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Extracellular polymeric substances in Antarctic environments: A review of their ecological roles and impact on glacier biogeochemical cycles

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Abstract
Antarctic continent comprises diverse ecosystems like snow, glacier ice, sea ice, melt pools, glacial soils, supraglacial and subglacial lakes that accommodate numerous microbial and algal communities. These biotic groups secrete matrices of biomacromolecules called Extracellular Polymeric Substances (EPSs), which enhance their ability to withstand extreme environmental conditions. EPSs are mainly composed of exopolysaccharides, proteins, lipids and nucleic acids, and exhibit assorted activities due to diverse functionalities of matrix like spatial conformation, molecular weight, rheology, charge, size and affinity. Several protective and ecological functions such as cryoprotection, anti-desiccation, buffering against high salinity and pH, trace metal uptake and binding, sequestration of dissolved organic matter and nutrients, aggregate formation and biofilm production have been attributed to microbial EPSs. They also contribute to production of dissolved and particulate organic matter and hence can influence biogeochemical cycling of elements, especially carbon and iron. Further, they aid in aggregation of dust particles on the glacier surface, substantially reducing the albedo of the ice and thereby accelerate melting of glaciers. This study provides an overview of role of EPS in Antarctic ecosystems, its ecological significance and chemical functionalities, and discusses the approach for a better understanding of relevance of EPS in biogeochemistry of Antarctic environments.

Keywords: EPS, exopolysaccharides, functionalities, dissolved organic matter, cryoconite,

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Abbreviations

CHO: carbohydrates; DFe: dissolved iron; DNA: deoxyribonucleic acid; DOC: dissolved organic carbon; DOM: dissolved organic matter; EPS(s): Extracellular Polymeric Substance(s); ExP(s): exopolysaccharide(s); IR: infrared; nanoSIMS: nanoscale secondary ion mass spectrometry; NMR: nuclear magnetic resonance; PFe: particulate iron; POC: particulate organic carbon; POM: particulate organic matter; TEP: transparent exopolymer particles; $T_g$: glass transition temperature; UV: ultraviolet; UVA: ultraviolet region A; UVB: ultraviolet region B.

1. Introduction

The cryosphere including its diverse components such as snow, glacier ice, sea ice, soils, fresh water, brackish and saline lakes; supraglacial and subglacial lakes are now considered a unique biome that are largely dominated by microorganisms, whose activity can impact biogeochemical processes on local and global scales (Anesio and Laybourn-Parry, 2012). The glacier biome occupies a significant portion of the Earth’s surface area, the majority of which comprises the Antarctic continent (Kennicutt et al., 2014). Among the various cryospheric components, snow and ice have the largest areal extent and blankets a major portion of the Antarctic continent (Goodison et al., 1999). The characteristics of these icy habitats can vary both temporally and spatially. For example, snow can change from white in the dry winter to different hues of green, brown, orange, purple-grey, pink, or red in summer (wet conditions) due to the growth of pigmented snow algae (Hoham and Remias, 2020). The ice surface can also change from white to a darker shade depending on the amount of organic and inorganic impurities that pockmark its surface (Wientjes et al., 2011). These mineral
deposits together with the organic impurities generated autochthonously on site or supplied allochthonously (Antony et al., 2014; Hu et al., 2013) can fuel the growth of cyanobacteria, algae, and heterotrophic bacteria (Anesio et al., 2017). Cyanobacteria are dominant and often the primary colonizers in several Antarctic ecosystems (Vincent, 2000). They are prolific producers of extracellular polymeric substances (EPS), which are matrices of exopolysaccharides (high molecular weight polymers composed of neutral and acidic monosaccharide residues), proteins, lipids, nucleic acids, extracellular enzymes and other secondary metabolites secreted by microbes (Decho and Gutierrez, 2017). The term EPS is used interchangeably in literature to denote both extracellular polymeric substances and its exopolysaccharide component. However, exopolysaccharides are distinct from extracellular polymeric substances as they represent one of the principal components of the later. To avoid confusion, in this paper, we use ‘EPS’ for extracellular polymeric substances and ‘ExP’ for exopolysaccharides. The sticky EPS extruded by cyanobacteria acts as a glue bundling together the inorganic and organic impurities on the ice surface (Cook et al., 2016). This biological darkening of the ice surface together with the presence of dark impurities, hasten the melting of the ice by reducing the albedo of the ice surface (Di Mauro et al., 2017; Takeuchi et al., 2001). The EPS enabled biological binding of organic matter and dust on the ice surface also provide a stable microhabitat for other microbes to colonize (Cook et al., 2016). These microbes benefit from the carbon and nitrogen fixed by the cyanobacteria on the outer surface of granules and that supplied by dead and degraded cyanobacteria in the granules’ interior (Segawa et al., 2020). The EPS itself can serve as a food source for bacteria (Decho and Gutierrez, 2017). Heterotrophic bacteria use the organic matter and generate inorganic nutrients specially $\text{NO}_3^-$ and $\text{NO}_2^-$ that are assimilated by cyanobacteria and support
specialist microbes like nitrifiers and denitrifiers (Segawa et al., 2020). Some of the colonizing bacteria and yeasts themselves produce EPS (Langford et al., 2010; Sanyal et al., 2020). EPS is generally produced by microbes in response to surrounding environmental stress and/or as metabolic by-product (Decho and Gutierrez, 2017) and in glacial habitats may serve a number of different purposes that assist microbes in survival under the harsh conditions typical of these environments. For example, EPS acts as a cryoprotectant preventing the disruption of cell membranes by freezing (Caruso et al., 2019; Madigan et al., 2017); it helps to retain water preventing cells from undergoing desiccation (Krembs et al., 2002) in the dry Antarctic climate; it can buffer cells against high salinity and pH conditions (Nichols et al., 2004) such as those experienced in Antarctic hypersaline lakes (Williams et al., 2014); contribute to the sequestration of metal ions, dissolved organic matter (DOM) and nutrients (Nichols et al., 2005a); shield microbes against antimicrobial compounds (Parrilli et al., 2019), and help cells adhere to surfaces (Decho and Gutierrez, 2017).

Although EPS is fundamentally involved in survival and adaptive strategies of microbes, their role goes beyond just shielding and sustaining microbes under harsh environmental conditions. They also contribute significantly to crucial ecological processes. For example, EPS due to its slimy nature and surface ionic charges, easily attach to sediment particles, holding them together (Chenu, 1995), playing a key role in sediment stabilization in the environment (Gerbersdorf et al., 2009). On the glacier surface, the ability to glue together mineral debris (cryoconite) is one of the key aspects that promotes the aggregation and formation of dark cryoconite granules on the glacier surface that have implications for surface albedo and melting of the glacier surface (Fountain et al., 2004). They play an important role in the formation of biofilms on glacier surfaces, promoting efficient transfer
and cycling of nutrients (Smith et al., 2016). They also contribute to mineral precipitation (Norman et al., 2015; Or et al., 2007), production of organic matter (Nichols et al., 2005a), and biogeochemical cycling of elements (Bhaskar and Bhosle, 2005; Janssens et al., 2018; van der Merwe et al., 2009) in the environment. EPS is not only produced by cyanobacteria and heterotrophs, but also consumed and re-assimilated by them (Stuart et al., 2016), thus fuelling the microbial web (Almela et al., 2019). More crucially, it provides a stable environment where cells carry out genetic exchange (Liao et al., 2016), and cooperative/antagonistic interactions that are difficult to accomplish efficiently by free-living cells (Decho and Gutierrez, 2017). Not surprisingly, EPS is produced by a range of organisms including bacteria (Antony et al., 2012; Nichols et al., 2005a), cyanobacteria (De Los Ríos et al., 2004; Wynn-Williams and Edwards, 2000), microalgae (Aslam et al., 2012), yeasts (Sanyal et al., 2020), and fungi (Selbmann et al., 2002) in diverse Antarctic habitats. Besides the diversity of functions, EPSs vary immensely in their composition and structures (Giudice and Poli, 2020). Most of the protective and ecological advantages that EPS offers are attributed to the specific physical and chemical properties of the EPS (Nichols et al., 2005a). However, as EPSs occur in a range of molecular sizes, conformations and physical/chemical properties, their structure-function relationships are often difficult to ascertain. Most reviews, therefore deal with EPS properties, ecological roles and applications, with limited focus on structure-property relationships of microbial EPSs (Giudice and Poli, 2020; Jadhav and West, 2017; Verdugo, 2012). The aim of this study is to collate the majority of research pertaining to chemical functionalities of exopolysaccharides viz-a-viz its relationships with EPS properties and evaluate the role of EPS in Antarctic ecosystems and its influence on broader biogeochemical processes in the cryosphere. This
study also discusses approaches for a better understanding of the structure-function relationship of EPS and its relevance in Antarctic environments.

2. Microbial EPS in Polar Regions

Under extreme environmental conditions, microbes try to stabilize their external environment by producing EPS either as capsules surrounding individual cells or as a larger matrix aggregating many microbial cells together (Figure 1).

The EPS matrix enables microbial communities to attach to living or inert surfaces in aquatic environments forming biofilms, facilitating the interaction of bacteria with the surrounding environment. EPS producing microbes are widespread in the Antarctic environment and include photoautotrophic cyanobacteria, heterotrophic bacteria, diatoms, and microalgae, inhabiting various environments like glacier ice, sea ice, snow, lakes, soils, cryoconite holes and melt pools on the glacier surface (Anesio et al., 2017; Boetius et al., 2015; Cook et al., 2016; Poli et al., 2010; Sanyal et al., 2020). Microbial EPS in the natural habitat and in microbes isolated from diverse Antarctic habitats are shown in figure 2.

The EPS biosynthesis, assembly and export in microbes are controlled by several gene clusters encoding various pathways. EPS biosynthesis is initiated within the cytoplasm of the cell, wherein oligosaccharides are synthesised and assembled into repeating units before being transferred by glycosyltransferases to lipid carrier molecules located in the plasma membrane. Finally, repeating units are polymerised, assembled and transported out of the cell, following mechanisms outlined in Pereira et al. (2015). Although EPS synthesis and export mechanisms are common among cyanobacteria, it is believed that the regulation of the various EPS biosynthesis pathways could occur at different levels under the specific
environmental constraints experienced in cold environments (Chrismas et al., 2016). However, how the regulation of these genes varies between closely related cyanobacterial lineages in the cryosphere is still unknown. Also, not much is known about the genes regulating the synthesis of EPS in other cryosphere inhabiting microbes.

The EPS facilitates cell survival, improves metabolic efficiency and provides microbes housed within it, the protection from a range of physical, chemical and biological stresses, enabling cells to cope with changing stress types and levels (Ricciardelli et al., 2019). For example, microbes within microbial mats (specialised biofilms comprising many microbial groups that are laterally compressed into a thin multilayered mat that range from several millimeters to a centimeter thick) that are exposed to high levels of direct UVA (320-400 nm) and UVB (280-320 nm) radiation, produce protective pigments (George et al., 2001) to counter UV induced cell damage (Rothschild, 1999). These mats are distributed on moist soils, streams, and lake bottoms (Stal, 2012), and are mainly crafted by photosynthetic cyanobacteria, green algae and golden algae, along with diatoms comprising about 70 % of the biomass (Fernández-Valiente et al., 2007; Rochera et al., 2013). The microbial mats in Antarctic soils and shallow ponds exhibit distinct colors (orange, green, black) due to these UV protective pigments (Fernández-Valiente et al., 2007). However, cyanobacterial species within these mats exhibit different tolerance levels to UV exposure (Hennion et al., 2006). For instance, cyanobacterial mats dominated by Leptolyngbya have lower biomass due to decrease in chlorophyll a under UV-B stress, while Phormidium mats are least affected as they secrete two folds more carotenoids than Leptolyngbya. Phormidium dominated mats also produce 25-fold higher mycosporine like amino acids for UV protection compared to other cyanobacteria (George et al., 2001). EPS synthesis is also believed to play an indirect
role in limiting UV damage to the cell. The matrix acts as a warehouse, where microbes slough and deposit mycosporine and melanin pigments after being stimulated by UV radiation wavelengths (Gorbushina et al., 2003). Other Antarctic organisms such as the moss Sanionia uncinate and black fungi Cryomyces antarcticus and Cryomyces minteri employ a multi-stress resistance strategy, wherein, in addition to EPS, they also produce trehalose, glycerol, protective proteins and mannitol to enhance resistance towards UV radiation (Wong et al., 2019). In Antarctic ecosystems, EPS also acts as a cryoprotectant, enabling survival in ice by inhibiting ice crystal nucleation (Krembs et al., 2002). This is discussed in more detail in section 4.1. Further, EPS helps secure the cell attachment to ice surface and sea-ice interfaces, facilitating microbial survival in these environments (Nichols et al., 2005a).

Though EPS biosynthesis is energetically expensive, microbes produce it as a trade-off to facilitate structural stability and cellular functions at the community level. This is evident in biofilms, where secreted biopolymers not only push the daughter cells to oxygenic sites for enhanced growth and proliferation, they also outcompete their non-EPS producing neighbours by consuming localized oxygen and suffocating them (Flemming and Wingender, 2010; Xavier and Foster, 2007). EPS also acts as a scaffold for carrying out extracellular digestion, gene exchange and quorum sensing (Decho and Gutierrez, 2017). The extracellular enzymes produced by microbes housed in the biofilm can degrade its own and other microbial matrices when in a starved state, in order to access some of the extracellular organic carbon (Stuart et al., 2016; Zhang and Bishop, 2003). These processes likely dictate the bioavailability of extracellular carbon to complex microbial communities in the biofilm (Stuart et al., 2016). Gene exchange among bacteria is an important process that is facilitated within the EPS matrix due to the fact that two cells remain in close proximity for a prolonged
period of time, thereby improving the chance of conjugative gene exchange (i.e., exchange of genetic material via a pilus connecting two cells). Also, extracellular DNA immobilized within EPS provides a large and accessible gene pool increasing the chance for transformational DNA exchange (i.e., active uptake of free DNA by bacterial cells) among cells (Decho and Gutierrez, 2017). Genetic material transfer between bacteria (Rahman et al., 2015; Romaniuk et al., 2019) and archaea (Demaere et al., 2013) has been observed in the Antarctic environment. In Deep Lake, Antarctica, members of Haloarchaea perform inter-genera gene exchange between halobacterial genera such as *Halobacterium, Haloquadratum, and Haloarcula*, with broad shared regions of DNA up to 35kb (Demaere et al., 2013). The EPS matrix also facilitates quorum sensing, which is a process by which bacteria communicate through exchange of chemical signals between nearby cells to coordinate cooperative activities and physiological processes in a collective manner (Fuqua et al., 1996). Quorum sensing regulates interactions between bacteria and higher organisms, biofilm formation, virulence, bioluminescence, synthesis of enzymes, toxins, antibiotics, and other secondary metabolites (Khmel et al., 2008). Figure 3 summarises the main roles of EPS in the polar environments. While little is known about its importance and scale in cold regions, active quorum sensing genes (Williamson et al., 2005) and signalling molecules involved in bacterial quorum sensing have been identified in bacteria such as *Pseudomonas, Pseudoalteromonas* and *Planococcus* from Antarctic (Kaur et al., 2019; See-Too et al., 2018, 2017, 2016) and non-Antarctic cold habitats (Schwenteit et al., 2011).

In addition to providing protection from environmental stressors, microbial biofilms and mats influence nitrogen biogeochemical cycles through nitrogen fixation, ammonia and nitrogen uptakes (Fernández-Valiente et al., 2007). The phototrophic members within the
microbial mats produce copious amounts of EPS providing an autochthonous nutrient source for heterotrophic bacteria and invertebrates in oligotrophic Antarctic lakes (e.g. Byers Peninsula, McMurdo Dry Valley lakes) (Toro et al., 2007; Zhang et al., 2015), thereby acting as a hotspot of microbial diversity and activity. The ecological functions of EPS are elaborated in further sections of this review.

3. Ecological Roles of EPSs

The polar biosphere, especially the microbial life is in constant interaction with the lithosphere (sediment stabilization, benthic biota, biological weathering), atmosphere (CO$_2$ sequestration, N$_2$ fixation, gaseous exchange) and hydrosphere (adding dissolved organic carbon and nitrogen, affecting pH of lakes, sea ice and brine channels) through numerous biogeochemical processes involving EPS, thereby recycling and replenishing biogeochemically crucial elements at each interface. The strikingly large spatial distribution of EPS producing bacteria in Antarctica spanning diverse ecological systems that are in constant interaction with the hydrosphere and atmosphere are highlighted in figure 4.

3.1. Sediment stabilization

EPS is a major biotic factor that affects sediment dynamics and plays a significant role in sediment stabilization in soils, cryoconite holes (meltwater pits on the ice surface containing sediment at the base) and other sediment laden shallow water habitats (Cook et al., 2016; Cowan and Tow, 2004; Wood et al., 2008; Wynn-Williams and Edwards, 2000). The EPS (especially uronic acids and protein components) exuded by microbes living outside or within biofilms and microbial mats in sediment ecosystems, glue the sediment particles together and enhance their cohesion and erosion thresholds (Baldi et al., 2010; Hubas et al.,
That is, the EPS matrix produced by these microbes bind the sediment grains together very strongly preventing them from moving independently, thereby suppressing sediment transport (Malarkey et al., 2015). Even very small amounts of EPS that are ubiquitously distributed in the sediment are sufficient to produce a substantial change in sediment property and bed dynamics (Malarkey et al., 2015). Interestingly, biofilms mask the sediment in layers, wherein the top layer is a glucose-rich neutral fraction formed by phototrophy (De Winder et al., 1999), while lower layers are composed of refractory acidic EPS that binds sediment particles consistently across sublayers, that proliferates as the biofilm matures (Gerbersdorf et al., 2009). This attachment across sediment depths is what improves critical shear stress of the sediment bed and its erosion threshold. On washing of the biofilm top layer, lower sublayers endure the adhesion with individual grains and prevent further erosion (Chen et al., 2017). Sediment binding and stabilization could be attributed to electrostatic interaction between carboxylate and sulfate groups of EPS and mineral cations (mainly Ca$^{2+}$ ions) of sediments, that further prevents sediment calcification (Decho and Gutierrez, 2017).

The rate of binding depends upon structural attributes of EPS, i.e. carbohydrate (CHO)/protein ratio, molecular weight and degree of branching. Intriguingly, experiments have demonstrated no rheological changes in sediment structures in presence and absence of EPS (De Brouwer et al., 2002; Stal, 2003), indicating that interactions between EPS and sediment particles might be reversible physical adsorption and not chemical bonding.

In arid Antarctic habitats such as the soils of the dry valley, or ice-free landscapes, adhesion of soil particles to EPS produced by cyanobacteria and algae colonizing the topsoil result in the formation of visible biological soil crusts (biocrusts) on the top few millimetres of soil (Büdel and Colesie, 2014; Jung et al., 2018). These biocrusts are eventually colonized
by other microbial species and more complex organisms like mosses, lichens and small plants (Büdel and Colesie, 2014). In such regions, sediment binding by EPS builds the physical structure of the soil, stabilizing the soil, retaining moisture and protecting it from erosion (Büdel and Colesie, 2014). Carbon and nitrogen fixing activities within the biocrust also helps improve its fertility and contributes to the carbon reservoir of the topsoil at a landscape level (Jung et al., 2018; Mergelov et al., 2020).

Of particular relevance on the glacier surface is the contribution of EPS to the aggregation of cryoconite granules. The term cryoconite refers to discrete, aggregated granules of mineral and organic matter (living and dead microbes, microbial exudates, decomposition products and organic matter), present within cryoconite holes or elsewhere in the supraglacial zone (Cook et al., 2016). Filamentous cyanobacteria on the glacier surface weave themselves around mineral dust particles from various local and distant sources, physically binding them together. Further, they produce EPS that binds to mineral surfaces amassing the cryoconite granules into a single large aggregate (Langford et al., 2010). Other microorganisms including EPS producing non-filamentous microalgae (Hodson et al., 2010) and yeasts (Sanyal et al., 2020) have also been detected within cryoconite granules, suggesting that they play an important role in cryoconite aggregation. These EPS-rich sticky aggregates grow further, driven by growth and proliferation of unicellular photoautotrophs and heterotrophic bacteria, together with entrapment of mineral particles and organic matter within the cohesive EPS matrix. As EPS provides substantially higher surface area for binding than individual cells (Liu and Fang, 2002), it enhances their ability to retain particulates and resist entrainment into flowing meltwater. Moreover, exopolymer sheaths of cyanobacteria are to some extent believed to be recalcitrant to mineralization, enabling them
to retain the ability for cohesively binding particulates (De Winder et al., 1999). Time-lapse studies suggest that cryoconite aggregates can persist for several years, enabling microbial communities to proliferate and evolve within them (Hodson et al., 2010). But as the granules grow beyond a certain size, they disintegrate into smaller fragments, which further develop into new granules. This process can occur repeatedly over a cycle of several years on the glacier surface (Fountain et al., 2004). Also, as granules grow, the accumulation of dark humic material driven by microbial activity (Takeuchi et al., 2010; Tedesco et al., 2013), darkens the ice surface locally and accelerates melting of the surrounding ice surface (Takeuchi et al., 2010). EPS therefore not only affects the formation process of mineral–organic aggregates, but also determines its stability and longevity, thereby influencing cryoconite coverage and albedo of the glacier surface.

3.2. Carbon cycling

As discussed earlier, microbial EPSs are widely distributed in various Antarctic environments where they are found in the dissolved form, colloids, and associated with particulate matter including cell aggregates, biofilms, microbial mats, biocrusts etc. Photoautotrophic algal and cyanobacterial communities drive primary production in glacier ecosystems transforming atmospheric CO$_2$ to organic matter (including EPS). Rates of primary productivity in these ecosystems are relevant on the scale of the global carbon cycle (e.g., Anesio et al., 2010, 2009). Antarctic green algae is estimated to produce $1.3 \times 10^3$ tonnes of dry biomass that corresponds to sequestration of 479 tonnes of carbon per growth season (Gray et al., 2020). In the Antarctic environment, where decomposition rates are low, biocrusts also act as an important CO$_2$ sink continuously enriching the soil with organic carbon contributing between 7 and 17% total organic carbon in the top soil (Jung et al.,
They therefore facilitate the build up of soil organic matter at a landscape level in depauperate Antarctic soils (Mergelov et al., 2020). Some of the organic carbon fixed by the autotrophic communities is eventually utilized by heterotrophic microbes and CO$_2$ is released back into the atmosphere through community respiration (Stibal et al., 2012).

As EPSs are organic rich material, they are an important high-density carbon and energy source for heterotrophic microorganisms and filter feeders (Arrigo, 2014). Microbial secretion of dissolved EPS, conversion of dissolved EPS to particulate organic matter and enzymatic breakdown of particulate EPS into dissolved organic matter by bacteria form an important pathway for organic carbon cycling in the Antarctic environment. In addition, viruses can depolymerize the EPS secreted by bacteria contributing to the degradation of DOM (Lelchat et al., 2019). Although viruses are abundant in glacier environments (Laybourn-Parry and Pearce, 2016, 2007) and influence DOM production through viral induced lysis of bacterial cells (Anesio et al., 2017), their potential role in contributing to EPS degradation in these environments is not known. It is worth mentioning that viruses also exhibit glycoprotein capsules that can be broken down and released into the environment during virophage (small viruses that infect giant viruses) mediated lysis, and could be an additional process that potentially contributes to the DOM pool. However, more research is required for an understanding of bacterial EPS-virus interactions in the Antarctic environments, inputs of viral capsular polysaccharides, and the composition and degradation (lysis) of viral capsular material in order to determine their role in organic matter transformation in the Antarctic environment. Irrespective of the source organism, both dissolved and particulate forms of EPS serve a source of carbon and nutrients to other organisms in the glacier environment (Nichols et al., 2005a). EPS and other organic matter
that are not degraded by heteroraphs are transported to englacial and subglacial habitats via
drain channels or melt runoff (Stibal et al., 2012), thereby acting as a carbon sink.
Additionally, during summer, glacial melt transport nutrients from supraglacial habitats to
the surrounding ocean leading to phytoplankton blooms, contributing further to EPS
synthesis. Marine EPSs in the sea surface microlayer may also result in the release of
atmospherically active aerosols with ice nucleating abilities, when sea-spray aerosols
enriched in EPS are ejected into the polar atmosphere during bubble bursting (Wilson et al.,
2015), potentially influencing the climate (Hong et al., 2020).

Antarctic sea ice supports abundant algal communities that produce dissolved and
particulate EPS (Underwood et al., 2010), possibly for cryo-protection and protection from
high salinity brines (Ewert and Deming, 2013). EPS in turn serves as a significant carbon
source contributing to > 68% of dissolved carbohydrates in Antarctic sea ice habitats, which
on ice melt influence the water column carbon cycling (Underwood et al., 2010) and vertical
carbon fluxes to deeper polar waters (Riedel et al., 2007). At Weddell Sea, total dissolved
carbohydrates (of which about 36% is EPS) constitute up to 90% of the DOC in bulk sea ice
brines. Further, particulate EPS contributes up to 30% of the particulate organic carbon
(POC) in sea ice indicating that EPS is subsidized nearly equally between dissolved and
particulate forms in sea ice (Underwood et al., 2010). POC in the environment are subject to
transitions between dissolved and particulate phases by various processes such as
aggregation (Bhaskar et al., 2005), photo-flocculation (Shammi et al., 2017), hydrolysis
(Underwood et al., 2010), and microbial degradation (Hofmann et al., 2009). The physical
aggregation of EPS produced by sea ice algae to form larger particles may promote the
sinking of particulate organic matter from the surface waters (Riebesell et al., 1991), with
potential implications for food webs and carbon turnover in the ocean. In the environment, EPSs are modified by physical, photochemical and enzymatic processes resulting in components, ranging from highly labile to relatively refractory (Decho and Gutierrez, 2017). The labile portion comprises high molecular weight EPS (\( > 1 \) kDa) with short residence time and is composed of deoxy and neutral monosaccharides, amino acids and labile peptides and glycoproteins, while the refractory part with low molecular weight (\(< 1 \) kDa), comprise uronic acids, aminosugars, amyloids and other recalcitrant proteins (Decho and Gutierrez, 2017). Recent studies have shown that green algal EPS is protein rich with a greater proportion of amino acids, while that produced by red algae has higher carbohydrate and lipid content (Davey et al., 2019). The compositional differences in EPS and modifications post-secretion will determine its transition from DOM to particulate organic matter (POM).

In the water column, labile EPS undergoes bio- and/or photo-degradation to form DOM, contributing to DOC and dissolved organic nitrogen, while the refractory fraction may undergo physicochemical association and/or photo-flocculation with other lithogenic and biogenic elements to form POM. It is mainly the resistant uronic acid content of refractory ExP that possesses stickiness enabling cross links with divalent ions to develop gels, transparent exopolymer particles (dense EPS gels > 0.4 \( \mu \)m formed by the aggregation of smaller EPS molecules), bioflocs, marine snow (aggregates of > 500 \( \mu \)m, comprising dead phytoplankton and other planktonic microorganisms within an EPS matrix), and marine oil snow (marine snow that contains oil droplets embedded within its matrix), which may sink to the bottom and fuel marine food webs (Shammi et al., 2017). This aggregation of DOM molecules into POM plays a key role in the redistribution of carbon in polar marine systems.
and affects the flux of carbon to the sea floor (Alldredge, 2000; Meiners et al., 2004), in turn impacting the global cycling of carbon (Krembs et al., 2002; Simon et al., 2002).

3.3. Iron biogeochemical cycle

Iron (Fe) is a vital element for primary productivity. In the remote Southern Ocean, where external inputs of Fe are low (de Baar et al., 1990), phytoplankton growth is limited by the availability of Fe. In Antarctic ecosystems, iron is present in ferrous (Fe$^{+2}$) and ferric (Fe$^{+3}$) states in dissolved (DFe < 0.2µ) and particulate (PFe > 0.2µ) forms, of which Fe$^{+2}$ in dissolved form is the most bioavailable. Concentrations of both DFe and PFe are at least a magnitude higher in sea ice than in typical Antarctic surface waters (Lannuzel et al., 2016). A large fraction of the Fe is bioavailable and therefore when released during sea ice melt can boost primary productivity in the Fe-depleted polar waters. However, during spring melt, a temporal decoupling between the release of DFe and PFe into the sea water has been observed with DFe being released first, followed by PFe (Lannuzel et al., 2013; van der Merwe et al., 2011). This delayed release of PFe is believed to involve the complexation of Fe with EPS in the brine phase of sea-ice (Krembs et al., 2002). The negatively charged surface of EPS (Decho, 1990; Nichols et al., 2005a) helps bind cationic metals like Fe$^{3+}$ and Fe$^{2+}$. This together with the fact that EPS in marine systems, can be several orders of magnitude more adhesive than other particles (Passow, 2002), facilitates the attachment and adhesion of PFe in sea ice (Janssens et al., 2018; Lannuzel et al., 2016). EPS thus serves as an organic ligand that controls DFe enrichment in sea ice (Lannuzel et al., 2015). After ligand saturation, a part of excess unbound Fe undergoes oxidative precipitation to PFe (Thuróczy et al., 2012), while the rest participates in a positive feedback cycle, wherein, high Fe release leads to enhanced primary productivity. This in turn contributes more organic
ligands that bind the excess Fe and maintains its soluble state (Lannuzel et al., 2015). Despite being a weak ligand, EPS competes with strong ligands like siderophores due to the fact that i) ExPs offers several binding sites-saccharides, uronic acid and sulfate groups (Hassler et al., 2011; Norman et al., 2015); ii) EPS is abundant in dissolved, particulate and colloidal forms in water column; iii) it is mass produced by algae and bacteria; iv) it can enhance Fe solubility and increase its residence time; and v) EPS adsorbs Fe oxyhydroxides and prevents their aggregation and subsequent conversion to PFe (Hassler et al., 2015). Further, Fe-EPS binding, in some cases may be higher than that of Fe-siderophore (Norman et al., 2015). The EPSs with more acidic groups can bind PFe by organic complexation and increase its residence time and bioavailability (Hassler et al., 2011). When zooplankton feed on organic matter (principally EPS) it releases Fe back into the system. Thus, EPS clearly plays a crucial role in the enrichment of Fe in sea ice and the cycling of Fe in polar waters. In the context of changing sea-ice conditions due to climatic perturbations, it is more critical now than ever to identify and quantify the links between EPS-Fe and sea-ice and how these are likely to be affected in the future.

3.4. Uptake and accumulation of contaminants

Human interference in pristine Antarctic environment has resulted in tangible contamination of the Antarctic environment (Aronson et al., 2011; Stark et al., 2014). A rapid increase in tourists and scientific crew, mainly during summers, when Antarctic ecosystems are most active, has ramified the impact and established non-ingenious flora and fauna in the Antarctic subcontinent (Convey et al., 2012; Frenot et al., 2005). Further, inflating human footprints, for research activities, has led to the import of nonnative microorganisms (Hughes et al., 2013). Apart from biological contamination, chemical pollutants include xenobiotics,
microplastics, oil spills, heavy metals, flame retardants and chemicals released from sewage disposal (Tin et al., 2009).

Microplastic particles have been reported in sediments at Terra Nova Bay (Munari et al., 2017), marine sediment at Ryder Bay (Reed et al., 2018), in scats of gentoo penguins (Bessa et al., 2019) and East Antarctic sea ice (Kelly et al., 2020) with the most prevalent microplastic material being polyethylene, polypropylene and polyamide. Recent studies have reported that the sea ice algae Fragillariopsis cylindrus produces sticky EPS that binds microplastic granules (Hoffmann et al., 2020) enabling the entry of microplastics into the food web. Further, microplastics stress the microbes and induces enhanced production of EPS (Lagarde et al., 2016), which in turn accumulates more microplastics, increasing its adverse effects. A comprehensive study of persistent organic pollutants in Antarctica, revealed the presence of a total of 70 persistent organic pollutants including pharmaceuticals, biocides and personal care products in phytoplankton cells (Duarte et al., 2021), sampled from Deception Island. It is possible that EPS being amphiphilic might have acted as a vehicle to transport the organic pollutants inside the cell membrane causing bioaccumulation of xenobiotics in primary producers. Such accumulation of highly toxic contaminants could have unforeseen impacts on marine food webs.

4. EPSs functionalities and chemistry behind them

4.1. Cryoprotection

Extremely low temperature freezes cellular fluids, decelerate metabolic activities and affects several key biological processes. Microbes residing in extreme cold Antarctic environments employ diverse adaptive strategies to contravene the deleterious effects of low
temperature (Caruso et al., 2019; Marx et al., 2009; Underwood et al., 2010). EPSs are involved in microbial survival and sustenance in Antarctica as they form a barrier around the cell preventing membrane disruption during freezing and protect it from thermal shock and frequent freeze/thaw cycles. The major component of EPS, i.e. ExP (Caruso et al., 2018b; Manca et al., 1996; Nichols et al., 2004) surrounds the microbial cells as a branched framework. The amorphous ExP possess a property called glass transition temperature ($T_g$), which is defined as a temperature at which polymer structure undergoes a transition from rigid to flexible state. When cells encased in the polymer matrix are exposed to subfreezing temperatures, water molecules in ExP starts freezing till a stage is reached where the polymer is in equilibrium with frozen ice crystals in the surrounding environment. This temperature at which the exopolymers undergo a conformational change from rigid to flexible, that allow water molecules around it to freeze till it attains a limiting glass transition state i.e. aqueous polymeric solution is in equilibrium with frozen ice crystals, is referred to as the limiting glass transition temperature. Polymers with limiting $T_g$ of ca. -20°C exhibit the best cryoprotectant abilities (Takahashi et al., 1988). As the amount of liquid water decreases, viscosity of exopolymer increases, which in response starts supercooling the cells moderately. As temperature further sink from $T_g$ value, this highly viscous layer solidifies and prevents loss or exchange of moisture between cells and extracellular ice, maintaining osmotic balance. Further, laboratory experiments illustrates that at temperatures below -70°C, formation of intracellular ice crystals is suppressed by high intracellular fluid viscosities and hence it allows formation of ice crystals ≤ 300 nm, which is tolerable to cell (Takahashi et al., 1988). While microbial cells in Antarctica do not frequently encounter such ultra-low
temperatures, microbial assemblages encased in EPS will be better equipped to tide over such temperature lows compared to other non-EPS producing microbes.

4.2. Anti-desiccation

In Antarctic terrestrial ecosystems, availability of moisture and liquid water is limited, due to extremely dry air and biological inaccessibility of water in the form of ice (Convey et al., 2014). The maintenance of water balance and adaptation to low moisture availability is critical for the survival of microbes living in extreme dry environments. Different organisms employ different strategies to circumvent desiccation. Lichens occupy endolithic, sublithic and interstitial sites in rocks to protect from low temperature, direct radiation and wind (Convey et al., 2014). *Nostoc* cyanobacterial mats in shallow aquatic systems that are subjected to desiccation on several time scales are capable of recovering from prolonged desiccation in as less as 10 minutes of rewetting (Hawes et al., 1992). Other microbes that do not have access to liquid water and/or endolithic refuge, produce EPS as a physiological response to reduce water loss from cells. As EPS comprises of different polymeric components of high viscosity, they tend to be hygroscopic, often containing more water than the bulk environment (Potts, 1994). EPS therefore provides continuous hydration by decreasing the rate of water loss from cells and helps prevent dehydration. Alginates (a component of EPS) from *Pseudomonas mandelii* inhabiting Antarctic marine sediments (Vásquez-Ponce et al., 2017) and Xanthan gum ExP isolated from *Parageobacillus thermoantarcticus* from Antarctic soil (Giudice and Poli, 2020), were found to hold water up to 4 and 18 times the weight of polymers, respectively (Horstmann et al., 2018). ExPs hold water molecules in their hydration layers. Water in these macromolecules can either be
present around the polymer in aqueous solution or bound within the 3D network of polymeric chains through H-bonding which in turn stabilizes the matrix. While the unbound water freezes at low temperature, bound water molecules neither participate in solvation, nor do they freeze at 0°C (Berendsen, 1975). Bound water does not freeze completely because the crystallization of water ceases at a point (ca. -30°C) when the vapour pressures of water in liquid and solid phases attains equilibrium (Belton, 1997). The glass transition property of carbohydrate polymers (as discussed above) also contributes to the co-existence of water in liquid and ice phases in biopolymeric matrix below freezing point. As temperature declines and approaches $T_g$, the system undergoes glass transition state, where the rate of diffusion of bound water molecules becomes slower than the cooling rate. Consequently, the composition of the matrix containing partial ice crystals and liquid water becomes static and further decrease in temperature does not alter hydration levels in EPS (Kocherbitov, 2016).

4.3. **Buffering against high salinity and pH**

Antarctica harbours many freshwater, acidic, alkaline, saline, and hypersaline lakes (Gilbert et al., 2004; Magesh et al., 2020; Murray et al., 2012). Hypersaline and alkaline environments present distinct challenges to resident microbes, especially fluctuations in ion concentrations and osmolarity. High salinity and pH may increase osmotic stress on cells and destabilize the cell membranes (Potts, 1994). Microbes in these systems often produce either capsular EPS or a biofilm matrix. Biofilms provide partial protection to embedded microbes against ion fluctuations by selectively precipitating salts on its surface (Decho and Gutierrez, 2017). Microbes living within brine inclusions in sea ice also experience extremes of salinity (Junge et al., 2001). These microbes produce EPS (Aslam et al., 2012; Marx et al., 2009), as
a mechanism to survive the cold and saline conditions of sea ice. Marine microbes exude mostly acidic EPS bearing functional groups—uronic acids, sulfates, pyruvates, succinyl, lactates, phosphates, and acetates (Nichols et al., 2005a). In Antarctica, acidic EPSs isolated till date contains mainly uronic acids and sulfates (Nichols et al., 2005b). EPS, due to the presence of carboxylic acid group on uronic acid moiety, acts as a weak acid and dissociates partially to maintain equilibrium (Equation 1), where R is a long EPS chain containing uronic acid residue with functional group COOH. In Equation 1, only one -COOH group has been shown for the sake of simplification. However, ExP actually contains multiple uronic acid moieties with numerous -COOH groups. In an alkaline environment (pH > 7), the equilibrium shifts to right resulting in higher degree of dissociation, conferring negative charge to EPS. At high salinity, salts such as NaCl, KCl, MgCl₂, CaCl₂, present in lacustrine water would dissociate (Equations 2 and 3) and EPS in its carboxylate form, binds with metal ions such as Na⁺, K⁺, Ca²⁺, Mg²⁺ to form esters (Equations 4 and 5). At pH >7.0, equation 1 would move in the forward direction (i.e., more EPS would dissociate and chelate cations), thereby preventing their diffusion across the cell wall, maintaining required osmotic levels for cell survival. Equations 4 and 5 are reversible and pH dependent. The pH depends upon factors such as dissolved CO₂ concentration in water, microbial respiration, photosynthesis and presence of other salts—carbonates, bicarbonates, phosphates, nitrates, sulfates and silicates. Under high/low pH conditions, microbial EPS counteracts the effect of high salinity and pH, as shown in equations 4 and 5 thereby helping microbes overcome its adverse effects.
Nature has engineered microbes with various organic ligands, including several primary and secondary metabolites like EPS, polyphenols, siderophores, flavonoids etc. that confer negative charge and bind metals reversibly. Major heavy metals reported from some Antarctic environments (soils, lakes, cryoconite holes, sediments and marine ecosystems) are iron, aluminium, manganese, mercury, lead, nickel, vanadium, thallium, cadmium, copper, zinc, cobalt and arsenic, originated and/or transported from different natural and anthropogenic sources (Buda et al., 2020; Chu et al., 2019; Majer et al., 2014; Metcheva et al., 2010). EPSs regulate oxidation states of metals and hence the toxicity. They encompass several binding sites including uronic acids, sulfates, hydroxyl groups, acetates, pyruvates, phosphates, etc., that can
chelate metals (Nichols et al., 2005a; Figure 5). These EPS ligand–metal attachment processes can also help bind to mineral metal oxide surfaces (Li and Logan, 2004). EPSs from Antarctic seawater microbes efficiently chelate heavy metals, enabling the cells to tolerate high concentrations (Caruso et al., 2018a, 2018b, 2019). Higher metal content triggers enhanced EPS production and hence, higher metal uptake capacity, leading to reduced free metals in surrounding environment, highlighting the role of EPS in counteracting metal stress (Caruso et al., 2018b). This has implications for bioremediation as these EPS producing microbes can be exploited as a valuable tool to remove heavy metal pollutants from water (Caruso et al., 2019).

On glacier surface environments, scavenging of trace elements by EPS produced by algae and bacteria result in elevated concentration of metals in cryoconite holes (Owens et al., 2019). As microbes inhabiting cryoconite produce copious quantities of EPS, EPS mediated cell–metal interactions may be significant within the cryoconite. This could have implications for aquatic ecosystems located downstream when glacial melt and the release of cryoconite contents can seed these environments with high metal loadings.

### 4.5. Surface adhesion, colonization and aggregate formation

Aggregate formation, surface adhesion and colonization are survival strategies of microorganisms as it prevents their direct exposure to extreme cold, toxic metal ions, ocean currents, UV radiations and predators. The stickiness of EPS owing to the uronic acids and sulfate groups on it, glues the cells, nutrients and organic matter together to form aggregates. Depending on the influence of local environment, different physicochemical transformations of EPS bound aggregates occur to form POM, transparent exopolymer particles, marine snow and marine oil snow (Decho and Gutierrez, 2017). EPS due to presence of hydrophilic components
(uronic acids, sulfates, polyhydroxyl groups), and hydrophobic components (methyls, acetylts, protein moieties, 3,6-anhydrous monosaccharides) interact with surrounding particles through electrostatic, covalent, vander waal, hydrogen-bonding, and cross linking. Such interactions enable EPS to undergo self-aggregation from molecular- to micro-scale to form self-assembled networks. For instance, transparent exopolymer particles are formed by cross linking Ca$^{2+}$ and Mg$^{2+}$ ions with sulfate (-$\text{OSO}_3^-$) groups of EPS and converting DOM to POM as marine gels within minutes (Decho and Gutierrez, 2017). Further, marine snow is formed by physisorption of dead or dying phytoplankton, fecal matter, microbes and inorganic particles on EPS and then sticking to it by intermolecular forces. Similarly, marine oil snow is formed when EPS component of marine snow build hydrophobic interactions with oil hydrocarbons.

### 4.6. Emulsifying properties

An emulsifier is a chemical that stabilizes lipophillic (oil) and hydrophillic (water) emulsion by reducing the surface tension of later. EPS extracted from *Pseudomonas sp.* ID1 from South Shetland Islands in Antarctica possesses excellent emulsifying properties against olive, sunflower and corn oils and n-hexadecane, which is equivalent and, in some cases higher than commercial emulsifiers- xanthan and arabic gums (Carrióń et al., 2015). Further, EPSs isolated from Antarctic *Marinobacter* sp., *Winogradskyella* sp., and *Shewanella* sp., strains exhibit high emulsifying activity against hexane, octane, hexadecane and tetradecane compared to the commercial emulsifiers Tween 80 and Triton X-100 (Caruso et al., 2018a, 2019). The emulsifying property of EPS enables the psychrophiles to degrade and utilize hydrocarbons (phenol, diesel, oil) as carbon source accumulated in Antarctic ecosystems due to accidental fuel spills (Abdulrasheed et al., 2020; Gentile et al., 2016; Roslee et al., 2020; Ruoppolo et al., 2013).
EPS demonstrates emulsifying activity due to presence of protic (uronic acid, sulfates) groups that bind to water and hydrophobic groups that bind to hydrocarbons. The emulsifying properties of EPS are further enhanced with molecular mass, protein content and degree of branching, which in turn increases hydrophobicity and reduces surface area of the exopolymer. The ecological roles and functionalities of extracellular polymeric substances in polar environments are summarised in Table 1.

5. EPS composition as a function of the surrounding environment

EPS composition was considered to be a function of microbial species i.e. microbes of the same species were believed to secrete EPS of same physical, chemical and rheological properties irrespective of their biotic and abiotic environments. The structural profiles of EPSs from various algal and microbial species, collected from different Antarctic environments show that ExPs are dominant constituents, followed by proteins and uronic acids (Figure 6; Caruso et al., 2019, 2018b; Manca et al., 1996; Nichols et al., 2004). Further ExP profiling reveals mannose and glucose as ubiquitous components of EPS in Antarctic microbes, followed by galactose and galacturonic acid (Figure 7). Higher amount of carbohydrates (CHO) is a result of unbalanced microbial growth in limited nutrient supply. The autotrophic algae and diatoms growing under adequate light and CO₂, but under nitrogen limitation continue to photosynthesize and produce glucose. However, the fixed carbon is not converted into amino acids and proteins but CHO (Stal, 2010). Conversely, EPSs extracted from sea water and marine sediments collected from higher latitudes along the Terra Nova Bay and east Weddell Sea region of Antarctica, contain high lipids and higher proteins than ExPs, with protein to CHO ratio in the range 2 to 12 (Baldi et al., 2010; Isla et al., 2006).
microbial assemblages in these sediments include aerobic and anaerobic heterotrophs belonging to *Gamma*- and *Delta*-proteobacteria, *Bacteroidetes* and *Acidobacteria*. Anaerobic conditions in sediments promote the production of amino acids over CHOs, since former has lower fermentation rates than later. As a result, the microbes feeding on these protein rich EPSs would take longer to degrade refractory protein than CHOs, leading to extended sustenance of EPS producing microbes (Baldi et al., 2010).

*Pseudoalteromonas* species isolated from an Antarctic marine habitat was found to produce 30-fold higher EPS at -2 and 10°C than at 20°C, suggesting that EPS is produced in retort to temperature stress (Nichols et al., 2004). Similarly, when sea ice diatoms *Synedropsis* sp., *Fragilariopsis curta* and *Fragilariopsis cylindrus* were exposed to conditions of nutrient limitation, increased salinity and addition of xanthan gum as an external cryoprotectant at -12°C, nutrient limitation was found to have more pronounced impacts on lowering the EPSs yields and EPS glucose content in all organisms. High salinity was found to reduce microbial growth but enhance EPS production. EPS production was reduced in presence of the cryoprotectant xanthan gum (Aslam et al., 2012). Therefore, the surrounding environmental conditions seem to influence EPS composition rather than the microbial species involved. The presence/absence of certain growth substrates also influenced EPS composition. For example, EPS of a haloalkalophilic *Halomonas* strain was composed of mannan and xylomannan when grown in the absence of 1% sodium acetate in complex media, and of fructo-glucan when grown in its presence (Poli et al., 2004). The EPS produced by this *Halomonas* strain when grown in the absence/presence of 1% sodium acetate were completely different in composition and bonding and required entirely different enzymes for degradation. This illustrates the unique versatility of microbial EPS producing
genes. These observations highlight the influence of localized environmental stress on microbial abilities to cope up with it. Such observations ascertain that EPS composition is a function of the surrounding environmental conditions. This is emphasized in a recent study (Blanco et al., 2019) where twenty phylogenetically disparate microbial mats, collected from five extreme habitats were tested for EPS composition and biochemical characterization. The results revealed that although biofilms collected from similar environments, had distinct phylogeny, they had similar EPS composition. The authors suggest that horizontal gene transfer between different species in the same environment could be responsible for similarities in the EPS profiles. Another possibility could be adaptability and expression of EPS producing gene clusters in microbes that are triggered by same environmental conditions. This implies that microbes from specific environments can be exploited to obtain EPS of desired functionalities. More studies in the field of EPS gene regulation is required to reach tangible conclusions.

6. Perspectives and Future Prospects

EPS plays central roles in assisting microbes survive in extreme climatic conditions of Antarctica and participates in several key ecological and biogeochemical processes. While current research highlights the properties, ecological functions and biotechnological applications of EPS, there is huge scope for further research in the following associated fields: 1) interaction of microbial EPS and virus; 2) role of viral ExPs in Antarctic ecosystems; 3) EPS gene regulation, pathways of EPS production and adaptive structural modification; and 4) interaction of polar microbial EPS with xenobiotics and microplastics.
Some recommendations to expand the knowledge database of polar EPS and develop a better understanding of its role in polar environments are as follows:

**Structural Characterization of EPS:** Very few microbial species, specifically members of the genera *Halomonas, Psychrobacter, Winogradskyella, Colwellia, Shewanella, Cryptococcus, Marinobacter, Parageobacillus, Pseudoalteromonas, Pseudomonas* and *Cytophaga-Flexibacter* have been investigated for their EPS structure. Thus, there is a vast scope for isolation and structural characterization of EPS from unexplored polar species to understand their uniqueness and participation in ecological processes. Amongst isolated and characterized Antarctic EPSs, very limited number of EPS structures and/or ExPs has been systematically investigated. In most cases, only primary structural data (CHO, protein, uronic acid, sulfate contents, monosaccharide composition) has been determined and vital information on molecular weight, linkage, conformations (α or β, pyranose or furanose), branching etc., is not available. Hence, a systematic approach for meticulous structure characterization of EPS is required to obtain a reliable and complete database. Chemical structural investigations of EPS involve chromatographic, spectroscopic and hyphenated mass spectrometric techniques. The isolation and purification of EPS prior to chromatographic measurements can result in changes in the chemical and physical properties of EPS due to degradation occurring during isolation and purification processes. The extraction conditions or the methods used for derivatizing the samples for determining monosaccharide composition, linkage analysis, uronic acid, CHO, protein and sulfate concentrations of EPS could cause the biochemical composition of EPS to change. This can be overcome by employing non-destructive spectroscopic approaches – NMR (nuclear magnetic resonance), Raman, IR (infrared) - to retain the integrity of samples and obtain
more reliable unbiased structural information of EPS. Further, *in situ* experiments designed to study factors controlling EPS abundance and distribution in the natural environment are required to understand the overall contribution of EPS to the glacier carbon budget. Small nano scale quantities of sample from the original habitat is adequate for advanced spectroscopic and chromatographic techniques with dynamic modules that allow monitoring of physicochemical changes in samples with time. Moreover, live interaction of EPS in an ecosystem can be studied by combining isotopic substitution and dynamic NMR and nanoSIMS (nanoscale secondary ion mass spectrometry) isotope ratio. Such approaches can be used to track the inputs of EPS to other biogeochemical processes.

**EPS as proxies for nutrient availability and environmental conditions:** It is evident that EPS composition is not a function of species, but of the surrounding environment, i.e. the microbes of different species in similar habitats secrete EPS of similar composition. Further, structure-nutrient relationship and horizontal gene transfer studies are required to substantiate this fact. The information can be used to develop new EPS based proxies viz. uronic acid/ExP, acidic EPS/neutral EPS, uronic acid/sulfate, molecular weight range, EPS/total CHO, that can be used to predict nutrient availability in a particular habitat.

**EPS for bioremediation in Antarctica:** A growing body of evidence has revealed the presence of chemical contaminants, especially xenobiotics, microplastics and oil spills in Antarctic subcontinent. Though recent studies have reported bioaccumulation of these pollutants in the Antarctic food web (Bessa et al., 2019; Duarte et al., 2021; Kosek et al., 2016; Raymond et al., 2017), further investigations are needed to study the interaction of microbial EPS with contaminants and to determine the role of EPS in circumventing the anthropogenic influence...
on polar microbiota. Laboratory based experiments have established a higher efficacy of marine and psychrophilic EPSs in degrading and utilizing hydrocarbons (Abdulrasheed et al., 2020; Gentile et al., 2016; Roslee et al., 2020) and xenobiotics (Kosek et al., 2016; Santschi, 2018). However, specific in situ case studies are required to substantiate this role in the Antarctic environment.

As evident from this study, despite a growing awareness of microbial EPS and their influences on ecological and biogeochemical processes, microbial EPS in Antarctic environments remains a less explored field of study with numerous research opportunities. The significance of EPS in Antarctic environments must be assessed not only in terms of how much EPS is present, but also what kinds of EPS are present, the processes that regulate its secretion, and its role in ecological and biogeochemical processes. In the context of a fast-changing climate, and potential large-scale disturbances to Antarctic landscapes and ecosystems, it is important to clearly identify the drivers and to quantify the processes that affect EPS biosynthesis and functions in cold regions.

Conflicts of interest
The authors declare that they have no conflict of interest.

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Author contributions

The paper is written by SN and RA, with inputs from MT.

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Table 1. Ecological roles and functionalities of extracellular polymeric substances in polar environments

<table>
<thead>
<tr>
<th>S.No</th>
<th>EPS functionality/Role</th>
<th>Chemistry behind functionality</th>
<th>Effect</th>
<th>Examples of microbial genera/species that exhibit this functionality</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1</td>
<td>Cryoprotection</td>
<td>Amorphous nature; possess limiting glass transition temperature of ca. -20°C</td>
<td>Microbes sustain repetitive freeze-thaw cycles</td>
<td>Winogradskyella, Colwellia, Shewanella</td>
<td>Caruso et al., 2018b; Takahashi et al., 1988</td>
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<td>2</td>
<td>Anti-desiccation</td>
<td>Presence of unfrozen bound water in hydration layers of 3D network</td>
<td>High water holding capacities</td>
<td>Pseudomonas mandelii, Acidobacteria, Nostoc commune</td>
<td>Horstmann et al., 2018; Kielak et al., 2017; Giudice and Poli, 2020; Novis and Smissen, 2006; Vásquez-Ponce et al., 2017</td>
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<td></td>
<td>Function</td>
<td>Mechanism</td>
<td>Organisms</td>
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<td>3</td>
<td>Buffering against high salinity and pH</td>
<td>EPS with uronic acid moiety act as weak acid and dissociates reversibly at pH &gt; 7.0</td>
<td>Maintains cellular osmotic levels by preventing ionic diffusion across the cell wall</td>
<td><em>Lentisphaera araneosa</em>, <em>Verrucomicrobium spinosum, Spirochaeta</em></td>
<td>Aslam et al., 2012; Decho and Gutierrez, 2017; Gilbert et al., 2004; Murray et al., 2012</td>
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<tr>
<td>4</td>
<td>Trace metal uptake and binding</td>
<td>Presence of binding sites- sulfates, uronic acid, acetates, phosphates</td>
<td>Regulates the toxicity and bioavailability of trace metals; Provides scaffold for binding silica and calcium carbonate</td>
<td><em>Pseudoalteromonas, Shewanella, Winogradskyella</em></td>
<td>Caruso et al., 2018b; Lee et al., 2016; Santschi, 2018</td>
</tr>
<tr>
<td>5</td>
<td>Surface adhesion, colonization and aggregate</td>
<td>Presence of uronic groups enhances “stickiness” of the biopolymer</td>
<td>Bioflocculation, biofilm formation, transparent</td>
<td><em>Oscillatoria, Actinobacteria, Proteobacteria</em></td>
<td>Decho and Gutierrez, 2017; Ricciardelli et al., 2019; Smith et al., 2016</td>
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<tr>
<td>Formation</td>
<td>Properties</td>
<td>Exopolymeric particles</td>
<td>Bacterial taxa</td>
<td>References</td>
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<tr>
<td>Emulsifying properties</td>
<td>Amphiphilic groups of EPS reduces the surface tension of hydrophilic layers and stabilize oil in water emulsion</td>
<td>Marine oil snow formation</td>
<td>Shewanella, Winogradskyella, Marinobacter, Winogradskyella</td>
<td>Carrión et al., 2015; Caruso et al., 2019, 2018b</td>
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<tr>
<td>Cryoconite granule formation</td>
<td>Dust, soot, microbes and mineral particles are glued together by EPS on the supraglacial surface</td>
<td>Cryoconite granules bound together by EPS reduces albedo and enhances melting</td>
<td>Cyanobacteria, Cytophaga-Flavobacteria, Gemmatinomonas</td>
<td>Cook et al., 2016; Laybourn-Parry and Pearce, 2016</td>
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<tr>
<td>DOM source</td>
<td>Labile fraction of high molecular weight EPS, composed of neutral</td>
<td>Fuels the food web; source of readily available</td>
<td>Cyanobacteria, Actinobacteria, Alphaproteobacteria</td>
<td>Decho and Gutierrez, 2017; Smith et al., 2016; Stibal et al., 2012</td>
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<tr>
<td>POM source</td>
<td>Low molecular weight refractory and labile EPS fractions, composed of uronic acids, amino sugars, neutral sugars and amyloids undergo physicochemically mediated self-association and binding with other</td>
<td>Transported through melt water to downstream supraglacial, englacial and subglacial habitats and acts as a carbon sink</td>
<td>Cyanobacteria, Actinobacteria, Alphaproteobacteria, Bacteroidetes, Betaproteobacteria</td>
<td>Decho and Gutierrez, 2017; Smith et al., 2016; Stibal et al., 2012</td>
<td><strong>Bacteroidetes, Betaproteobacteria</strong></td>
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Monosaccharides, amino acids, labile peptides and glycoproteins undergoes abiotic and biotic degradation to form low molecular weight fractions that could be labile or refractory carbon substrate for heterotrophs.
<table>
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<tr>
<th></th>
<th>Protection against UV</th>
<th>UV active pigments are secreted and accumulated in biofilm by microbes.</th>
<th>UV protection to snow and ice algae</th>
<th>Sanionia uncinate, Cyanobacteria, Mesotaenium berggrenii,</th>
<th>Anesio et al., 2017; Foreman et al., 2013; Lud et al., 2003</th>
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<tr>
<td>10</td>
<td>Sediment stabilization</td>
<td>Negatively charged groups of EPS attach to sediment sublayers via reversible physical adsorption or ionic interactions.</td>
<td>Improved sediment erosion threshold</td>
<td>Cyanobacteria, Bacillus subtilis</td>
<td>Chen et al., 2017; Stal, 2003</td>
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</table>
Figure 1. Bacterial capsular polysaccharides and exopolysaccharides [Image courtesy, Aritri Sanyal]. A) Capsular polysaccharides form a capsule structure (stained blue with alcian blue) enveloping the cell (stained pink with safranin). Capsular polysaccharides form strong associations with the cell. B) Secreted exopolysaccharides (blue) lack robust cell (stained pink) surface associations and are released into the immediate environment. Both polysaccharides play critical roles in interaction between microbes and the surrounding environment.

Figure 2. Microbial EPS from different Antarctic environments. A) Slimy green algal mat from an Antarctic lake [Image courtesy, Abhijit Patil and Pratibha Gupta], B) Cryoconite granules formed by the bundling of dust and organic material by sticky EPS extruded by cyanobacteria, C) a close up of the EPS-rich micro-aggregates, D) EPS producing bacteria isolated from Antarctic cryoconite holes exhibit a glossy and slimy appearance on nutrient media plates [Image courtesy, Aritri Sanyal], E) Microscopic images of yeast cells (stained pink with safranin) embedded within extracellular polymeric substance (stained blue with alcian blue) [Image courtesy, Aritri Sanyal], F) An EPS film produced by bacteria isolated from Antarctic ice and G) Scanning electron microscopy image of uncultured bacteria in Antarctic snow enveloped in a layer of EPS (See arrow).

Figure 3. Various modes of EPS mediated intra- and inter- microbial interactions with the polar environment.

Figure 4. Schematic illustrating the distribution of extracellular polymeric substances in major Polar habitats; In supraglacial environments (snow, meltpools), EPS is secreted by
photoautotrophic snow algae, cyanobacteria and heterotrophic microbes. This EPS acts as a

glue that coalesce other microbes, dust, soot, minerals and organic matter on the ice

surface, to form dark cryoconite granules. These aggregates owing to darkening effect

reduce albedo of snow, absorb more heat and melt the ice below forming cryoconite holes.

The microbes residing at the bottom of cryoconite holes and meltpools produce biofilms

that aid in colonizing and stabilizing the sediments. During melting and run off, the organic

matter, microbes, EPS etc. from cryoconite holes drain into englacial environments and

subglacial lakes through hydrological networks. EPS produced by microbes in hypersaline

lakes aids in buffering of excess of salt in water and maintains adequate osmotic levels for

their survival. In the marine environment, sea ice algae produce copious amounts of EPS

that when released into the water column, combine with dead microorganisms,

phytoplankton, zooplankton, feces, organic and inorganic matter to form marine snow that

sinks down the bottom of sea floor and acts as energy reservoir for benthic organisms.

Exopolysaccharides also crosslink abiotically with calcium and magnesium ions in the

water column forming colloidal gel particles called transparent exopolymer particles. In

events of oil slick, EPS acts as an emulsifier binding oil droplets (hydrocarbon) with other

organic matter forming marine oil snow.

**Figure 5.** Adsorption of metals by the interaction between metal cations and negative

functional groups such as phosphates, sulfates carboxyl and hydroxyl groups on the EPS

surface.

**Figure 6.** EPS composition of Antarctic microbes. The data taken from different sources

has been normalized for comparison (Carrión et al., 2015; Caruso et al., 2018a, 2018b,

2019; Corsaro et al., 2004; Daglio et al., 2018; Finore et al., 2019; Manca et al., 1996;
Figure 7. Exopolysaccharide compositions of different Antarctic microbial species. The data taken from different sources has been normalized for comparison (Bai et al., 2012; Carrión et al., 2015; Caruso et al., 2018b, 2018a, 2019; Chen et al., 2015; Corsaro et al., 2004; Daglio et al., 2018; Finore et al., 2019; Manca et al., 1996; Mukhopadhyay et al., 2014; Nichols et al., 2004; Nichols et al., 2005a; Norman et al., 2015; Pavlova et al., 2009; Poli et al., 2004, 2007; Selbmann et al., 2002; Vásquez-Ponce et al., 2017; Vlaev et al., 2013; Yu et al., 2016).
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: