





REVIEW ARTICLE

Applications of microbial bioplastic polyhydroxyalkanoates as biosignatures for astrobiological detection

Justin Lorence Wang^{1,2} , Stephanie Hikari Schubert², Nicholas Brady Dragone³ ,
Sydney Marie Ciechanowicz^{2,4} and Brian Michael Hynek^{2,4}

¹Keck School of Medicine of the University of Southern California, 2020 Zonal Avenue, Los Angeles, CA 90033, USA

²Laboratory for Atmospheric and Space Physics, University of Colorado, 3665 Discovery Dr, Boulder, CO 80303, USA

³Cooperative Institute for Research in Environmental Science, University of Colorado, 216 UCB, Boulder, CO 80309, USA

⁴Department of Geological Sciences, University of Colorado, Campus Box 399, Boulder, CO 80309, USA

Corresponding author: Justin Lorence Wang; Email: justinlw@usc.edu

Received: 6 November 2023; **Revised:** 8 July 2024; **Accepted:** 19 August 2024

Keywords: biomarker, bioplastic, biosignature, *Dragonfly*, Europa, *ExoMars*, extremophile, Mars, *Perseverance*, polyhydroxyalkanoate

Abstract

Determining a reliable method to detect life on another planet is an essential first step in the pursuit of discovering extraterrestrial life. Polyhydroxyalkanoates (PHAs), bioplastic polymers created by microorganisms, are strong candidates for defining the presence of extraterrestrial life due to their water insolubility, strong ultraviolet resistance, high melting points and high crystallinity, amongst other qualities. PHAs are abundant on Earth, and their chemical properties can easily be distinguished from non-biological matter. Their widespread distribution and conferred resistance to astrobiologically relevant extreme environments render PHAs highly favourable candidates for astrobiological detection. Integrating detection of PHA biosignatures into current and future life-detection instruments would be useful for the planetary search for life. PHAs are analysed and characterized in laboratories by gas chromatography-mass spectrometry, infrared spectroscopy, Raman spectroscopy and immunoassay analysis in addition to other methods. We outline a path forward to integrate PHA detection in astrobiology missions to aid the search for extraterrestrial life.

Contents

Introduction	1
PHA production, degradation and material properties	3
PHA-producing microbes in extreme environments	6
Astrobiology detection and instrumentation	9
Conclusions and future directions	12

Introduction

The pursuit of extraterrestrial life is often likened to searching for a needle in a haystack. While scientists understand and have characterized life on Earth to a great extent, it is still unknown if life has ever existed on extraterrestrial worlds. Moreover, if life existed on other worlds, we do not know if it would even be recognizable. Because Earth is the only current example of how life could manifest in the Universe, an underlying assumption of many astrobiology missions past, present and future is that extraterrestrial organisms are carbon-based with similar chemistry and structures as terrestrial life. The limitations of this assumption, compounded with the habitability limitations of nearby worlds

and the long-term preservation potential of biological signatures (i.e. biosignatures), is why searching for extraterrestrial life feels like searching for a needle in a haystack on a literal universal scale.

To date, the only missions directly looking for life on other worlds have been conducted on the surface of Mars. NASA's *Viking* missions in the 1970s were the first (and only) to have tested for evidence of extant life (Klein, 1979a). Of the multiple experiments that *Viking* performed to test for life, the Labeled Release experiment remains the most noteworthy. A significant increase in the radioactive carbon dioxide production of Martian soil samples, as compared to a heat-sterilized negative control, led the lead scientists to believe that this was a sign of biological metabolism on Mars (Levin and Straat, 2016). Nevertheless, this claim has since been extensively disputed. Because of the unique quality of Martian soils, these results could be recreated abiotically (Klein, 1979b), by geological and chemical means without processes. Refining life detection techniques is an important task in order to avoid these confusions in the future. A chief lesson learned was that future astrobiological experiments should be capable of discriminating between biotic and abiotic processes and should also consider the distinct geology and chemistry that might bias any instruments (Klein, 1979b).

Since the *Viking* missions, *Mars Science Laboratory* (i.e. the *Curiosity* rover) has confirmed the presence of complex organic compounds in Gale crater (Eigenbrode *et al.*, 2018) and reported a strong seasonal variation in atmospheric methane (Webster *et al.*, 2018). While neither finding is definitive evidence of past or present life on Mars, life could be a contributor to these effects (Eigenbrode *et al.*, 2018; Yung *et al.*, 2018), necessitating further scrutiny. One of the key goals of the *Perseverance* rover, which arrived on Mars in early 2020, is to search for signs of past life. Still, signs of life are yet to be detected; however, there have been recent *in situ* data consistent with the presence of diverse organic molecules (Sharma *et al.*, 2023). The currently delayed *ExoMars* rover, *Rosalind Franklin*, is planned to arrive at Oxia Planum sharing *Perseverance's* goal of searching for signs of past life while also searching for extant life on the Red Planet (Quantin-Nataf *et al.*, 2021).

While Mars remains the only extraterrestrial environment where life has been specifically investigated, other worlds in the solar system have been discussed as potentially hosting habitable environments such as Europa, Enceladus, Ceres, Titan and Venus. The *Europa Clipper* mission, currently slated to reach Jupiter's tidally heated, icy moon in 2030, will fly close enough to Europa to sample potential plumes of ejected water in order to identify organic and inorganic compounds (Howell and Pappalardo, 2020), which could better characterize the potential for this environment to host life. In fact, a number of icy bodies in the outer solar system are now thought to host subsurface oceans that could potentially be habitable, or even inhabited (e.g. Hand *et al.*, 2020; Postberg *et al.*, 2023). In 2020, Venus was in the spotlight for the detection of relatively high concentrations of phosphine in its atmosphere (Greaves *et al.*, 2020), a potential biosignature. An independent analysis reports no finding of phosphine in Venus' atmosphere (Villanueva *et al.*, 2021), but Greaves *et al.* (2020) assert that they still discovered phosphine in Venus' atmosphere even though their initial methods required a recalibration (Greaves *et al.*, 2021a, 2021b). Nevertheless, altitudes of Venus' atmosphere where temperatures and pressures match those in Earth's atmosphere remain an interesting astrobiological target for future missions (Izenberg *et al.*, 2021).

In addition to providing unequivocal evidence of a biological process, the highest regarded biosignatures are those that are also highly common to life on Earth. If the search for life takes place in relict extraterrestrial environments (e.g. Martian palaeolacustrine features and hydrothermal systems), then the preservation potential of molecular biosignatures is key to whether they can be discovered presently (Farmer and Des Marais, 1999). For example, lipids and biopolymers (and their oxidized and reduced products) are expected to be the best preserved biosignatures on Mars if life was present early in its history $\sim 3.5\text{--}4$ Ga (Simoneit, 2002).

A bioplastic is an overarching term for a plastic-like biopolymer produced from a biological source. Many of the well-described bioplastics can only be derived in manufacturing settings as they are derivatives of other biological products (e.g. corn, food waste, oils), but some, like polyhydroxyalkanoates (PHAs), are produced naturally by microorganisms from a host of different environments (Raza *et al.*, 2018). Many biosignatures, such as lipids and sugars, are well understood and studied for

astrobiological purposes (Simoneit, 2002). In contrast, scientific literature for detecting PHAs *in situ* in natural environments, as opposed to in manufacturing or laboratory settings, is exceedingly rare.

To our knowledge, this paper is the first incidence that any bioplastic material has been assessed for its astrobiological potential. As PHAs are only known to be formed by biological processes, have unique preservation qualities, are produced by microorganisms in numerous planetary analogue environments on Earth and can likely be detected extraterrestrially using current instruments and techniques, we argue that PHAs may be a useful and viable biosignature in the search for extraterrestrial life.

PHA production, degradation and material properties

PHAs are a type of polyester, meaning that the ester functional group links chains of hydroxyalkanoate groups together (Raza *et al.*, 2018). PHAs are classified by the length of the carbon chain: short-chain length PHAs (scl-PHAs) have less than five carbon atoms, medium-chain length PHAs (mcl-PHAs) have between 5 and 14 carbon atoms and the less-studied long-chain length PHAs have more than 14 carbon atoms (Raza *et al.*, 2018). PHAs can be copolymerized, and there are over 150 different monomers that are known to be incorporated into PHA chains, which makes PHAs the largest group of natural polyesters (Kim *et al.*, 2007). The first discovered, and the most common, PHA is the scl-PHA polyhydroxybutyrate (PHB) (Fig. 1) (Raza *et al.*, 2018).

Ongoing research efforts into PHAs have focused on both nanobiotechnology applications and using them to manufacture more environmentally safe alternatives to petroleum-derived plastics (Raza *et al.*, 2018). PHAs are hypothesized to be created and utilized as energy and carbon stores in times of stress and starvation in microorganisms (Urtuvia *et al.*, 2014). PHAs are synthesized in both nutrient-rich and nutrient-poor environments by at least 92 genera of Gram-positive and Gram-negative bacteria (Alamgeer, 2019; Vicente *et al.*, 2023) including both heterotrophic and autotrophic bacteria as well as cyanobacteria living under aerobic and anaerobic conditions (Palleroni and Palleroni, 1978; Xiao and Jiao, 2011), and in extreme environments of astrobiology relevance (Wang *et al.*, 2022a). PHAs are also synthesized by archaea and eukaryotes, including plants and recombinant yeast (Terentiev *et al.*, 2004; Xiao and Jiao, 2011).

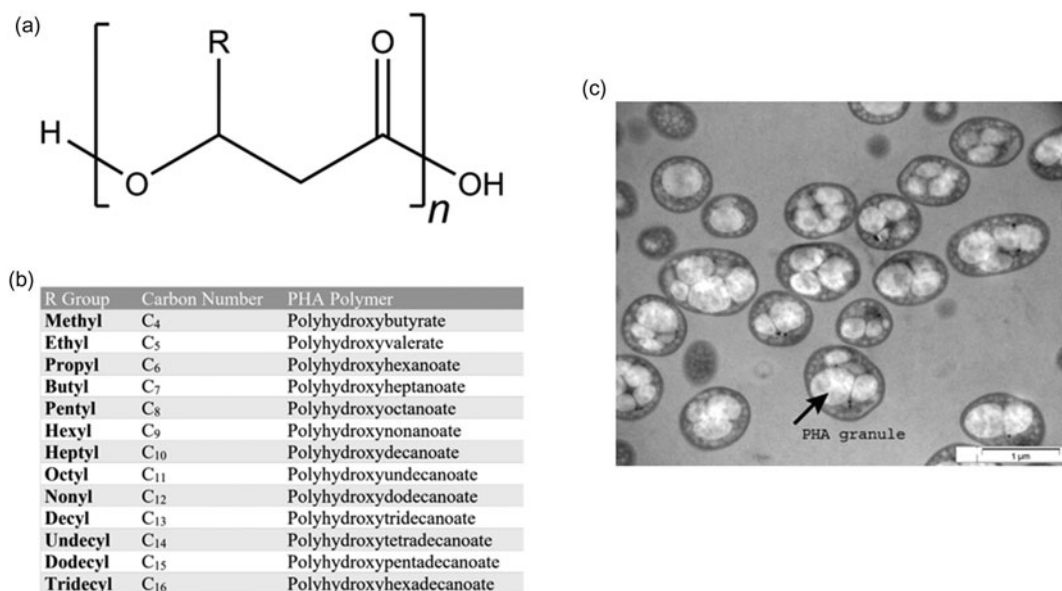
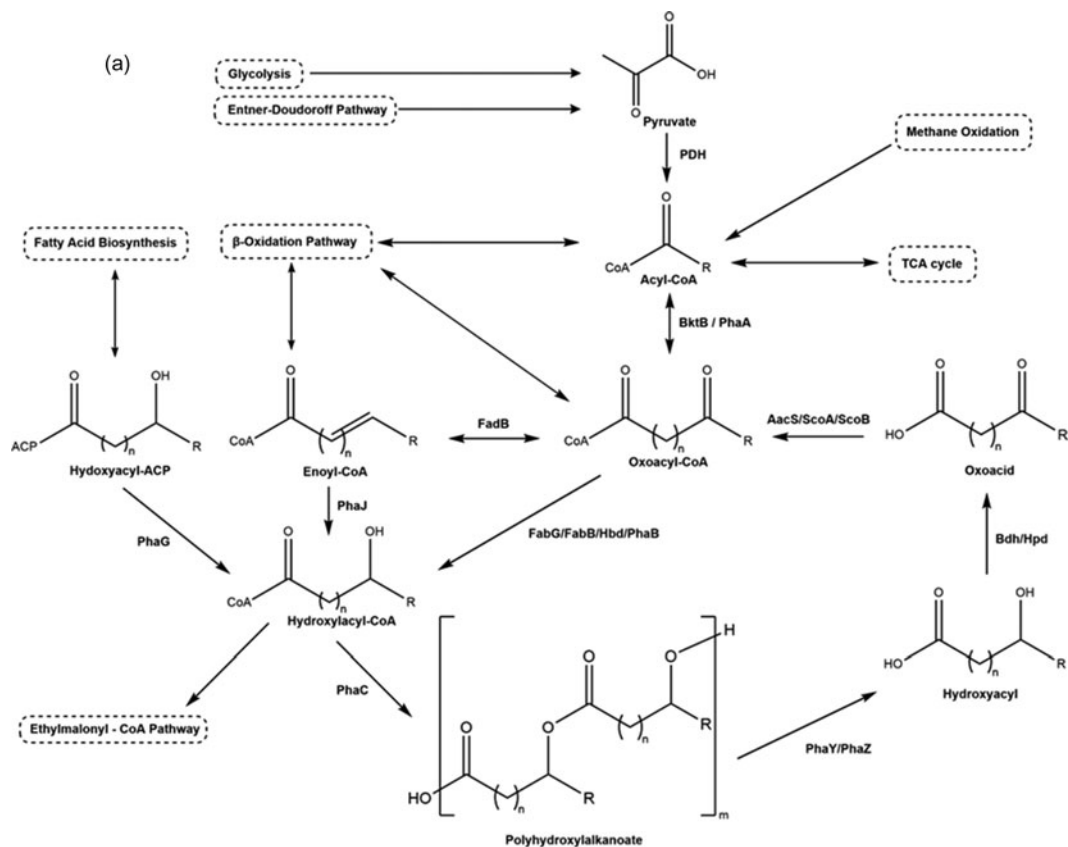


Figure 1. (a) General chemical structure of PHAs with (b) nomenclature and carbon number determined by the functional alkyl R group (adapted from Tan *et al.*, 2014a). (c) *Pseudomonas putida* CA-3 cells with PHA granules from a transmission electron micrograph (Ward Patrick *et al.*, 2005).

PHAs are synthesized and degraded in the PHA cycle (Fig. 2). The PHA cycle branches off from many well understood and common carbon metabolisms including glycolysis, the citric acid cycle, fatty acid biosynthesis, the β -oxidation pathway, the glyoxylate cycle, the ethylmalonyl-coenzyme A pathway, the Entner–Doudoroff pathway and methane oxidation (Urtuvia *et al.*, 2014; Liu *et al.*, 2020; de Vogel *et al.*, 2021; Wang *et al.*, 2022a).



(b)

Enzyme Abbreviations	Enzyme Names
AacS	Acetoacetyl-CoA synthetase
Bdh	3-hydroxybutyrate dehydrogenase
BktB	β -ketothiolase
FabG	3-oxoacyl-[acyl-carrier-protein] reductase
FadB	Multifunctional enoyl-CoA hydratase and hydroxyacyl-CoA dehydrogenase
Hbd	3-hydroxybutyryl-CoA dehydrogenase
Hpd	3-hydroxypropionate dehydrogenase
PDH	Pyruvate Dehydrogenase
PhaA	Acetyl-CoA acetyltransferase
PhaB	Acetoacetyl-CoA reductase
PhaC	PHA synthase
PhaG	Hydroxyacyl-CoA-[acyl-carrier-protein] transferase
PhaJ	(R) – specific enoyl-CoA hydratase
PhaY	PHA oligomer hydrolase
PhaZ	PHA depolymerase
ScoA/ScoB	3-oxoacid CoA-transferase subunit A and B

Figure 2. (a) Overview of the PHA cycle with chemical structures, connected metabolic pathways, and (b) enzyme-catalysing reactions. CoA, coenzyme A; ACP, acyl carrier protein. Adapted from de Vogel *et al.* (2021).

PHAs form insoluble, complex and well-organized granule structures inside cells measuring from 0.2 to 0.5 μm in diameter intracellularly (Jendrossek *et al.*, 2007) and from ~ 100 nm to several micrometres extracellularly (Bäckström *et al.*, 2007). PHA granules (Fig. 3) are commonly surrounded by proteins bound to the core, with PHA granule-associated proteins (PhaP or ‘phasins’) being the most common. Phasins are a group of amphipathic proteins that bind specifically and hydrophobically with the PHA core, and they can coat up to 54% of the PHA granule (Tian *et al.*, 2005; Bäckström *et al.*, 2007). A phospholipid membrane surrounding the PHA core is heavily debated (Maestro and Sanz, 2017); while original studies detected phospholipids attached to the PHA granule, Wahl *et al.* (2012) and Bresan *et al.* (2016) argue that prior findings of phospholipids were due to contamination in PHA extraction and preparation techniques.

Bacteria have been able to produce up to 90% (w/w) PHAs of their cell dry weight (Tan *et al.*, 2014b). PHAs are water insoluble (resistant to hydrolytic degradation), and they sink in aqueous environments (Noreen *et al.*, 2020), which could help with biopreservation through burial. As a material, PHAs are easily attacked by acids and alkalis and dissolve in chlorinated solvents; nevertheless, PHAs have strong ultraviolet (UV) resistance (Bugnicourt *et al.*, 2014). PHAs have exhibited melting points between 50 and 180°C and crystallinity between 30 and 70% (Bäckström *et al.*, 2007; Rehm, 2010). Zhang *et al.* (2022) reported thermal and mechanical properties for multiple scl-PHAs and mcl-PHAs.

Photolysis of biodegradable plastics, including PHAs, has been shown in experiments to result in photodegradation and certain structural changes (Kaing *et al.*, 2024). For example, UV photodegradation has been shown to cause some chemical and structural changes to PHBs in experiments, leading to decreased biodegradability from increased crystallinity (Sadi *et al.*, 2010). Experiments investigating abiotic hydrolysis of PHAs discovered that longer side-chain lengths and higher crystallinity (>40%)

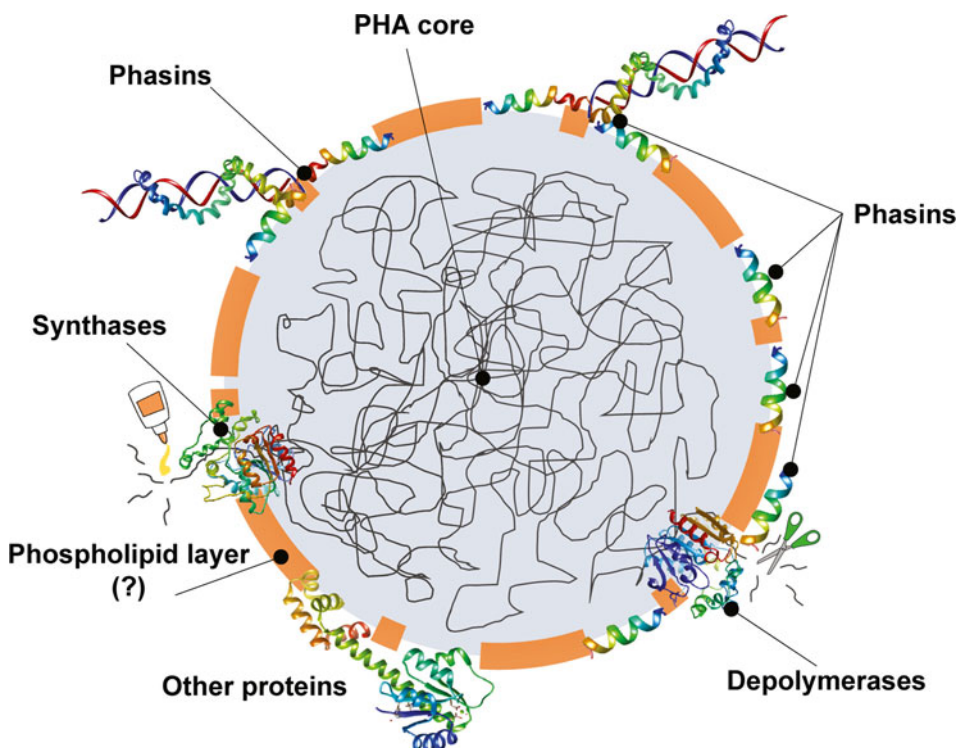


Figure 3. Schematic of a PHA granule structure from Maestro and Sanz (2017).

interfere with abiotic degradation under alkaline conditions, providing a higher stability against hydrolysis (Tarazona *et al.*, 2020).

Since most research into PHAs has been focused on enhancing biodegradability in industrial settings, there is limited knowledge on PHA's preservation potential extracellularly and its long-term stability without microbial degraders in various environments, particularly for geologic timescales when searching for relict life. PHA's weak resistance to solvents would make biopreservation a challenge in aqueous environments with non-neutral pHs, however it is possible that the cell membrane and protein layer surrounding PHA granules could help improve preservation, especially if the cell membrane is composed of certain phospholipids from extremophiles that are temperature and pH resistant (Sollich *et al.*, 2017). Encapsulation from extremophile phospholipids also would inherently assist biopreservation of any other biomolecule. It should be noted that extremophiles producing PHAs have been found in many extremophile environments (Gambarini *et al.*, 2022), though the literature is scarce on PHA biosignature detection in these environments and the material's stability extracellularly.

The strong UV resistance of PHAs makes them an attractive candidate when looking for biosignatures in planetary environments with high radiation, like Mars and Europa, where radiation can degrade molecules (Merino *et al.*, 2019). While PHAs may not be as well preserved as some other biosignatures (e.g. lipids, pigments, biomembranes), the high crystallinity, melting temperature, water insolubility and binding with proteins (and potentially lipids) may make PHAs a stronger biosignature than other labile compounds (e.g. amino acids, sugars, DNA/RNA). While long-term stability is challenged until further experimentation, the material properties of PHAs combined with the diversity of the polymer and the many ways in which they can bind and be preserved by other biomolecules make them a candidate biosignature for astrobiological purposes, especially in the search for extant life.

PHA-producing microbes in extreme environments

PHAs are not only found in all three branches of life (bacteria, archaea and eukarya), but they are found in many extremophiles living in astrobiological-relevant environments. The adaptability of some microorganisms to their extreme environments has even been attributed to PHAs and their material properties, as summarized in Table 1.

Acidic environments are astrobiologically relevant, as life is hypothesized to have been harboured in hydrothermal environments on Mars (Wang *et al.*, 2022a) and perhaps currently on the moons of Europa and Enceladus where hydrothermal activity could provide heat and nutrients for life (Deamer and Damer, 2017). Sulphur discovered via remote sensing on Europa could be associated with sulphur-metabolizing microorganisms (Carlson *et al.*, 1999; Trivedi Christopher *et al.*, 2020) and life on Venus is even hypothesized to live in the sulphuric acid clouds where Earth temperatures and pressures are found (Izenberg *et al.*, 2021). Thus, biosignatures generated by acidophiles may be of importance in future astrobiology space missions.

PHA metabolism is a key feature of microbes in the *Acidiphilium* genus, an aerobic acidophile capable of a wide array of metabolisms and adaptations including sulphur oxidation and metal detoxification (Li *et al.*, 2020). Wang *et al.* (2022a) noted that genes allowing for PHB metabolism, a type of PHA, were present in their *Acidiphilium*-dominated metagenome of microorganisms from the crater lake of the Poás Volcano. The Poás Volcano is an extreme Martian acid-sulphate hydrothermal analogue exhibiting frequent phreatomagmatic eruptions, with pH measurements as low as -0.87 , and temperatures as high as boiling (Hynek *et al.*, 2018). While low pH fluids easily react with PHAs making them unstable in these kinds of environments, Wang *et al.* (2022a) suggested PHB metabolism may actually be one of the key factors allowing for the persistence of *Acidiphilium* spp. in this extremely acidic and dynamic environment. The authors hypothesized that the presence of PHB metabolism genes in the *Acidiphilium* spp. bacteria survived the frequent phreatomagmatic eruptions between their multiple field campaigns using PHB metabolism to manage periods of carbon starvation (in either the crater lake, connected groundwater or the surrounding soils) thus allowing future recolonization of a locale if it was sterilized. Note that the authors did not find nor search for PHB's in their field samples,

Table 1. Summary of various PHA-producing microorganisms from varying environments

	Microorganisms	Conditions	Environment
Acidophiles	<i>Acidiphilium</i> spp.	Low pH, high temperature, phreatomagmatic eruptions	Poás Volcano, Costa Rica
Alkaliphiles	<i>Serpentinimonas raichei</i> sp. nov. <i>Serpentinimonas barnesii</i> sp. nov. <i>Serpentinimonas maccroryi</i> sp. nov.	High pH, serpentine reactions	The Cedars serpentization site in Northern California
Thermophiles	<i>Geobacillus stearothermophilus</i> <i>Cupriavidus cauae</i>	High temperature	Hot springs
Aerobic soil microbes	<i>Alcaligenes eutrophus</i> <i>Alcaligenes latus</i> <i>Pseudomonas oleovorans</i>	Aerobic	Soil
Anaerobic soil microbes	<i>Clostridium botulinum</i> <i>Clostridium acetireducens</i>	Anaerobic	Soil
Methanotrophs	<i>Methylosinus trichosporium</i> <i>Methylocystis hirsute</i>	High methane, nutritional imbalance	Obtained from culture collections
Psychrophiles	<i>Pseudomonas extremaustralis</i> <i>Janthinobacterium</i> spp. <i>Pseudomonas</i> spp.	Cold and variable temperature, low nutrient availability, oxidative stress, radiation resistance	Antarctic freshwater sources
Halophiles	<i>Natrinema altunense</i> <i>Haloterrigena jeotgali</i>	High salinity	Chott El Jerid, Tunisia

so even if PHAs may be an integral feature of acidophilic microorganisms in extraterrestrial environments, further studies into PHA preservation in low pH would need to be conducted to assess their functionality as a biosignature in acidic environments.

Hydrothermal environments undergoing serpentinization reactions are also of astrobiological relevance as they are hypothesized to have been important for the origin of life on Earth (e.g. Russell *et al.*, 2010) and potentially extraterrestrial life in environments on Europa, Enceladus, Titan and Mars (Vance and Melwani Daswani, 2020), including Jezero crater (Simon *et al.*, 2023), *Perseverance*'s main target. Serpentinization is a water-driven reaction of mafic and ultramafic minerals (i.e. olivine and pyroxene) which generates serpentine, hydrogen and methane amongst other products. This can occur at temperatures between ambient and 400°C and at high pH values (Ehlmann *et al.*, 2010); moreover, the alkalinity of Enceladus' Ocean, pH ~11–12, as inferred from *Cassini* spacecraft data, is attributed to serpentinization processes (Glein *et al.*, 2015). In a study performed by Bird *et al.* (2021), PHA accumulations were produced by three cultures of facultative autotrophic, highly alkaliphilic bacterial strains (pH ~9.0–12.5) that were isolated from terrestrial serpentinizing springs. The cultures were dominated by members of the *Comamonadaceae* family, which have also been characterized for their poly(3-hydroxybutyrate-co-3-hydroxyvalerate)-degrading denitrifying activity

(Khan Shams *et al.*, 2002; Bird *et al.*, 2021). Thus, it is conceivable that microbial life in extraterrestrial environments dominated by serpentinization processes may also metabolize PHAs.

In addition to acidity and alkalinity, hydrothermal environments frequently exhibit high temperatures. Many thermophiles are capable of producing PHAs, and they have been reported to do so in temperatures of up to 80°C (Obulisamy and Mehariya, 2021). For example, *Geobacillus stearothermophilus* strain K4E3_SPR_NPP was shown to produce a PHA copolymer containing poly(3-hydroxybutyrate)-poly(3-hydroxyvalerate)-poly(3-hydroxyhexanoate) (Rodge *et al.*, 2023), and *Cupriavidus cauae* PHS1 was able to produce a 47% cell dry weight of PHB at a temperature of 47°C (An *et al.*, 2023). Considering that the last universal common ancestor of life on Earth is hypothesized to be a thermophile or hyperthermophile (Nisbet and Sleep, 2001; Giulio, 2003; Gaucher *et al.*, 2008), high-temperature compatibility is crucial when assessing PHA's astrobiological potential.

One of Mars *Perseverance*'s top-level goals is to search for biosignatures in a palaeo-fluviolacustrine environment by searching for biomarkers at or near the surface (Farley *et al.*, 2020). PHAs are found in a variety of soil environments utilizing either aerobic metabolism (e.g. *Alcaligenes eutrophus*, *Alcaligenes latus*, *Pseudomonas oleovorans*) or anaerobic metabolism (e.g. *Clostridium botulinum*, *Clostridium acetireducens*) (Santhanam and Sasidharan, 2010; Flüchter *et al.*, 2019). Since the majority of extraterrestrial astrobiology targets contain low concentrations of oxygen gas or are completely oxygen-free (Cockell *et al.*, 2018), the ability for PHA metabolism to be compatible with both aerobic and anaerobic respiration better supports its use as a biosignature for a variety of environments. Note that the aerobic soil bacteria *A. latus* was studied extensively by Palleroni and Palleroni (1978) and was found to have optimal growth at low oxygen levels (atmospheric O₂ at ~8%) and could utilize molecular hydrogen for autotrophic growth.

PHA metabolism has also been found in *Methylosinus trichosporium* and *Methylocystis hirsute*, methanotrophic bacterial strains that convert methane to PHB when nutrient supplies are 'imbalanced' (Liu *et al.*, 2020). Methanotrophs and methanogens have been postulated on Mars as a source of seasonal variation in atmospheric methane (Webster *et al.*, 2018; Seto *et al.*, 2019). Methanogens generate methane, often live in symbiosis with methanotrophs (Wang *et al.*, 2022b), and have been postulated to live on the surface of Saturn's moon Titan (McKay and Smith, 2005). While Titan's ultra-low temperatures allowing for liquid nitrogen and frozen water on the surface would likely make life on the moon very different for any terrestrial organisms (McKay, 2016), any discovery of PHAs on Titan would warrant an astrobiological interpretation.

While low temperatures resembling that of Titan are unfathomable on Earth, the survival mechanisms of PHA-producing psychrophiles are very well studied. PHAs in *Pseudomonas* sp. 14-3 were shown to protect the cells from oxidative stress (Ayub *et al.*, 2007), and an increased motility in biofilms developed by *Pseudomonas extremaustralis* was attributed to PHA accumulations (Tribelli and López, 2011). In another study, Tribelli *et al.* (2020) reported the bacterial diversity of cultivation-dependent freshwater samples from the Ecology Glacier foreland in King George Island, Antarctica. They noted that genes for PHA synthesis were present in all five sampling sites (temperatures ranging from 2.8 (±1.8) to 6.1 (±2.1)°C) in more than half of their isolated strains. In particular, PHA synthesis genes were found in six strains of *Pseudomonas* and four strains of *Janthinobacterium*. In their natural environments, these organisms were subject to very cold and variable temperatures, low nutrient availability and low water quality; however, it was hypothesized that PHAs helped the bacteria survive longer by providing a reserve source for when there were low nutrient and carbon supplies (Tribelli *et al.*, 2020). Similarly, Ciesielski *et al.* (2014) isolated the psychrophile *P. extremaustralis*, a producer of PHBs and mcl-PHAs, from a temporary water pond in Antarctica and subjected it to extreme cold and radiation. The authors found that higher PHB accumulation conferred with colder temperature tolerances and higher radiation resistance after a lethal ultraviolet-A (UVA) exposure (Ciesielski *et al.*, 2014). These studies suggest that PHAs are not only present in these psychrophilic and radiation-resistant microorganisms, but that PHAs confer better survival in both cold and high radiation

environments. PHAs would then be beneficial to any microorganisms living in cold and/or high radiation environments (e.g. on Mars, Europa and Enceladus).

Two archaeal halophilic strains, *Natrinema altunense* and *Haloterrigena jeotgali*, found in the extremely salty lake, Chott El Jerid (salt concentration up to 330 g l^{-1}), are producers of PHB and polyhydroxyoctanoate (Abdallah *et al.*, 2016, 2020). Briny hydrothermal activity has been postulated on Mars (e.g. Schmidt *et al.*, 2008) making halophilic compatibility an interesting consideration for PHAs astrobiological potential. A leading hypothesis for the recurring slope lineae (RSLs) on Mars is the presence of briny water, and thus RSLs have been given special consideration under many space agencies' planetary protection protocols as life could potentially live in this kind of environment (McEwen *et al.*, 2021). Moreover, halopsychrophiles, living in both cold and briny environments, have been proposed to possibly be capable of inhabiting hypothesized saline subsurface oceans of the dwarf planet Ceres, those of the moons Titan and Europa, and in Cl-rich subsurface brines on Mars (Merino *et al.*, 2019).

While PHAs are not ubiquitous in all living organisms, they are found in all three branches of life, in both heterotrophs and autotrophs, in aerobes and anaerobes and in a wide array of environments (i.e. cold, hot, acidic, basic, briny, high radiation). Not only are PHAs (or PHA genes) found in these extreme environments, but the adaptability of these microorganisms to these extremes are also attributed to the presence and utilization of PHAs in the case of nutrient imbalance (Tribelli *et al.*, 2020), cold temperatures and lethal UVA radiation (Ciesielski *et al.*, 2014), oxidative stress (Ayub *et al.*, 2007) and survival in an acidic environment exhibiting frequent phreatomagmatic eruptions (Wang *et al.*, 2022a). Thus, it is conceivable that microorganisms on other worlds may exploit PHAs, perhaps utilizing it as a primary metabolic and survival strategy.

Astrobiology detection and instrumentation

While the use of PHAs would be beneficial to microorganisms living in many extreme environments and potentially extraterrestrial worlds, the ability to detect them using current and upcoming techniques searching for biosignatures comes with limitations. The two primary technical limitations to detecting PHAs on other worlds we have identified are that: (i) the samples taken for analysis may contain impurities so that they interfere with or mask the PHA signal, and (ii) there may not be high enough PHA mass in samples to reach minimum detection limits. However, these limitations are problems for all molecular biosignatures, not just for PHAs.

To the knowledge of the authors, literature on detecting PHAs *in situ* is extremely limited, and this is the first paper that discusses using PHAs as an astrobiological biosignature. To determine whether certain instruments and techniques could be utilized to detect PHAs for astrobiology purposes, we review and report numerous methods that are utilized to measure PHAs in modern laboratories that are analogous to current or upcoming astrobiological instrumentation. Samrot *et al.* (2021) also discuss many characterization techniques for analysing PHAs beyond those of astrobiological relevance. Note from the section 'PHA production, degradation and material properties' that PHAs are diverse and can range from short-chain to long-chain lengths. This inherently results in varying detection signals for different kinds of PHAs. In this section, we review the most cited and relevant techniques for studying varying PHAs (Table 2) and then later provide future recommendations for furthering PHA detection technology for astrobiological purposes in the section 'Conclusions and future directions'.

Gas chromatography-mass spectrometry (GC-MS) is a popular method for identifying molecular compounds, and instruments have flown on various spacecraft. In GC-MS, a mass spectrometer uses an ionized beam of electrons to break the GC output to calculate a mass to charge ratio (m/z) with a relative abundance. Four GC-MS experiments have been sent to Mars: one on each Viking lander, the Sample Analysis at Mars (SAM) instrument on *Mars Science Laboratory* and the Mars Organic Molecule Organizer (MOMA) instrument on *ExoMars* (Klein, 1979a; Mahaffy *et al.*, 2012; Arevalo *et al.*, 2015). As PHAs are composed of multiple polyester units and do not have volatile tendencies, the PHA biopolyester must be broken down into volatile (or semi-volatile), low molecular weight and

Table 2. Summary of techniques to identify PHA's *in situ* for astrobiology missions

Analytical method	Detection range	Sample preparation	Analogous planetary instrument
Gas chromatography-mass spectrometry	Mass-to-charge ratio (m/z) between 40 and 600 depending on preparation	Methanolysis, pyrolysis with TMAH and MTBSTFA derivatization	SAM on <i>Curiosity</i> MOMA on <i>ExoMars</i> DraMS on <i>Dragonfly</i>
Infrared spectroscopy	Absorption peak at 1728 cm^{-1} and absorption band at $5.80\text{ }\mu\text{m}$	Pure and mixed microbial biomasses collected through centrifugation	Current planetary IR spectrometer (e.g. ISEM on <i>ExoMars</i>) do not cover the appropriate spectral range
Fourier-transform infrared spectroscopy	$1165\text{--}2925\text{ cm}^{-1}$ depending on type of PHA and intracellular versus extracellular	Pure and mixed microbial biomasses collected through centrifugation	No current planetary FRIT instruments; prototype from Anderson <i>et al.</i> (2005)
Raman spectroscopy	$800\text{--}1800\text{ cm}^{-1}$ with a 785 nm laser	Cell suspensions were analysed and calibrated with DNA and amide I to obtain reported wavenumbers	SHERLOC on <i>Perseverance</i> RLS on <i>ExoMars</i>
Immunoassay	N/A	Liquid or solid sample	SOLID3

thermally stable compounds prior to being analysed by any GC system (Tan *et al.*, 2016). Of the ways to prepare PHAs for GC-MS analysis, methanolysis, pyrolysis using tetramethylammonium hydroxide (TMAH) and derivatization using *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide (MTBSTFA) are techniques utilized by both SAM and MOMA. Both SAM and MOMA can perform MTBSTFA derivatization prior to GC-MS and TMAH pyrolysis prior to GC-MS (i.e. pyr-gc-ms), and SAM can also perform methanolysis (Mahaffy *et al.*, 2012; Arevalo *et al.*, 2015). The mass to charge ratio for PHAs depends on the type of PHA, the method of preparation and the ionic source, but m/z values have been reported to range from 40 to 600 (Tan *et al.*, 2014c), which overlaps well with the detectable ranges of both SAM and MOMA. In addition to these Martian rovers, NASA's *Dragonfly* mission, which is a rotorcraft poised to arrive on Titan in 2034, will carry a Dragonfly Mass Spectrometer (DraMS). DraMS uses heritage hardware from SAM and MOMA and is capable of performing pyrolysis and derivatization GC-MS (Trainer *et al.*, 2018; Barnes *et al.*, 2021). It must be noted that while GC-MS can identify and quantify PHAs in samples, the retention time and m/z values will depend on the individual instrument, sample preparation and internal standards. GC-MS was one of the first astrobiological relevant techniques used to search for life when it was utilized in the Viking landers; it is currently being utilized by spacecraft, and it will be exploited in future astrobiological missions. With access to and testing with SAM-, MOMA- and DraMS-analogues, it would be beneficial to determine the signals for various PHAs for each of these instruments.

Infrared (IR) spectroscopy is used to characterize absorptions in particular wavelengths that can be mapped to specific molecules based on characteristic vibrations released from the molecular bonds or angles shifting in space (e.g. Stuart, 2015). Poly(3-hydroxybutyrate) (P(3HB)) has a notably strong carbonyl absorption peak at 1728 cm^{-1} in the IR spectrum and a sharp absorption band at $5.80\text{ }\mu\text{m}$

(Godbole, 2016). Compared to current instrumentation, Infrared Spectrometer for *ExoMars* (ISEM) has a spectral range between 1.15 and 3.30 μm with a spectral resolution varying from 3.3 nm at 1.15 μm to 28 nm at 3.30 μm (Korablev *et al.*, 2017), which is beyond the range of what can detect P(3HB). Yet there are no technical issues in sending mid-to-thermal IR instruments to Mars, which have already been demonstrated on orbiters including *Viking*, *Mars Global Surveyor* and *Mars Odyssey*.

Fourier-transform infrared (FTIR) spectroscopy instruments, however, have the appropriate spectral ranges to detect PHAs. FTIR employs a mathematical process known as Fourier transformation to interpret the interference of two beams of radiation to generate an interferogram which achieves high-resolution spectral ranges (e.g. Stuart, 2015). The FTIR spectra of a pure PHA containing short-chain length and medium-chain length monomers such as hydroxybutyrate, hydroxyoctoate and hydroxydecanoate have shown strong detection bands at 1728, 1740 and 1732 cm^{-1} , respectively (Hong *et al.*, 1999). Additionally, the intensity of the methylene band near 2925 cm^{-1} is very strong which helps confirm PHA detection, and accompanying bands near 1280 and 1165 cm^{-1} help identify the type of PHA (Hong *et al.*, 1999). When analysing PHAs within bacterial cells, the strong marker bands were 1732, 1744 and 1739 cm^{-1} corresponding to intracellular PHB, mcl-PHA and P (HB + mcl-PHA), respectively (Hong *et al.*, 1999). While no FTIR instruments have been used or developed for an upcoming space mission, Anderson *et al.* (2005) proposed an FTIR for Mars research instrument with a resolution between 700 and 5000 cm^{-1} , which covers the appropriate spectral range for PHA detection. Note that in addition to the limitations as discussed in the beginning of this section, the IR and FTIR detection bands for PHAs are non-specific to PHA and can also be interpreted as another compound. Thus, we propose if any type of IR analysis is used for PHA detection that it be followed up with another technique more sensitive to PHAs for biosignature confirmation.

Raman spectroscopy analyses the lattice vibrations of molecules by measuring the shift in frequency by inelastically scattered light when incident photons hit the sample (Moon *et al.*, 2023). Raman spectroscopy is similar to IR spectroscopy; however, instead of measuring infrared absorption lines, Raman spectroscopy focuses on the dipole moment that is induced in the molecule by the electric field of the photons which generates Raman emission lines (Moon *et al.*, 2023). Samek *et al.* (2016) identified the relevant spectral region for a PHB as 800–1800 cm^{-1} utilizing a 785 nm laser. They calibrated their Raman emission lines for a PHB line at 1736 cm^{-1} using two internal standards (DNA at 786 cm^{-1} and amide I at 1662 cm^{-1}) by performing gas chromatography of PHB samples ranging in concentration from 12 to 90% (w/w) (Samek *et al.*, 2016). This preparation resulted in several Raman peaks at 837, 1455 and 1736 cm^{-1} . In comparison, the Scanning Habitable Environments with Raman & Luminescence for Organics & Chemicals instrument (SHERLOC) on *Perseverance* has a Raman region between 800 and 4000 cm^{-1} with a 248.6 nm laser (Bhartia *et al.*, 2021) and the Raman Laser Spectrometer (RLS) on *ExoMars* has a Raman region between 70 and 4200 cm^{-1} with a spectral resolution of 6–10 cm^{-1} with a 532 nm laser (Veneranda *et al.*, 2020). Inherently, the purity of the sample in Samek *et al.* (2016) allows for distinct Raman lines, and the calibration, internal standards and laser wavelength used by the authors resulted in the specific peaks for their PHB sample. For Raman spectroscopy to be used to detect PHAs on Mars or other planetary bodies, more studies working to identify PHA Raman lines using rover equivalent instrumentation and impure PHA samples should be conducted.

Another method, which we argue would be the most definitive, to detect PHAs in extraterrestrial worlds would be to exploit the binding between phasins and PHAs. Recall that phasins (PHA granule-associated proteins or PhaP) are a group of amphipathic proteins that bind specifically and hydrophobically with the PHA core. An instrument capable of detecting phasin–PHA associations in a sample would be a more definitive method to detect PHAs. The ‘Signs Of Life Detector’ 3rd generation (SOLID3) utilizes the LDChip300, a sandwich microarray immunoassay detection tool that analyses hundreds of different biosignatures from a small sample (Parro *et al.*, 2011). It employs fluorescent-based immunoassay antibodies (or antibody-like probes) to microscopically detect and report the presence of biosignatures. SOLID3 has been tested in multiple field campaigns and it was one of the instruments included in a proposed mission to Mars (Fairén *et al.*, 2020). If a phasin

could be added as an affinity tag, then SOLID3 may be able to detect PHAs on other worlds, in addition to the hundreds of different biomarkers it is already searching for.

There are many other methods to identify, quantify and analyse various types of PHAs including high-performance liquid chromatography, UV–visible spectroscopy, scanning and transmission electron microscopy, nanomagnetic resonance and other techniques as summarized by Koller and Rodríguez-Contreras (2015), Godbole (2016) and Samrot *et al.* (2021). These methods may require either heavier machinery or more human input and customization that is not conducive to planetary mission instrumentation. Nevertheless, considering NASA's plans for a Mars 2020 sample return mission (Haltigin *et al.*, 2021) and plans for a human mission to Mars, these other techniques may soon be able to scrutinize returned samples.

Conclusions and future directions

We argue that bioplastics could be a useful tool to search for extraterrestrial life. PHAs are a class of bioplastic that have unique properties already exploited in nanobiotechnologies and environmentally friendly manufacturing. They come in varying chain lengths and structures resembling a granule core surrounded by proteins (i.e. phasins) and potentially a heavily debated phospholipid membrane. Properties such as water insolubility, strong UV resistance, sinking in aqueous environments, high melting points, high crystallinity and being able to occupy a high percentage of bacterial cell weight make PHAs a strong candidate biosignature. Still, more experiments need to be conducted to determine PHA's preservation potential and degradability for geologic timescales in a variety of environmental conditions. This will help scrutinize whether we can expect PHAs to be detected in all environments of astrobiological relevance or only some (i.e. relict versus extant life and neutral versus non-neutral pH's).

Despite not being universal in all terrestrial life like some other biosignatures (e.g. DNA/RNA, certain types of lipids and amino acids), PHAs are found in all three branches of life, in both heterotrophs and autotrophs, in aerobes and anaerobes and in a wide variety of extreme environments with astrobiological relevance. Additionally, it has been noted in numerous cases that PHAs (or PHA genes) may contribute to the adaptability of some extremophiles to their environments. Considering that many potentially past or present habitable planetary environments host extreme and hostile environmental conditions, evolution of a stable carbon and energy store such as PHAs would be considerably beneficial to extraterrestrial organisms.

Discussions of incorporating PHA detection in astrobiology space missions are under-developed and almost non-existent outside of this review. We have identified five experimental methods in which they could be incorporated in current and future space missions (Table 2) with varying levels of technical maturity. While more work needs to be done to incorporate PHA detection and characterization in these methods, only one method would require a redesign (i.e. adding phasin as an affinity tag to SOLID3) while the others only require more laboratory testing to determine the various mass to charge ratios and retention times (for GC-MS) and wavelength absorption ranges (for FTIR and Raman spectroscopy) for different PHAs using the standards and sample preparations found on relevant rover instruments (i.e. SAM, MOMA, DraMS, SHERLOC and RLS).

To successfully implement PHA detection into current and future astrobiology missions, we recommend first starting by performing laboratory tests using rover analogous instruments and conditions to determine the appropriate detection values for various types of PHAs. With this knowledge, we recommend scrutinizing previous and current rover data for signal evidence of PHAs using GC-MS and Raman spectroscopy (e.g. from *Viking 1 and 2*, *Curiosity*, *Perseverance*). This would allow future results from *Curiosity*, *Perseverance*, *ExoMars* and *Dragonfly* to immediately be able to detect for PHAs without redesigning the instruments. Next, we recommend adding phasin as an affinity tag to SOLID3 to allow for PHA detection in future experiments and its maiden voyage. To help mature PHA detection with GC-MS, Raman spectroscopy, IR spectroscopy and immunoassay analysis, we suggest that these instruments test field samples from astrobiology analogue environments and taken

into the field to perform *in situ* detection of PHAs. Results from these laboratory experiments and field campaigns can inform and help optimize future astrobiology instrumentation for PHA detection. We argue that given the unique properties and preservation potential of microbial bioplastic PHAs, their consideration in the search for life beyond Earth will only strengthen our chances of success.

Financial support. This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Competing interests. None.

References

- Abdallah MB, Karray F, Mhiri N, Mei N, Quéménéur M, Cayol J-L, Erauso G, Tholozan J-L, Alazard D and Sayadi S (2016) Prokaryotic diversity in a Tunisian hypersaline lake, Chott El Jerid. *Extremophiles* **20**, 125–138.
- Abdallah MB, Karray F and Sayadi S (2020) Production of polyhydroxyalkanoates by two halophilic archaeal isolates from Chott El Jerid using inexpensive carbon sources. *Biomolecules* [Online] **10**.
- Alamgeer M (2019) Polyhydroxyalkanoates (PHA) genes database. *Bioinformation* **15**, 36–39.
- An J, Ha B and Lee SK (2023) Production of polyhydroxyalkanoates by the thermophile *Cupriavidus cauae* PHS1. *Bioresource Technology* **371**, 128627.
- Anderson MS, Andringa JM, Carlson RW, Conrad P, Hartford W, Shafer M, Soto A, Tsapin AI, Dybwad JP, Wadsworth W and Hand K (2005) Fourier transform infrared spectroscopy for Mars science. *Review of Scientific Instruments* **76**, 034101.
- Arevalo R, Brinckerhoff W, Amerom FV, Danell R, Pinnick V, Xiang L, Getty S, Hovmand L, Grubisic A, Mahaffy P, Goesmann F and Steininger H (2015) Design and demonstration of the Mars Organic Molecule Analyzer (MOMA) on the ExoMars 2018 rover. 2015 IEEE Aerospace Conference, 7–14 March 2015, pp. 1–11.
- Ayub ND, Pettinari MJ, Méndez BS and López NI (2007) The polyhydroxyalkanoate genes of a stress resistant Antarctic *Pseudomonas* are situated within a genomic island. *Plasmid* **58**, 240–248.
- Bäckström BT, Brockelbank JA and Rehm BHA (2007) Recombinant *Escherichia coli* produces tailor-made biopolyester granules for applications in fluorescence activated cell sorting: functional display of the mouse interleukin-2 and myelin oligodendrocyte glycoprotein. *BMC Biotechnology* **7**, 3.
- Barnes JW, Turtle EP, Trainer MG, Lorenz RD, Mackenzie SM, Brinckerhoff WB, Cable ML, Ernst CM, Freissinet C, Hand KP, Hayes AG, Hörst SM, Johnson JR, Karkoschka E, Lawrence DJ, Le Gall A, Lora JM, McKay CP, Miller RS, Murchie SL, Neish CD, Newman CE, Núñez J, Panning MP, Parsons AM, Peplowski PN, Quick LC, Radebaugh J, Rafkin SCR, Shiraishi H, Soderblom JM, Sotzen KS, Stickle AM, Stofan ER, Szopa C, Tokano T, Wagner T, Wilson C, Yingst RA, Zacny K and Stähler SC (2021) Science goals and objectives for the Dragonfly Titan rotorcraft relocatable lander. *The Planetary Science Journal* **2**, 130.
- Bhartia R, Beegle LW, Deflores L, Abbey W, Razzell Hollis J, Uckert K, Monacelli B, Edgett KS, Kennedy MR, Sylvia M, Aldrich D, Anderson M, Asher SA, Bailey Z, Boyd K, Burton AS, Caffrey M, Calaway MJ, Calvet R, Cameron B, Caplinger MA, Carrier BL, Chen N, Chen A, Clark MJ, Clegg S, Conrad PG, Cooper M, Davis KN, Ehlmann B, Facto L, Fries MD, Garrison DH, Gasway D, Ghaemi FT, Graff TG, Hand KP, Harris C, Hein JD, Heinz N, Herzog H, Hochberg E, Houck A, Hug WF, Jensen EH, Kah LC, Kennedy J, Krylo R, Lam J, Lindeman M, Mcglown J, Michel J, Miller E, Mills Z, Minitti ME, Mok F, Moore J, Neelson KH, Nelson A, Newell R, Nixon BE, Nordman DA, Nuding D, Orellana S, Pauken M, Peterson G, Pollock R, Quinn H, Quinto C, Ravine MA, Reid RD, Riendeau J, Ross AJ, Sackos J, Schaffner JA, Schwochert M, O Shelton M, Simon R, Smith CL, Sobron P, Steadman K, Steele A, Thiessen D, Tran VD, Tsai T, Tuite M, Tung E, Wehbe R, Weinberg R, Weiner RH, Wiens RC, Williford K, Wollonciej C, Wu Y-H, Yingst RA and Zan J (2021) Perseverance's scanning habitable environments with Raman and luminescence for organics and chemicals (SHERLOC) investigation. *Space Science Reviews* **217**, 58.
- Bird LJ, Kuenen JG, Osburn MR, Tomioka N, Ishii SI, Barr C, Neelson KH and Suzuki S (2021) *Serpentinimonas* gen. nov., *Serpentinimonas raichei* sp. nov., *Serpentinimonas barnesii* sp. nov. and *Serpentinimonas maccrorryi* sp. nov., hyperalkaliphilic and facultative autotrophic bacteria isolated from terrestrial serpentinizing springs. *International Journal of Systematic and Evolutionary Microbiology* **71**.
- Bresan S, Sznajder A, Hauf W, Forchhammer K, Pfeiffer D and Jendrosseck D (2016) Polyhydroxyalkanoate (PHA) granules have no phospholipids. *Scientific Reports* **6**, 26612.
- Bugnicourt E, Cinelli P, Lazzeri A and Alvarez V (2014) Polyhydroxyalkanoate (PHA): review of synthesis, characteristics, processing and potential applications in packaging. *EXPRESS Polymer Letters* **8**, 791–808.
- Carlson RW, Johnson RE and Anderson MS (1999) Sulfuric acid on Europa and the radiolytic sulfur cycle. *Science* **286**, 97–99.
- Ciesielski S, Górnica D, Możejko J, Świątecki A, Grzesiak J and Zdanowski M (2014) The diversity of bacteria isolated from Antarctic freshwater reservoirs possessing the ability to produce polyhydroxyalkanoates. *Current Microbiology* **69**, 594–603.
- Cockell CS, Schwendner P, Perras A, Rettberg P, Beblo-Vranesevic K, Bohmeier M, Rabbow E, Moissl-Eichinger C, Wink L, Marteinson V, Vannier P, Gomez F, Garcia-Descalzo L, Ehrenfreund P, Monaghan EP, Westall F, Gaboyer F, Amils R, Malki M, Pukall R, Cabezas P and Walter N (2018) Anaerobic microorganisms in astrobiological analogue environments: from field site to culture collection. *International Journal of Astrobiology* **17**, 314–328.

- Deamer D and Damer B (2017) Can life begin on Enceladus? A perspective from hydrothermal chemistry. *Astrobiology* **17**, 834–839.
- de Vogel FA, Schlundt C, Stote RE, Ratto JA and Amaral-Zettler LA (2021) Comparative genomics of marine bacteria from a historically defined plastic biodegradation consortium with the capacity to biodegrade polyhydroxyalkanoates. *Microorganisms* [Online] **9**.
- Ehlmann BL, Mustard JF and Murchie SL (2010) Geologic setting of serpentine deposits on Mars. *Geophysical Research Letters* **37**.
- Eigenbrode JL, Summons RE, Steele A, Freissinet C, Millan M, Navarro-González R, Sutter B, Mcadam AC, Franz HB, Glavin DP, Archer PD, Mahaffy PR, Conrad PG, Hurowitz JA, Grotzinger JP, Gupta S, Ming DW, Sumner DY, Szopa C, Malespin C, Buch A and Coll P (2018) Organic matter preserved in 3-billion-year-old mudstones at Gale crater, Mars. *Science* **360**, 1096–1101.
- Fairén AG, Gómez-Elvira J, Briones C, Prieto-Ballesteros O, Rodríguez-Manfredi JA, López Heredero R, Belenguer T, Moral AG, Moreno-Paz M and Parro V (2020) The complex molecules detector (CMOLD): a fluidic-based instrument suite to search for (bio)chemical complexity on Mars and icy moons. *Astrobiology* **20**, 1076–1096.
- Farley KA, Williford KH, Stack KM, Bhartia R, Chen A, De la Torre M, Hand K, Goreva Y, Herd CDK, Hueso R, Liu Y, Maki JN, Martinez G, Moeller RC, Nelessen A, Newman CE, Nunes D, Ponce A, Spanovich N, Willis PA, Beegle LW, Bell JF, Brown AJ, Hamran S-E, Hurowitz JA, Maurice S, Paige DA, Rodriguez-Manfredi JA, Schulte M and Wiens RC (2020) Mars 2020 mission overview. *Space Science Reviews* **216**, 142.
- Farmer JD and Des Marais DJ (1999) Exploring for a record of ancient Martian life. *Journal of Geophysical Research: Planets* **104**, 26977–26995.
- Flüchter S, Follonier S, Schiel-Bengelsdorf B, Bengelsdorf FR, Zinn M and Dürre P (2019) Anaerobic production of poly(3-hydroxybutyrate) and its precursor 3-hydroxybutyrate from synthesis gas by autotrophic clostridia. *Biomacromolecules* **20**, 3271–3282.
- Gambarini V, Pantos O, Kingsbury JM, Weaver L, Handley KM and Lear G (2022) PlasticDB: a database of microorganisms and proteins linked to plastic biodegradation. *Database* **2022**.
- Gaucher EA, Govindarajan S and Ganesh OK (2008) Palaeotemperature trend for Precambrian life inferred from resurrected proteins. *Nature* **451**, 704–707.
- Giulio MD (2003) The universal ancestor was a thermophile or a hyperthermophile: tests and further evidence. *Journal of Theoretical Biology* **221**, 425–436.
- Glein CR, Baross JA and Waite JH (2015) The pH of Enceladus' ocean. *Geochimica et Cosmochimica Acta* **162**, 202–219.
- Godbole S (2016) Methods for identification, quantification and characterization of polyhydroxyalkanoates – a review. *International Journal of Bioassays* **5**.
- Greaves JS, Richards AMS, Bains W, Rimmer PB, Sagawa H, Clements DL, Seager S, Petkowski JJ, Sousa-Silva C, Ranjan S, Drabek-Maunder E, Fraser HJ, Cartwright A, Mueller-Wodarg I, Zhan Z, Friberg P, Coulson I, Lee EL and Hoge J (2020) Phosphine gas in the cloud decks of Venus. *Nature Astronomy* **5**, 655–664.
- Greaves JS, Richards AMS, Bains W, Rimmer PB, Clements DL, Seager S, Petkowski JJ, Sousa-Silva C, Ranjan S and Fraser HJ (2021a) Reply to: No evidence of phosphine in the atmosphere of Venus from independent analyses. *Nature Astronomy* **5**, 636–639.
- Greaves JS, Richards AMS, Bains W, Rimmer PB, Sagawa H, Clements DL, Seager S, Petkowski JJ, Sousa-Silva C, Ranjan S, Drabek-Maunder E, Fraser HJ, Cartwright A, Mueller-Wodarg I, Zhan Z, Friberg P, Coulson I, Lee EL and Hoge J (2021b) Addendum: Phosphine gas in the cloud deck of Venus. *Nature Astronomy* **5**, 726–728.
- Haltigin T, Hauber E, Kminek G, Meyer MA, Agee CB, Busemann H, Carrier BL, Glavin DP, Hays LE, Marty B, Pratt LM, Udry A, Zorzano M-P, Beaty DW, Cavalazzi B, Cockell CS, Debaille V, Grady MM, Hutzler A, Mccubbin FM, Regberg AB, Smith AL, Smith CL, Summons RE, Swindle TD, Tait KT, Tosca NJ, Usui T, Velbel MA, Wadhwa M and Westall F (2021) Rationale and proposed design for a mars sample return (MSR) science program. *Astrobiology* **22**, S-27–S-56.
- Hand KP, Sotin C, Hayes A and Coustenis A (2020) On the habitability and future exploration of ocean worlds. *Space Science Reviews* **216**, 95.
- Hong K, Sun S, Tian W, Chen GQ and Huang W (1999) A rapid method for detecting bacterial polyhydroxyalkanoates in intact cells by Fourier transform infrared spectroscopy. *Applied Microbiology and Biotechnology* **51**, 523–526.
- Howell SM and Pappalardo RT (2020) NASA's Europa Clipper – a mission to a potentially habitable ocean world. *Nature Communications* **11**, 1311.
- Hynek BM, Rogers KL, Antunovich M, Avarado G and Alvarado GE (2018) Lack of microbial diversity in an extreme Mars analog setting: Poás Volcano, Costa Rica. *Astrobiology* **18**, 923–933.
- Izenberg NR, Gentry DM, Smith DJ, Gilmore MS, Grinspoon DH, Bullock MA, Boston PJ and Słowik GP (2021) The Venus life equation. *Astrobiology* **21**, 1305–1315.
- Jendrossek D, Selchow O and Hoppert M (2007) Poly(3-hydroxybutyrate) granules at the early stages of formation are localized close to the cytoplasmic membrane in *Caryophanon latum*. *Applied and Environmental Microbiology* **73**, 586–593.
- Kaing V, Guo Z, Sok T, Kodikara D, Breider F and Yoshimura C (2024) Photodegradation of biodegradable plastics in aquatic environments: current understanding and challenges. *Science of the Total Environment* **911**, 168539.

- Khan Shams T, Horiba Y, Yamamoto M and Hiraishi A (2002) Members of the family Comamonadaceae as primary poly(3-hydroxybutyrate-co-3-hydroxyvalerate)-degrading denitrifiers in activated sludge as revealed by a polyphasic approach. *Applied and Environmental Microbiology* **68**, 3206–3214.
- Kim DY, Kim HW, Chung MG and Rhee YH (2007) Biosynthesis, modification, and biodegradation of bacterial medium-chain-length polyhydroxyalkanoates. *Journal of Microbiology* **45**, 87–97.
- Klein HP (1979a) The Viking mission and the search for life on Mars. *Reviews of Geophysics* **17**, 1655–1662.
- Klein HP (1979b) Simulation of the Viking biology experiments: an overview. *Journal of Molecular Evolution* **14**, 161–165.
- Koller M and Rodríguez-Contreras A (2015) Techniques for tracing PHA-producing organisms and for qualitative and quantitative analysis of intra- and extracellular PHA. *Engineering in Life Sciences* **15**, 558–581.
- Korablev OI, Dobrolensky Y, Evdokimova N, Fedorova AA, Kuzmin RO, Mantsevich SN, Cloutis EA, Carter J, Poulet F, Flahaut J, Griffiths A, Gunn M, Schmitz N, Martín-Torres J, Zorzano M-P, Rodionov DS, Vago JL, Stepanov AV, Titov AY, Vyazovetsky NA, Trokhimovskiy AY, Sapgir AG, Kalinnikov YK, Ivanov YS, Shapkin AA and Ivanov AY (2017) Infrared spectrometer for ExoMars: a mast-mounted instrument for the rover. *Astrobiology* **17**, 542–564.
- Levin GV and Straat PA (2016) The case for extant life on Mars and its possible detection by the Viking labeled release experiment. *Astrobiology* **16**, 798–810.
- Li L, Liu Z, Zhang M, Meng D, Liu X, Wang P, Li X, Jiang Z, Zhong S, Jiang C and Yin H (2020) Insights into the metabolism and evolution of the genus *Acidiphilium*, a typical acidophile in acid mine drainage. *mSystems* **5**. doi: 10.1128/mSystems.00867-20.
- Liu L-Y, Xie G-J, Xing D-F, Liu B-F, Ding J and Ren N-Q (2020) Biological conversion of methane to polyhydroxyalkanoates: current advances, challenges, and perspectives. *Environmental Science and Ecotechnology* **2**, 100029.
- Maestro B and Sanz JM (2017) Polyhydroxyalkanoate-associated phasins as phylogenetically heterogeneous, multipurpose proteins. *Microbial Biotechnology* **10**, 1323–1337.
- Mahaffy PR, Webster CR, Cabane M, Conrad PG, Coll P, Atreya SK, Arvey R, Barciniak M, Benna M, Bleacher L, Brinckerhoff WB, Eigenbrode JL, Carignan D, Cascia M, Chalmers RA, Dworkin JP, Errigo T, Everson P, Franz H, Farley R, Feng S, Frazier G, Freissinet C, Glavin DP, Harpold DN, Hawk D, Holmes V, Johnson CS, Jones A, Jordan P, Kellogg J, Lewis J, Lyness E, Malespin CA, Martin DK, Maurer J, Mcadam AC, McLennan D, Nolan TJ, Noriega M, Pavlov AA, Prats B, Raaen E, Sheinman O, Sheppard D, Smith J, Stern JC, Tan F, Trainer M, Ming DW, Morris RV, Jones J, Gundersen C, Steele A, Wray J, Botta O, Leshin LA, Owen T, Battel S, Jakosky BM, Manning H, Squyres S, Navarro-González R, McKay CP, Raulin F, Sternberg R, Buch A, Sorensen P, Kline-Schoder R, Coscia D, Szopa C, Teinturier S, Baffes C, Feldman J, Flesch G, Forouhar S, Garcia R, Keymeulen D, Woodward S, Block BP, Arnett K, Miller R, Edmonson C, Gorevan S and Mumm E (2012) The sample analysis at Mars investigation and instrument suite. *Space Science Reviews* **170**, 401–478.
- McEwen AS, Schaefer EI, Dundas CM, Sutton SS, Tamppari LK and Chojnacki M (2021) Mars: abundant recurring slope lineae (RSL) following the planet-encircling dust event (PEDE) of 2018. *Journal of Geophysical Research: Planets* **126**, e2020JE006575.
- McKay CP (2016) Titan as the abode of life. *Life* [Online] **6**.
- McKay CP and Smith HD (2005) Possibilities for methanogenic life in liquid methane on the surface of Titan. *Icarus* **178**, 274–276.
- Merino N, Aronson HS, Bojanova DP, Feyhl-Buska J, Wong ML, Zhang S and Giovannelli D (2019) Living at the extremes: extremophiles and the limits of life in a planetary context. *Frontiers in Microbiology* **10**.
- Moon J, Li M, Ramirez-Cuesta AJ and Wu Z (2023) Raman spectroscopy. In Wachs IE and Bañares MA (eds). *Springer Handbook of Advanced Catalyst Characterization*. Cham: Springer International Publishing, pp. 75–110.
- Nisbet EG and Sleep NH (2001) The habitat and nature of early life. *Nature* **409**, 1083–1091.
- Noreen A, Sultana S, Sultana T, Tabasum S, Zia KM, Muzammil Z, Jabeen M, Lodhi AZ and Sultana S (2020) Chapter 3 – Natural polymers as constituents of bionanocomposites. In Mahmood Zia K, Jabeen F, Anjum MN and Ikram S (eds). *Bionanocomposites*. Amsterdam: Elsevier, pp. 55–85.
- Obulisamy PK and Mehariya S (2021) Polyhydroxyalkanoates from extremophiles: a review. *Bioresource Technology* **325**, 124653.
- Palleroni NJ and Palleroni AV (1978) *Alcaligenes latus*, a new species of hydrogen-utilizing bacteria. *International Journal of Systematic and Evolutionary Microbiology* **28**, 416–424.
- Parro V, De Diego-Castilla G, Rodríguez-Manfredi JA, Rivas LA, Blanco-López Y, Sebastián E, Romeral J, Compostizo C, Herrero PL, García-Marín A, Moreno-Paz M, García-Villadangos M, Cruz-Gil P, Peinado V, Martín-Soler J, Pérez-Mercader J and Gómez-Elvira J (2011) SOLID3: a multiplex antibody microarray-based optical sensor instrument for *in situ* life detection in planetary exploration. *Astrobiology* **11**, 15–28.
- Postberg F, Sekine Y, Klenner F, Glein CR, Zou Z, Abel B, Furuya K, Hillier JK, Khawaja N, Kempf S, Noelle L, Saito T, Schmidt J, Shibuya T, Srama R and Tan S (2023) Detection of phosphates originating from Enceladus's ocean. *Nature* **618**, 489–493.
- Quantin-Nataf C, Carter J, Mandon L, Thollot P, Balme M, Volat M, Pan L, Loizeau D, Millot C, Breton S, Dehouck E, Fawdon P, Gupta S, Davis J, Grindrod PM, Pacifici A, Bultel B, Allemand P, Ody A, Lozach L and Broyer J (2021) Oxia Planum: the landing site for the ExoMars 'Rosalind Franklin' rover mission: geological context and prelanding interpretation. *Astrobiology* **21**, 345–366.

- Raza ZA, Abid S and Banat IM (2018) Polyhydroxyalkanoates: characteristics, production, recent developments and applications. *International Biodeterioration & Biodegradation* **126**, 45–56.
- Rehm BHA (2010) Bacterial polymers: biosynthesis, modifications and applications. *Nature Reviews Microbiology* **8**, 578–592.
- Rodge SP, Shende KS and Patil NP (2023) Polyhydroxyalkanoate biosynthesis and optimisation of thermophilic *Geobacillus stearothermophilus* strain K4E3_SPR_NPP. *Extremophiles* **27**, 13.
- Russell MJ, Hall AJ and Martin W (2010) Serpentinization as a source of energy at the origin of life. *Geobiology* **8**, 355–371.
- Sadi RK, Fechine GJM and Demarquette NR (2010) Photodegradation of poly(3-hydroxybutyrate). *Polymer Degradation and Stability* **95**, 2318–2327.
- Samek O, Obruča S, Šiler M, Sedláček P, Benešová P, Kučera D, Márova I, Ježek J, Bernatová S and Zemánek P (2016) Quantitative Raman spectroscopy analysis of polyhydroxyalkanoates produced by *Cupriavidus necator* H16. *Sensors* [Online] **16**.
- Samrot AV, Samanvitha SK, Shobana N, Remitta ER, Senthilkumar P, Kumar SS, Abirami S, Dhiva S, Bavanilatha M, Prakash P, Saigeetha S, Shree KS and Thirumurugan R (2021) The synthesis, characterization and applications of polyhydroxyalkanoates (PHAs) and PHA-based nanoparticles. *Polymers* **13**, 3302.
- Santhanam A and Sasidharan S (2010) Microbial production of polyhydroxy alkanotes (PHA) from *Alcaligenes* spp. and *Pseudomonas oleovorans* using different carbon sources. *African Journal of Biotechnology* **9**.
- Schmidt ME, Ruff SW, McCoy TJ, Farrand WH, Johnson JR, Gellert R, Ming DW, Morris RV, Cabrol N, Lewis KW and Schroeder C (2008) Hydrothermal origin of halogens at Home Plate, Gusev crater. *Journal of Geophysical Research: Planets* **113**.
- Seto M, Noguchi K and Cappellen PV (2019) Potential for aerobic methanotrophic metabolism on Mars. *Astrobiology* **19**, 1187–1195.
- Sharma S, Roppel RD, Murphy AE, Beegle LW, Bhartia R, Steele A, Hollis JR, Siljeström S, Mccubbin FM, Asher SA, Abbey WJ, Allwood AC, Berger EL, Bleefeld BL, Burton AS, Bykov SV, Cardarelli EL, Conrad PG, Corpolongo A, Czaja AD, Deflores LP, Edgett K, Farley KA, Fornaro T, Fox AC, Fries MD, Harker D, Hickman-Lewis K, Huggett J, Imbeah S, Jakubek RS, Kah LC, Lee C, Liu Y, Magee A, Minitti M, Moore KR, Pascuzzo A, Rodriguez Sanchez-Vahamonde C, Scheller EL, Shkolyar S, Stack KM, Steadman K, Tuite M, Uckert K, Werynski A, Wiens RC, Williams AJ, Winchell K, Kennedy MR and Yanchilina A (2023) Diverse organic-mineral associations in Jezero crater, Mars. *Nature* **619**, 724–732.
- Simon JJ, Hickman-Lewis K, Cohen BA, Mayhew LE, Shuster DL, Debaille V, Hausrath EM, Weiss BP, Bosak T, Zorzano MP, Amundsen HEF, Beegle LW, Bell III JF, Benison KC, Berger EL, Beyssac O, Brown AJ, Calef F, Casademont TM, Clark B, Clavé E, Crumpler L, Czaja AD, Fairén AG, Farley KA, Flannery DT, Fornaro T, Forni O, Gómez F, Goreva Y, Gorin A, Hand KP, Hamran SE, Henneke J, Herd CDK, Horgan BHN, Johnson JR, Joseph J, Kronyak RE, Madariaga JM, Maki JN, Mandon L, Mccubbin FM, McLennan SM, Moeller RC, Newman CE, Núñez JJ, Pascuzzo AC, Pedersen DA, Poggiali G, Pinet P, Quantin-Nataf C, Rice M, Rice Jr JW, Royer C, Schmidt M, Sephton M, Sharma S, Siljeström S, Stack KM, Steele A, Sun VZ, Udry A, Vanbommel S, Wadhwa M, Wiens RC, Williams AJ and Williford KH (2023) Samples collected from the floor of Jezero crater with the Mars 2020 Perseverance rover. *Journal of Geophysical Research: Planets* **128**, e2022JE007474.
- Simoneit BRT (2002) Molecular indicators (biomarkers) of past life. *The Anatomical Record* **268**, 186–195.
- Sollich M, Yoshinaga MY, Häusler S, Price RE, Hinrichs K-U and Bühring SI (2017) Heat stress dictates microbial lipid composition along a thermal gradient in marine sediments. *Frontiers in Microbiology* **8**.
- Stuart B (2015) Infrared Spectroscopy. Kirk-Othmer Encyclopedia of Chemical Technology.
- Tan G-YA, Chen C-L, Li L, Ge L, Wang L, Razaad IM, Li Y, Zhao L, Mo Y and Wang J-Y (2014a) Start a research on biopolymer polyhydroxyalkanoate (PHA): a review. *Polymers* [Online] **6**.
- Tan D, Wu Q, Chen J-C and Chen G-Q (2014b) Engineering *Halomonas* TD01 for the low-cost production of polyhydroxyalkanoates. *Metabolic Engineering* **26**, 34–47.
- Tan G-YA, Chen C-L, Ge L, Li L, Wang L, Zhao L, Mo Y, Tan SN and Wang J-Y (2014c) Enhanced gas chromatography-mass spectrometry method for bacterial polyhydroxyalkanoates analysis. *Journal of Bioscience and Bioengineering* **117**, 379–382.
- Tan G-YA, Ge LG, Pan C, Ngim Tan S and Wang J-Y (2016) Current and emerging advanced analytical technologies for biopolyesters characterization. In Koller M (ed). *Recent Advances in Biotechnology Microbial Biopolyester Production, Performance and Processing Bioengineering, Characterization, and Sustainability*. Sharjah, United Arab Emirates: Bentham Science Publisher, pp. 303–402.
- Tarazona NA, Machatschek R and Lendlein A (2020) Unraveling the interplay between abiotic hydrolytic degradation and crystallization of bacterial polyesters comprising short and medium side-chain-length polyhydroxyalkanoates. *Biomacromolecules* **21**, 761–771.
- Terentiev Y, Breuer U, Babel W and Kunze G (2004) Non-conventional yeasts as producers of polyhydroxyalkanoates – genetic engineering of *Arxula adenivorans*. *Applied Microbiology and Biotechnology* **64**, 376–381.
- Tian J, He A, Lawrence Adam G, Liu P, Watson N, Sinskey Anthony J and Stubbe J (2005) Analysis of transient polyhydroxybutyrate production in *Wautersia eutropha* H16 by quantitative western analysis and transmission electron microscopy. *Journal of Bacteriology* **187**, 3825–3832.
- Trainer MG, Brinckerhoff WB, Freissinet C, Lawrence DJ, Peplowski PN, Parson AM, Zacny K, Turtle EP, Barnes JW, Lorenz RD, Hörst SM, Stickle AM and Team D (2018) Dragonfly: investigating the surface composition of titan. 49th Lunar and Planetary Science Conference. Woodlands, TX, USA: Lunar and Planetary Institute.

- Tribelli PM and López NI (2011) Poly(3-hydroxybutyrate) influences biofilm formation and motility in the novel Antarctic species *Pseudomonas extremaustralis* under cold conditions. *Extremophiles* **15**, 541.
- Tribelli PM, Pezzoni M, Brito MG, Montesinos NV, Costa CS and López NI (2020) Response to lethal UVA radiation in the Antarctic bacterium *Pseudomonas extremaustralis*: polyhydroxybutyrate and cold adaptation as protective factors. *Extremophiles* **24**, 265–275.
- Trivedi Christopher B, Stamps Blake W, Lau Graham E, Grasby Stephen E, Templeton Alexis S and Spear John R (2020) Microbial metabolic redundancy is a key mechanism in a sulfur-rich glacial ecosystem. *mSystems* **5**. doi: 10.1128/mSystems.00504-20.
- Urtuvia V, Villegas P, González M and Seeger M (2014) Bacterial production of the biodegradable plastics polyhydroxyalkanoates. *International Journal of Biological Macromolecules* **70**, 208–213.
- Vance SD and Melwani Daswani M (2020) Serpentinite and the search for life beyond Earth. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **378**, 20180421.
- Veneranda M, Lopez-Reyes G, Manrique-Martinez JA, Sanz-Arranz A, Lalla E, Konstantinidis M, Moral A, Medina J and Rull F (2020) ExoMars Raman laser spectrometer (RLS): development of chemometric tools to classify ultramafic igneous rocks on Mars. *Scientific Reports* **10**, 16954.
- Vicente D, Proença DN and Morais PV (2023) The role of bacterial polyhydroxyalkanoate (PHA) in a sustainable future: a review on the biological diversity. *International Journal of Environmental Research and Public Health* **20**, 2959.
- Villanueva GL, Cordiner M, Irwin PGJ, De Pater I, Butler B, Gurwell M, Milam SN, Nixon CA, Luszcz-Cook SH, Wilson CF, Kofman V, Liuzzi G, Faggi S, Faucher TJ, Lippi M, Cosentino R, Thelen AE, Moullet A, Hartogh P, Molter EM, Charnley S, Arney GN, Mandell AM, Biver N, Vandaele AC, de Kleer KR and Kopparapu R (2021) No evidence of phosphine in the atmosphere of Venus from independent analyses. *Nature Astronomy* **5**, 631–635.
- Wahl A, Schuth N, Pfeiffer D, Nussberger S and Jendrosseck D (2012) PHB granules are attached to the nucleoid via PhaM in *Ralstonia eutropha*. *BMC Microbiology* **12**, 262.
- Wang JL, Dragone NB, Avard G and Hynek BM (2022a) Microbial survival in an extreme Martian analog ecosystem: Poás Volcano, Costa Rica. *Frontiers in Astronomy and Space Sciences* **9**.
- Wang L, Ge J, Feng L, Liu Y, Li Y, Wang J, Xiao X and Zhang Z (2022b) The synergism between methanogens and methanotrophs and the nature of their contributions to the seasonal variation of methane fluxes in a wetland: the case of Dajiuhu sub-alpine peatland. *Advances in Atmospheric Sciences* **39**, 1375–1385.
- Ward Patrick G, De Roo G and O'Connor Kevin E (2005) Accumulation of polyhydroxyalkanoate from styrene and phenylacetic acid by *Pseudomonas putida* CA-3. *Applied and Environmental Microbiology* **71**, 2046–2052.
- Webster CR, Mahaffy PR, Atreya SK, Moores JE, Flesch GJ, Malespin C, McKay CP, Martinez G, Smith CL, Martin-Torres J, Gomez-Elvira J, Zorzano M-P, Wong MH, Trainer MG, Steele A, Archer D, Sutter B, Coll PJ, Freissinet C, Meslin P-Y, Gough RV, House CH, Pavlov A, Eigenbrode JL, Glavin DP, Pearson JC, Keymeulen D, Christensen LE, Schwenzer SP, Navarro-Gonzalez R, Pla-García J, Rafkin SCR, Vicente-Retortillo Á, Kahanpää H, Viudez-Moreiras D, Smith MD, Harri A-M, Genzer M, Hassler DM, Lemmon M, Crisp J, Sander SP, Zurek RW and Vasavada AR (2018) Background levels of methane in Mars' atmosphere show strong seasonal variations. *Science* **360**, 1093–1096.
- Xiao N and Jiao N (2011) Formation of polyhydroxyalkanoate in aerobic anoxygenic phototrophic bacteria and its relationship to carbon source and light availability. *Applied and Environmental Microbiology* **77**, 7445–7450.
- Yung YL, Chen P, Neelson K, Atreya S, Beckett P, Blank JG, Ehlmann B, Eiler J, Etiopie G, Ferry JG, Forget F, Gao P, Hu R, Kleinböhl A, Klusman R, Lefèvre F, Miller C, Mischna M, Mumma M, Newman S, Oehler D, Okumura M, Oremland R, Orphan V, Popa R, Russell M, Shen L, Sherwood Lollar B, Staehle R, Stamenković V, Stolper D, Templeton A, Vandaele AC, Viscardi S, Webster CR, Wennberg PO, Wong ML and Worden J (2018) Methane on Mars and habitability: challenges and responses. *Astrobiology* **18**, 1221–1242.
- Zhang X, Liu X-Y, Yang H, Chen J-N, Lin Y, Han S-Y, Cao Q, Zeng H-S and Ye J-W (2022) A polyhydroxyalkanoates-based carrier platform of bioactive substances for therapeutic applications. *Frontiers in Bioengineering and Biotechnology* **9**.