate hydrothermal environments on Mars, PHB metabolism genes were found in the metagenomes of lake fluid and soil samples [5]. The potential functionality of PHB metabolism in lake microorganisms were hypothesized to be a key factor that allowed for their persistence in an environment exhibiting frequent phreatic eruptions [5]. Additionally, if integrating bioplastics in current instruments and techniques can be done without requiring significant redesign, searching for PHAs in future astrobiology missions would come at little hindrance to other mission objectives.

We have outlined many of the technologies used to identify and/or analyze PHAs in manufacturing and research laboratories. While more technology and/or procedural progress needs to be made to allow for PHA detection in exoplanetary missions, we argue that bioplastic is a viable biomarker and thus should be considered for astrobiology investigations. Integrating bioplastic detection in the science goals of current and future missions would be seamless, feasible, and beneficial to any astrobiology mission.

Future Steps: We outline future steps which we propose would be necessary to allow for the successful implementation of searching for bioplastic biomarkers in future astrobiology missions.

- Review planetary environments where PHAs could be preserved. Document benefits and limitations.
- Compare PHA literature to rover technology to identify best instruments and practices.
- If applicable, scrutinize previous and current rover data to search for signal of bioplastics (*e.g., Viking 1 and 2, Curiosity, Perseverance*).
- Optimize tools and techniques for PHA detection (*e.g.,* IR spectroscopy, GC-MS, Raman spectroscopy, SOLID3) from field samples.
- Test PHA detection in planetary analog environments (*e.g.,* Mars surface, Europa & Enceladus plume, and Venus atmosphere analogs) where missions may be searching for biosignatures.
- Integrate researched methodologies and/or instrument parameters that allow for the detection of PHA with astrobiology missions.

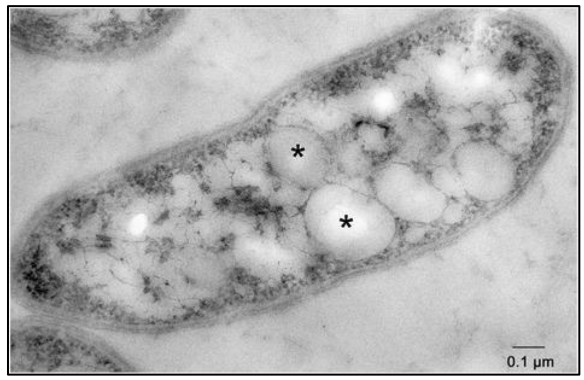

Fig. 2. *Acidiphilium* spp. with polyhydroxybutyrate (PHB) granules marked by asterisks, from [19]

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